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INFLUENCE OF BODY POSITION, EMOTIONS, PLACEBO AND COGNITIVE MODULATION ON PAIN EXPERIENCE AND PAIN-RELATED SOMATOSENSORY ERPs

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ENGLISH SUMMARY

The present work contributed to our understanding of the neurocognitive mechanisms underlying pain modulation through sensory, attentional, emotional and cognitive processes. We used subjective, behavioral, and electrophysiological indexes to reveal the effects of body position, emotions, placebo expectations and cognitive reappraisal on subjective pain experience and pain-related somatosensory potentials. Four studies were conducted to investigate different forms of pain modulation. Study 1 tested the hypothesis that the horizontal body position reduces pain perception and cortical pain processing. We demonstrated that the supine vs. sitting body position was associated with dampened perception of non-painful stimuli and inhibited cortical late processing (300-600 ms) of non-painful and painful stimuli, related to neural activity within frontal right regions (anterior cingulate cortex and superior frontal gyrus). Study 2 investigated gender differences in the emotional modulation of pain. Although males and females did not differ at the behavioral level and reported reduced pain ratings only during the visual perception of erotic pictures, striking gender differences emerged in the N2 and P2 potentials, elicited by painful stimuli. Males showed inhibited cortical processing of pain stimuli when viewing erotic pictures only, whereas females showed a differentiated cortical pain modulation for each emotional content took into consideration (erotic vs. sport/adventure vs. neutral vs. fear/threat vs. mutilation pictures), in particular for N2 potentials. In Study 3, we examine the role of individual beliefs on the effectiveness of a traditional and a homeopathic analgesic treatment. We utilized a deceptive paradigm, i.e., neither the participants nor the experimenters were aware that the administered treatment was an inert substance. We found that only the participants who took a treatment that was coherent with their beliefs showed a reduced cortical pain processing, indicated by dampened P2 amplitudes. Finally, Study 4 demonstrated that healthy participants are able to modify their pain experience using an imaginary-based reappraisal strategy. Perceived pain intensity and unpleasantness were either reduced or enhanced with respect to a neutral condition, and an effective pain inhibition was associated with increased N2 and decreased P2 amplitudes.

ITALIAN SUMMARY

Il presente lavoro di ricerca ha contribuito alla comprensione dei meccanismi neurocognitivi sottostanti alla modulazione del dolore da parte di processi sensoriali, attenzionali, emozionali e cognitivi. Abbiamo preso in considerazione indici soggettivi, comportamentali ed elettrofisiologici per rilevare gli effetti della posizione del corpo, delle emozioni, delle aspettative legate al placebo, e del reappraisal cognitivo sull'esperienza soggettiva del dolore e sui potenziali somatosensoriali dolore-relati. Quattro studi sono stati condotti per indagare differenti tipologie di modulazione del dolore. Lo Studio 1 ha testato l'ipotesi che la posizione orizzontale del corpo riduca la percezione e l'elaborazione corticale del dolore. Abbiamo dimostrato che la posizione del corpo supina vs. seduta era associata ad una diminuita percezione di stimoli non dolorosi e ad una inibita elaborazione corticale tardiva (300-600) di stimoli dolorosi e non dolorosi, relata ad attività neurale in regioni frontali destre (corteccia cingolata anteriore e giro frontale superiore). Lo Studio 2 ha indagato le differenze di genere nella modulazione emozionale del dolore. Sebbene maschi e femmine non differissero a livello comportamentale e mostrassero ridotti punteggi di dolore solamente durante la visione di immagini erotiche, delle notevoli differenze di genere sono emerse nei potenziali N2 e P2 elicitati da stimoli dolorosi. I maschi avevano mostrato una inibita elaborazione corticale del dolore solamente durante la visione di immagini erotiche, mentre le femmine hanno mostrato una modulazione corticale del dolore diversificata per ogni contenuto emozionale preso in considerazione (immagine erotiche vs. sport/avventura vs. neutre vs. paura/minaccia vs. mutilazione), in particolare per la N2. Nello Studio 3, abbiamo esaminato il ruolo delle credenze individuali nell'efficacia di un trattamento analgesico tradizionale e di uno omeopatico. Abbiamo utilizzato un paradigma decettivo, i.e., né i partecipanti, né le sperimentatrici erano a conoscenza che il trattamento somministrato era una sostanza inerte. Abbiamo trovato che solamente i partecipanti che assumevano un trattamento che era coerente con le loro credenze mostravano una ridotta elaborazione corticale del dolore, indicata da diminuite ampiezze della P2. Infine, lo Studio 4 ha dimostrato che i partecipanti sani sono in grado di modificare la propria esperienza del dolore, utilizzando una strategia di reappraisal cognitiva che fa uso di immagini mentali. L'intensità di dolore percepita era o diminuita o aumentata rispetto ad una condizione neutra e un'efficace inibizione del dolore era associata ad incrementate ampiezze N2 e diminuite ampiezze P2.

LIST OF ABBREVIATIONS

ACC Anterior Cingulate Cortex

aMCC anterior MidCingulate Cortex

BA Brodmann Area

BOLD Blood Oxygen Level Dependent

BR Bed Rest

CBF Cerebral Blood Flow

CIP Congenital Insensitivity to Pain

CnF Nucleus Cuneiformis

CNS Central Nervous System

DBS Deep Brain Stimulation

DLPFC Dorsolateral Prefrontal Cortex

EBA Extrastriate Body Area

EEG Electroencephalogram

ERF Event Related Field

ERP Event Related Potential

fMRI functional Magnetic Resonance Imaging

HDBR Head Down Bed Rest

IAPS International Affective Picture System

IASP International Association for the Study of Pain

IC Insular Cortex

ICA Independent Component Analysis

ISI Inter Stimulus Interval

LEF Laser Evoked Field

LEP Laser Evoked Potential

LPP Late Positive Potential

MC1Rs melanocortin 1 receptor

MCC MidCingulate Cortex

MEG Magnetoencephalogram

MRI Magnetic Resonance Imaging

N1 Primary Nociceptive Cortex

NMDA N-Methyl-D-Aspartic acid

NMDAR NMDA-type glutamate receptor

OFC orbitofrontal cortex

P1 Primary Pain Cortex

PAG PeriAcqueductal Gray

PB Parabrachial Nucleus

PET positron emission tomography

PFC prefrontal cortex

pgACC pregenual Anterior Cingulate Cortex

PICA Probabilistic Independent Component Analysis

pMCC posterior MidCingulate Cortex

rACC rostral Anterior Cingulate Cortex

RVM Rostral Ventromedial Medulla

S1 Primary Somatosensory Cortex

S2 Secondary Somatosensory Cortex

S3 Third Somatosensory Cortex

sgACC subgenual Anterior Cingulate Cortex

SAM Self-Assessment Manikin

SEF Somatosensory Evoked Fiel

SEP Somatosensory Evoked Potential

SIA Stress-Induced Analgesia

STAI State Trait Anxiety Inventory

SMT Spino-Mesencephalic Tract

SRT Spino-Reticular Tract

STT Spino-Thalamic Tract

tDCS transcranial Direct Current Stimulation

VMpo posterior part of the Ventral Medial nucleus of the thalamus

VTA Ventral Tegmental Area

WDR Wide Dynamic Range

INTRODUCTION

If you are distressed by anything external, the pain is not due to the thing itself, but to your estimate of it; and this you have the power to revoke at any moment.
(Marcus Aurelius, Meditations, 170-180 A.C.)

The questions "what is pain" and "what are the psycho-biological mechanisms causing it" have challenged scholars since antiquity. The importance of answering these questions relates to the fundamental role pain phenomena plays in our everyday survival and experienced quality of life. Accordingly, the opportunity to discover strategies and treatments that relieve pain sensations and suffering has fascinated generations of thinkers and researchers. Improved solutions to managing acute and chronic pain have valuable implications not only for patients' well-being, quality of life and the prevention of physical disabilities, but also for national health care systems in terms of the vast socioeconomic costs of attempting to mediate pain without success.

Pain represents the mechanism through which individuals become immediately aware of an actual or potential lesion that may lead to tissue damage and unpleasant experiences if not stopped or prevented. In most of the cases, pain is inseparable from a strong motivation to avoid it and its consequences. Indeed, pain differs from the exteroceptive senses (i.e., vision, hearing, touch, taste, and smell) because it elicits a motivational drive aimed at avoiding or limiting pain sensations. This motivational function indicates that a primary role of pain is to protect the body from damage, preventing negative conditions that may threaten the individual's long-term survival. An emblematic case is found in patients suffering a rare condition of insensitivity to pain (Congenital Insensitivity to Pain, CIP). These patients cannot be subjectively aware of any damage of their body if not with other senses such as vision and touch, and so small undetected lesions can lead to fatal consequences yet in childhood. Thus, low levels of nociceptive activity and pain are fundamental in daily life, by signaling also when a certain movement or posture may be harmful for the body.

In acute states, pain disappears immediately after the source of potential or actual damage is removed and the body has healed. When pain sensations stop, the

relief is pervasive and comforting. However, in chronic diseases such as headaches, backaches, and neuropathic pain, painful sensations can persist beyond the expected period of healing or fluctuating over long periods of time. In such cases, the long-term management of pain symptoms is a crucial aspect of patients' treatment. Common approaches include pharmacological and chirurgical1 treatments that lead to temporary but typically non-lasting moments of pain relief. Such approaches are often expensive, and can be severely contraindicated by ever-present mechanisms of dependence, habituation, and other ill-advised side effects leading to a serious reduction in patient well-being. In the present thesis, we speculate that individuals and patients can profit greatly from non-pharmacological "treatments" based on emotional, attentional and cognitive manipulations. Psychological strategies alone cannot resolve acute and chronic pain states, but in combination with other pharmacological interventions, can be decisive for comprehensive, effective intervention and for limiting the negative sensations associated to pain conditions.

The complexity of pain experience is also replicated at the neuroanatomical level, which is characterized by diverse and highly distributed neural components ranging from peripheral structures to higher cortical areas associated with emotion and attention regulation. The introduction of non-invasive brain imaging techniques such as EEG, MEG, PET, and fMRI has revolutionized pain studies, establishing the primary role of cerebral cortex for the conscious experience of pain. Before the advent of such techniques, pain was supposed to result from thalamic processing (Head & Holmes, 1911) and the participation of the cortex in pain processing was questioned (Penfield & Boldrey, 1937). Imaging technologies also altered our understanding of chronic pain states. Chronic conditions, initially conceived as deregulation of the somatosensory system, are now recognized as true clinical syndromes related to the dysfunction of emotional and cognitive neural pain modulation, and are neurally degenerative processes (Borsook, Sava, & Becerra, 2010). The past three decades have witnessed the exponential growth of studies investigating the neural structures and mechanisms underlying pain processing. While researchers generated a vast array of novel data using these new research paradigms, quite surprisingly the neural processing responsible of acute and chronic pain conditions is still not completely known (Tracey, 2011).

1 e.g., ablative procedures targeting either the peripheral nerves (such as neurectomies,

During the three years PhD program, we contributed to the comprehension of neurocognitive mechanisms underpinning pain phenomenon, by investigating the modulatory effects of body position, emotion, placebo and cognitive reappraisal on pain experience and pain-related cortical processing mechanisms. This thesis thus synthesizes four experiments aimed to determine the extent to which such manipulations modulate both subjective pain experience and specific ERP deflections elicited by painful and tactile electrical stimulation.

The first and second chapters provide a theoretical and experimental framework for pain studies. Pain is defined as an extreme "malleable" experience, highly susceptible to contextual modulation (Tracey & Dickenson, 2012), which is best understood within a multi-factorial framework. Indeed, both acute and chronic pain experience depend upon the biological, psychological and socio-cultural context in which the pain occurs. This framework is built upon critical findings regarding the neuroanatomical structures and neurophysiological mechanisms underlying nociception and pain. Given the importance of methodological clarity and real-world application for novel research, we consider primary experimental paradigms and results from both experimental and clinical studies. The relevant findings regarding pain modulation are organized in four macro areas: sensory-motor, attentional, emotional, and cognitive pain modulation. In the third chapter, we report the methods, results and discussions of four studies, conducted during the PhD period: (1) *Horizontal body position reduces late cortical pain-related processing;* (2) *Gender differences in pain responses under emotional stimulation;* (3) *Placebo effect in participants with high and low confidence in homeopathy*; (4) *Reappraisal of pain and Mental Imagery induce hypoalgesic and allodynic effects*. Collectively, these studies attempt to elucidate the effects of four domains of pain modulation on pain experience and cortical processing, differentiated depending on the involvement of primarily bottom-up (body position) or top-down strategies (emotional and cognitive manipulations). Finally, the last chapter summarizes relevant implications and conclusions that can be drawn from the present studies.

CHAPTER I

PAIN AND ITS PSYCHOBIOLOGICAL MECHANISMS. THE STATE OF THE ART

"Pain - an emergent, malleable experience rather than a single, static entity."
(Tracey and Dickenson, 2012)

"It remains an act of faith to continue searching the brain for some still undiscovered nest of cells whose activity reliably triggers pain."
(Patrick Wall, 1995)

1.1 PAIN AND NOCICEPTION

The interpretation of "what is pain" followed a tortuous historical pathway, assuming diverse meaning across medical and metaphysical domains. Nowadays, the commonly accepted definition has been provided by the International Association for the Study of Pain (IASP, 1979) who describes pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". According to this definition, the core features of a pain experience include both sensorial (i.e., perceived sensory stimulation) and affective aspects (i.e., hurting feeling). Noteworthy, this IASP statement has the advantage of including those situations in which the physical cause of pain is undetected ("described in terms of such damage"), officially overtaking the past tradition to interpret the lack of biological markers as an index of a psychiatric disorder. For instance, Freud and Breuer (1895) described cases of non-organic pain as hysterical or malingering; for a review, see Tyrer (2006). Currently, it is commonplace to distinguish between nociception and pain and to relate to these concepts to explain cases of dissociation where pain self-reports appear incongruent with physical signs.

Nociception consists of the "neural process of encoding noxious stimuli" (IASP, 1994), namely the neural processes underlying the transfer of information about tissue damage or bodily inflammation from the periphery to the brain. Nociception does not necessarily implicate pain sensations. Instead, pain refers to the conscious experience,

which is often but not necessarily triggered by nociception, and depends upon cortical activity (Treede, Kenshalo, Gracely, & Jones, 1999). Nociception and pain, even if strictly related, are dissociable processes. Nociception without pain occur, for example, during Stress-Induced Analgesia (SIA), when individuals are not aware of severe tissue damage until (for example) the athletic contest or the combat situation is over. Pain without nociception is supposed to emerge in at least some chronic pain states, supporting the notion that the brain can generate pain experience even when sensory inputs are lacking.

In summary, the transition from nociception to pain is non-linearly modulated by biological, psychological or social factors, but depends upon the context-dependent relationship among these aspects. This definition explains why the same twist of the ankle may be felt as an unbearable pain when walking home after a day in the office, or may not be felt at all, when running in alarm. As such, in pain research and clinical practice, the pain phenomenon needs to be understood in a biopsychosocial framework. In the following section (see Chapter II), we discuss separately the principal constituents of pain perception, classified in terms of sensory-motor, attentional, emotional and cognitive aspects. However, one must keep in mind that these dimensions are integrated, reciprocal, and virtually dialectic parts of the individual's holistic pain experience (Lewontin, 1978).

1.2 PAIN THERMINOLOGY

Pain can be classified as acute or chronic, depending on the duration of the symptoms. Acute pain is limited in time to specific events that cause tissue damage or inflammation. Instead, chronic pain refers to persistent and long-lasting conditions that exceed the expected period of healing. The DSM-IV suggests a criterion of six months to differentiate between acute and chronic pain (First & Gibbon, 1997). However, this time limit should be considered as indicative, since the diagnosis of acute and chronic states should depend upon the expected time of healing, which can differ according to the specific pain condition.

Anti- and pro-nociceptive phenomena can be described in terms of analgesia, hypoalgesia, hyperalgesia and allodynia. Analgesia refers to the absence of pain sensations, despite a stimulation which would be normally perceived as painful, whereas hypoalgesia represents diminished pain sensations. Hyperalgesia consists of

increased pain sensations induced by painful stimulation, whereas allodynia refers to pain sensations induced by normally innocuous stimulation (e.g., subjective painful feeling from the touch of sunburned skin). In these definitions, "normally" is defined by the usual subjective effects in response to pain stimulation (IASP, 1994). Both hyperalgesia and allodynia are processes that promote healing after an injury or tissue damage. Primary hyperalgesia refers to the increased pain sensations occurring in the damaged tissues, whereas secondary hyperalgesia consists of increased pain sensations occurring in the surrounding areas of the damaged tissues. However, hyperalgesic and allodynic phenomena can emerge in chronic pain states, without an evident purpose for the individual's healing, as consequence of dysregulated central mechanisms.

1.3 THEORIES OF PAIN

The definition of pain has been fervently debated from ancient times. For instance, Socrates famously contemplated the similarity of pain and pleasure2. Historically, pain has been conceived as a specific sense conveyed by specialized peripheral and central structures ("Specificity theory", Fig. 2a). This theory opposed the "Intensity theory" (Fig. 2b), which argues for a definition of pain as any intense stimulation irrespective of the sensory modality (e.g., Goldscheider's intensity theory). More recently, the "pattern theory" (Fig. 2c) has established pain as a pattern of convergent and integrated somatosensory activity within a distributed spinal ("Gate control theory", Fig. 2d) or brain network ("Neuromatrix theory", Par. 1.2.2).

2 "How singular is the thing called pleasure, and how curiously related to pain, which might be thought to be the opposite of it; for they never come to a man together, and yet he who pursues either of them is generally compelled to take the other. They are two, and yet they grow together out of one head or stem; and I cannot help thinking that if Aesop had noticed them, he would have made a fable about God trying to reconcile their strife, and when he could not, he fastened their heads together; and this is the reason why when one comes the other follows, as I find in my own case pleasure comes following after the pain in my leg, which was caused by the chain." Plato's Phaedo (Bostock, 1986)

1.3.1 CLASSIC THEORIES

The traditional "specificity theory" (see Fig. 2a) finds its roots in Descartes' work *Traite de l'homme* (1664; for an illustration, see Fig. 1), and defines the pain as a specific sensorial phenomenon, occurring when an external stimulus activates peripheral structures and nerve fibers which specifically transmit inputs to a pain centre in the brain. According to Descartes, a pain nerve fiber resembles a rope with a bell at its extreme, so that when the stimulus hurts the skin, the rope is pulled and the bell rings. Descartes identified the pineal gland, which he considered "le siège de l'âme", as the brain structure responsible of the pain experience. Later, the specific pain centre in the brain was postulated to be the thalamus, since alteration of pain sensations were observed in patients with thalamic lesions (i.e., central pain syndrome), but not after lesions of the somatosensory cortex (Head & Holmes, 1911).

Fig.1. Illustration of Descartes' pain theory (Descartes, *Traite de l'homme*, 1664)

The "specificity theory" gained favor, compared to the "intensity theory" (see Fig. 2b), which posits pain as the product of an intense stimulation in any sensory modality, due to several observations regarding the peculiar anatomy and physiology of pain, such as the spinal dissociation between touch vs. temperature and pain. Inspired by the specificity theory, investigations in the early twentieth century aimed to elucidate the pain mechanisms associated to each level of the pathway from transducing peripheral receptors to the brain and to produce analgesic drugs capable of interfering with this pathway. However, such progress in pain physiology and pharmacology would run into difficulty in attempting to explain and treat chronic pain conditions (Melzack, 2008).

Melzack and Wall (1965) acknowledged the limits of the specificity theory. For example, they argued that this theory does not explain the occurrence of pain after anterolateral cordotomy3, and proposed instead the "gate control theory" of pain (Fig.

3**Anterolater cordotomy** consists of the resection of the specific ascending pain pathway (for details on the ascending pain pathway, see Par. 1.3.1.3)

2d), providing a new conceptual framework to address unresolved questions about pain. The authors postulated the existence of a gateway in the spinal cord, consisting of a network of inhibitory and excitatory wide-dynamic-range neurons (WDR, Par. 1.3.1.3), where large Aβ and small Aδ/C fibers converge. The interplay of their activity determines the flow of the nociceptive information to the brain, so that the gate can be "opened" or "closed" by both ascending and descending projections. Thus, the theory sustains a definition of pain as result of intensity and patterned activity within a convergent somatosensory subsystem, denying specific neural elements. Curiously, the assumption that the "gate is closed" when large-diameter afferents inhibit the small-diameter fiber activation of WDR neurons, explains the effects of scratching the skin for a temporary pain relief.

Importantly, the gate control theory proposed a bidirectional model where, for the first time, interactions between top-down psychological and bottom-up sensory processes are taken into account to explain how pain is generated. In line with the new proposal, Melzack and Casey (Melzack & Casey, 1968) defined pain as a multidimensional experience and postulated three main constituents: sensory-discriminative, affective-motivational and cognitive-evaluative aspects. The sensory-discriminative dimension refers to the processing of physical features of the stimulus such as location, duration and intensity. The affective-motivational dimension includes cultural learning, past experience and personality variables. Finally, the cognitive-evaluative component depends on higher-order psychological processes such as attention, anxiety and expectation.

The introduction of the "gate control theory" and the notion of pain as a multidimensional phenomenon must be recognized as a crucial paradigm shift in the history of pain. This theory went beyond the classic conception of a rigid and passive bottom-up pathway, and extended the focus of the investigations from peripheral and spinal mechanisms to the multidimensional aspects underlying pain experiences. Remarkably, Melzack and his colleagues, by recognizing the role of the "top-down" in pain processing, "opened the gate" for a new generation of pain studies to identify how the brain generates pain experience. Further, their work has led to the development of new psychological interventions and effective pharmacological treatments, such as antidepressant and anti-epilepsy, to contain chronic pain (Mao, 2012; Melzack, 2008). In clinical settings, this revolutionary framework abandoned the traditional conceptions

of pain as physical symptom or hysterical manifestation and implicated the necessity to consider pain as an autonomous clinical entity, which requires a multidisciplinary approach to be effectively treated.

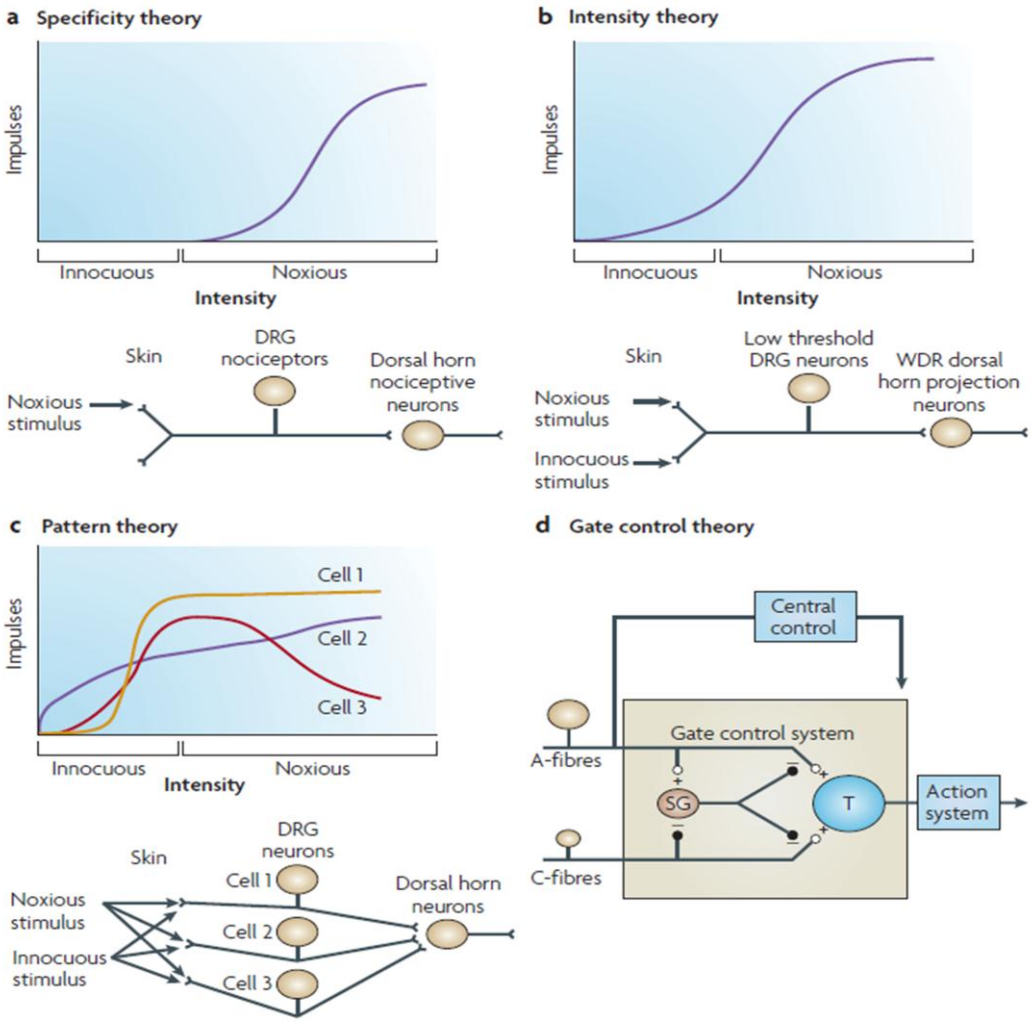

Fig. 2. Theories of Pain. From Perl (2007).

(a) Specificity Theory: Pain depends upon the activation of specific peripheral structures, nerve fibers and a pain center in the brain.

(b) Intensity Theory: Pain is determined by an exceeding intensity of a sensory modality, usually felt as innocuous.

(c) Pattern Theory: Pain is produced by patterns of neural activity in a distribute spinal or brain network.

(d) Gate Control Theory: Pain depends on the activity in the spinal cord, where a "gate" either blocks pain signals or allows them to continue on to the brain.

1.3.2 THE NEUROMATRIX THEORY

According to Melzack (1999), the multidimensional pain experience is produced by "neurosignature" patterns of neural activity in a widely distributed brain network called the "body-self neuromatrix". Sensory inputs, as well as activity in the CNS, may trigger these neural patterns independently of inputs from the periphery. The outputs of the body-self neuromatrix induce awareness, overt action response patterns, and homeostatic modifications to predispose the individual to cope with injury, stress, or other pathology. The brain processes in the neuromatrix are genetically determined, but they can be modified by sensory experience. In this framework, acute pain is conceived as the product of the neural patterns related to the activation of peripheral structures and thus of the ascending system. In contrast, chronic pain may be produced by altered processes occurring at higher levels of the pain system. The pathogenesis of chronic pain may be related to psychological and physical stress, as well as to genetic predisposition. Thus, the neuromatrix theory posits an equal importance of neural mechanisms of sensory transmission, genetic contributions, and neural-hormonal mechanisms of stress in the generation of acute and chronic pain states.

1.3.3 THE HOMEOSTATIC EMOTION THEORY

Craig (2003) defined pain as an interoceptive and homeostatic emotion. In such a context, interoception is one aspect of body awareness that refers to the sensory element of pain sensation, whereas a homeostatic emotion is a feeling associated with motivation, such as temperature, itch, thirst and hunger. The author proposed the homeostatic emotion theory , which describes pain as depending upon the activity of a well-organized and hierarchical system subserving homeostasis. The activity of the system depends on the integration of multiple spino-thalamo-cortical pathways, which convey both specific interoceptive information, through the lamina I spino-thalamo-cortical pathway, and convergent patterned somatosensory activity, through the lamina V spino-thalamo-cortical pathway. Thus, according to the homeostatic emotion theory, pain is a subjective meta-representation of the state of the body, experienced in terms of feelings and motivation for action. It is strictly associated with autonomic, neuroendocrine, and behavioral homeostatic mechanisms, needed to guarantee an optimal physiological balance. In this framework, chronic pain syndrome is conceived as a homeostatic dysfunction.

1.3.4 THE "PAIN MATRIX" AND THE "SALIENCE" THEORY

The advent of advanced functional neuroimaging techniques, in combination with adequate experimental paradigms, disclosed crucial findings for the understanding of central mechanisms underpinning pain perception and modulation in humans. These techniques, such as PET and fMRI, lead to the description of a neural network involved in pain processing labeled the "pain matrix". The pain matrix has been considered a "representation" (Treede et al., 1999) or a "signature" (Tracey & Mantyh, 2007) of cortical pain processing, which hold a key role in the study of pain in health and disease (Apkarian, Bushnell, Treede, & Zubieta, 2005). This theory argues that pain derives from the processing and integration of nociceptive and complex emotional and cognitive processes, implicating the participation of several pain-specific brain structures. The main components of the pain matrix include primary and secondary somatosensory (S1 and S2), insular (IC), anterior cingulate (ACC) cortices, as well as prefrontal and parietal areas. Other activations are observed in subcortical regions, such as thalamus, basal ganglia, amygdala, hippocampus, and in cerebellum. S1, S2 and posterior IC are thought to serve the processing of sensory-discriminative features of pain stimuli, whereas anterior IC and ACC are supposed to mediate the affective-motivational processing of pain. According to Tracey and Mantyh (2007), "pain perception, similar to many complex experiences, emerges from the flow and integration of information among specific brain areas" constituting the pain matrix. For further details on the pain matrix and the functional role of the different structures of the network, see Par. 1.5.2. Accordingly with the notion of pain matrix, EEG and MEG studies identified electrophysiological pain correlates, namely Event-Related Potentials (ERPs) or Event-Related scalp Fields (ERFs) whose modulation is supposed to index the activity within the pain matrix and reflect pain-specific processes. The high temporal resolution offered by such techniques permits description of pain-related temporal patterns with a millisecond precision, described in terms of P1, N1, N2, P2, and other late positive potentials, where the alphabetic prefix denotes the signal polarity (positive or negative) and the numeral suffix provides information about the signal latency (first positive, first negative, second negative, second positive component). The amplitudes of these components have been considered direct measures of brain activity responsible of pain sensations (Bromm & Lorenz, 1998). For further details on pain-related potentials and their functional role, see Par. 2.1.1.6 and 2.1.1.7.

Iannetti and Mouraux (2010) identified three main arguments used by researchers to support the role of the activity in the pain matrix as pain specific: (1) high correlations between perceived intensity ratings and the magnitude of the neural responses in the pain matrix; (2) observations of factors modulating both pain perception and the magnitude of neural responses in the pain matrix; (3) observations of painful sensations evoked by epilectic seizures or direct electrical stimulation of areas of the pain matrix. Noteworthy, the first two arguments are also used by researchers to conclude that certain ERP or ERF components specifically reflect pain processing. However, the authors speculate that such evidence is insufficient to conclude that the activity in the pain matrix, as well as the magnitude of ERP/ERF elicited by noxious stimulation, demarcates a pain-specific activity. Indeed, several studies demonstrate that the activity of the pain matrix (1) is dissociable from the perception of pain intensity (Iannetti, Hughes, Lee, & Mouraux, 2008), (2) is influenced by attentional and salience processes (Iannetti et al., 2008), and (3) is also evoked by non-nociceptive and non-painful stimulation (Downar, Crawley, Mikulis, & Davis, 2000). However, these arguments do not deny the involvement of cortical processing in pain experience, but question the notion that the so-called pain matrix reflects pain-specific processes (Iannetti et al., 2008; Legrain, Iannetti, Plaghki, & Mouraux, 2011). Thus, the activity within the pain matrix cannot be considered a specific cortical representation of pain, and cannot be directly interpreted as an objective index of the subjective dimension of experienced pain. Conversely, the authors proposed an alternative explanation expressed in terms of attentional and salience processing. The activity within the pain matrix would rather reflects "a system involved in detecting, processing and reacting to the occurrence of salient sensory events regardless of the sensory channel through which these events are conveyed. Such a network could reflect some of the basic operations by which the brain detects stimuli that can represent a potential threat for the integrity of the body" (Legrain et al., 2011).

Since the pain matrix and the salience theory imply a psychobiological perspective on pain, these concepts will be further explained in the following paragraphs (Par. 1.5.2).

1.4 PSYCHOBIOLOGICAL MECHANISMS OF PAIN

The anatomical and physiological correlates of pain, the so-called nociceptive system, consist of cutaneous and visceral nociceptors and spinal cord neurons which transmit input from the periphery to supraspinal structures, such as brainstem, thalamus, limbic system and neocortex (i.e., ascending system or bottom-up pain pathway). Projections of supraspinal structures send outputs to motor neurons and autonomic efferents (i.e., descending system or top-down pain pathway), which elicit avoidance and protective reflexes, produce actions to stop or prevent the pain, and adjust physiological activity, such as heart rate and respiratory rhythm.

1.4.1 THE ASCENDING PAIN PATHWAY

1.4.1.1 Nociceptors And Fibers

Nociceptors, namely the sensory receptors that respond to noxious stimulation, consist of cutaneous (somatic) and visceral free nerve endings of thinly myelinated and unmyelinated fibers. A broad range of tissue damage or inflammation, caused by mechanical stimulation, extreme temperature, oxygen deprivation and chemical, can lead to stimulation of these receptors. Nociceptors have been identified throughout the body, including skin, cornea, viscera, muscles, joints, bones, tendons, blood vessels and meninx; with the only exception of the white and grey matter.

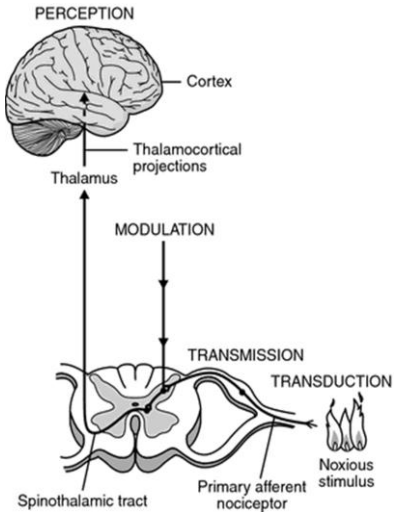

Fig. 3. The four processes of nociception: (1) Transduction; (2) Transmission (3) Modulation; (4) Perception

(Ferrante & VadeBoncouer, 1993)

Nociceptors can selectively respond to stimuli capable of tissue lesions, such as mechanical, thermal or chemical stimulation. Mechanical nociceptors show a specific response to strong pressure, thermal nociceptors show selective response to extremely high (> 45°) or low (< 5°) temperature and chemical nociceptors respond only to substances such as acids. Other nociceptors, called polymodal nociceptors, have no selective response and they may be activated by all stimuli, irrespectively of their nature. The polymodal nociceptors are activated also by substances, released when a tissue is damaged, like bradichinin, serotonin, histamine, prostaglandins, H+ and K+

ions. Finally, there are classes of primarily visceral nociceptors, referred as to "silent" or "sleeping", which may respond to noxious stimulation under peculiar conditions such as inflammation. Thus, noxious stimuli activate nociceptors and are transduced into electrical impulses, which induce action potentials when a certain threshold is exceeded. The transduction of noxious stimuli (Fig. 3, "Transductic") leads to trains of events that transmit (Fig. 3, "Transmission") the input towards higher synaptic stations until the brain for further nociceptive processing and for the emergence of conscious pain percepts (Fig. 3, "Modulation" and "Perception").

Inputs generated by activated nociceptors are driven to the central nervous system through myelinated Aδ (diameter: 1-5 μm; speed: 5-30 meters/second) and unmyelinated C fibers (diameter: 0.2-1.5 μm; 0.5-2 meters/second). The two different types of conduction are expressed in qualitatively different phenomenological aspects, the so-called "first" and "second pain". First pain, related to activation of fast-conducting Aδ fibers, is felt as brief, well localized, sharp and stinging. In contrast, second pain is associated with activation of the C fibers, and consists of longer lasting and less well-localized sensations, described as slow, dull and burning.

1.4.1.2 The Anterolateral System

Noxious inputs are transmitted to the CNS along a pathway referred to as "antero-lateral". The afferent nociceptive fibers, namely the first order nociceptive neurons, enter in the spinal cord through the dorsal horns, where they immediately form synapses with the cell bodies located in Rexed laminae I and V. The cells of laminae I consist of thermoreceptive-specific neurons and are selectively activated by thermal stimulation. Instead, the cells of laminae V, known as wide and dynamic range neurons (WDR), respond to different stimulation and receive convergent inputs from Aβ, Aδ and C fibers (Bromm & Lorenz, 1998; Craig, 2003). Thus, the second order nociceptive neurons immediately cross the midline and their projections reach the thalamic nuclei, travelling contralaterally to the stimulated side along the antero-lateral pathway (Fig. 3). The antero-lateral pathway comprises three main systems of fibers: the Spino-Thalamic (STT), the Spino-Reticular (SRT) and the Spino-Mesencephalic (SMT) Tracts. In addition, the nociceptive and thermal information from the face and head are conveyed through the trigeminal pathway. The first order neurons form synapsis with the cells of

the ipsilateral spinal trigeminal nucleus, whereas the second order neurons cross the midline and ascend through the trigeminal lemniscus to the contralateral thalamus.

The multiple tracts, differing in the lamina origin and in the central destinations, are supposed to differently contribute to pain perception. The fibers of the STT project to thalamic nuclei located in the postero-lateral (e.g., posterior part of the ventral medial nucleus, VMpo) and medial (e.g., intralaminar, central lateral, dorsal medial) areas of the structure (Craig, 2003). VMpo receives projections from lamina I neurons and its stimulation in awake patients leads to discrete and well-localized painful, thermal or visceral sensations. On the other hand, intralaminar thalamic nuclei receive projections from lamina V neurons and send outputs to the basal ganglia and to frontal and parietal cortices (e.g., ACC). Several authors made a distinction between a "lateral" and a "medial pain system", consistently with the differentiated thalamic projections. The lateral thalamus and in particular the VMpo nucleus, also referred to as "somatosensory thalamus", is considered a relay station which forms a network with the somatosensory cortices. The lateral network is supposed to subserve pain discriminative aspects and to render an individual capable to process precise properties of the noxious stimulus, such as its location, intensity and quality. Conversely, a network comprising the medial thalamus and its connections to ACC and limbic system is supposed to be associated to the motivational and emotional domains of pain processing, such as emotional interpretation of a noxious stimulus, unpleasantness feelings, protective and withdrawal behaviors. The fibers of the SRT project to the reticular formation, then from this area to the intralaminar thalamic nuclei, hypothalamus and limbic system. The pathway is supposed to play a role in affective-emotional, as well as cardiovascular and endocrine responses associated to pain. Finally, the fibers of the SMT, projecting to the Periacqueductal Gray (PGA), the mesencephalic reticular formation and the parabrachial nuclei, are considered to be involved in the descending pain modulation (Par 1.4) and in the affective components of pain processing. In conclusion, the joint activity of the antero-lateral systems, as well as the parallel activity of the lemniscal system (Par. 1.3.1.2), are integrated to elicit a unique pain percept, involving both sensory and emotional features. The contribution of the lemniscal system will be addressed in the next paragraph.

1.4.1.3 The Lemniscal System

Nociception and mechanoception differ in basic anatomical and functional proprieties. As described before, nociception refers to the processing of noxious information and relies on activity in the anterolateral pathway (Par. 1.3.1.2). Instead, mechanoception denotes the processing of tactile and vibratory mechanical information, processed along the lemniscal pathway. Mechanoceptors are the specific receptors that respond to low-intensity tactile, vibratory and proprioceptive stimulation, and are associated to Aβ fibers (diameter: 6-12 μm; 30-60 meters/second). The first order fibers enter the dorsal horns of the spinal cord and ascend ipsilaterally, within the dorsal column, until forming synapses in the gracile and cuneatus nuclei in the medulla oblongata. The second order fibers, called "medial lemniscus tract", decussate in the brainstem and reach the contralateral ventral posterolateral and posteromedial thalamic nuclei. From the thalamus, the third-order neurons continue on to the primary somatosensory cortex (S1) in the post-central gyrus.

The lemniscal system provide the sensory-discriminative quality of somatic sensations, such as location of the tactile stimuli on the body surface, the frequency of vibratory stimuli or the position of the limbs in the space. It also contributes to the discriminative aspects of pain processing. For instance, when the activity of Aα and Aβ fibers is pharmacologically blocked, the puncture of a needle is indistinguishable from a pinch stimulus.

1.5 THE DESCENDING PAIN PATHWAY

1.5.1 THE BRAINSTEM

The role of Periacqueductal Gray (PGA) in pain processing was first disclosed by Sherrington in 1906, who observed analgesia induced by the electrical stimulation of this brainstem area. Further studies corroborated the finding in animals and humans (Bantick et al., 2002; Dunckley et al., 2005; Reynolds, 1969; Tracey & Iannetti, 2006; Valet et al., 2004), and identify additional neuronal populations in the Rostral Ventromedial Medulla (RVM), Parabrachial Nucleus (PB), Ventral Tegmental Area (VTA), and the Nucleus Cuneiformis (CnF). Altogether, these brainstem areas constitute the descending modulatory system, which is the "final common output" receiving descendent outputs from cortical and subcortical regions, such as ACC, insula, hypothalamus, and amygdala (Fields, 1999). The functional connectivity

between these rostral areas of the brain and the brainstem is likely to constitute the neural basis of the cognitive and emotional modulation of pain. The descending pain pathway consists of a "bidirectional central control of nociception", which mediates the inhibition and the facilitation of ascending nociceptive inputs within the dorsal horn of the spinal cord (Fields, 1999). Indeed, Tracey and Mantyh (2007) wrote that "the brainstem plays a pivotal role in gating the degree of nociceptive transmission so that the resultant pain experienced is appropriate for the particular situation of the individual". Thus, the descending system could either reduce pain "switching on" anti-nociceptive mechanisms when analgesia is necessary for survival or facilitate pain "switching on" pro-nociceptive mechanisms when healing and recovery are needed. An alteration of the balance between inhibitory and facilitatory brainstem responses is thought to constitute a key mechanism in chronic pain states and central sensitization (Bingel & Tracey, 2008). Since the activity of this system is predominantly mediated by endogenous opioids, it is also referred to as the "brainstem opioid system". In particular, opioid-dependent neurons have been found in PAG, RVM and PB (Fields, 1999).

Importantly, the study of the functional role of the brainstem and midbrain structures in pain modulation has been hindered by the availability of techniques able to reliably capture the activity called into question. An elective method is the high spatial-resolution functional Magnetic Resonance Imaging (fMRI). In such a case, the imaging sequence is adjusted to be sensitive to the peculiar features of the brainstem. Indeed, the signal originating from this area is highly susceptible to hemodynamic and pulsation-related artifacts, which must be controlled through cardiovascular monitoring and use of selective anatomical masks.

Recent fMRI studies show brainstem activity throughout anticipatory periods prior to pain stimulation (Fairhurst, Wiech, Dunckley, & Tracey, 2007; Wager et al., 2004). Fairhurst and collaborators (2007) reported increased activity in PAG during the anticipation period and also in other brainstem structures (i.e., VTA, RVM, PB) during the actual pain stimulation period. Interestingly, the subjective anticipation ratings were predictive of the perceived pain intensity. Moreover, the neural activity in PAG, VTA and entorhinal cortex during anticipation was correlated with the anticipation ratings and with the posterior insula activity during the pain stimulation period. Thus, this study suggests that anticipatory mechanisms play a crucial role in pain modulation, by tuning the nociceptive system to deal with forthcoming pain.

1.5.2 THE SO-CALLED PAIN MATRIX

The identification of specific pain centers in the brain is an enquiry, which has largely failed to generate conclusive and fulfilling results compared to other sensory modalities, such as vision and audition. To identify which cortical area selectively responded to pain, Penfield and Boldrey (1937) carried on pioneering studies on epileptic patients who were resistant to pharmacological treatments. These scientists electrically stimulated surface cortical areas and asked conscious patients to report their experiences. Unexpectedly, this work did not succeed in detecting any pain cortical area, failing to corroborate the hypothesis regarding crucial involvement of primary somatosensory cortex in nociception. The notion that pain is not elicited by focal stimulation of cortical areas (Penfield & Faulk, 1955; Penfield & Jasper, 1954; Penfield & Perot, 1963) led Wall (1995) to claim that "it remains an act of faith to continue searching the brain for some still undiscovered nest of cells whose activity reliably triggers pain".

However, the advent and use of neuroimaging techniques based on hemodynamic features of the cerebral blood flow (PET and fMRI) permitted the identification of several cortical regions involved in pain processing, often referred to as the "pain matrix". The pain matrix includes primary and secondary somatosensory cortices, insula, anterior and mid-cingulate cortex, frontal and parietal cortices (Apkarian et al., 2005; Peyron, Laurent, & Garcia-Larrea, 2000). The activity of this this network of regions is supposed to be at least partially pain-specific and to subserve the integration of different domains of pain processing, such as sensorial-discriminative, cognitive, emotional, motor and vegetative aspects (Apkarian et al., 2005; Peyron et al., 2000; Treede et al., 1999). However, recent findings have suggested that these activations cannot be considered pain-specific, but constitute a cortical network involved in bottom-up attentional mechanisms induced by salient stimuli, irrespectively of their sensory modality (Iannetti & Mouraux, 2010; Legrain et al., 2011).

1.5.2.1 Primary Somatosensory Cortex

The primary somatosensory cortex (S1) is located in the lateral post-central gyrus, behind the central sulcus, and comprehends the Brodmann areas (BAs) 3, 1, and 2 (regions are listed along the anterior-posterior axis). S1 is considered the main cortical area involved in the processing of the sense of touch and the specific contribution of this area in the processing of noxious stimuli is still under debate. Lesions in S1 can lead to either analgesia or hyperalgesia in some cases, and do not generate evident signs of altered pain perception in other cases (Peyron et al., 2000; Schnitzler & Ploner, 2000). Regardless of contradictory results, many researchers continue to attribute a functional role of the region to sensory and discriminative pain processing, such as intensity codification, stimulus location, and spatial discrimination. However, since the perceived pain intensity is preserved after lesions in S1, other cerebral areas may contribute in parallel to the sensory-discriminative processing (Coghill, Sang, Maisog, & Iadarola, 1999).

Functional MRI and PET studies found S1 activations when a large amount of body surface was stimulated or when attention to noxious stimulation was required, suggesting that S1 activity may reflect spatial and temporal summation, as well as attentional processing (Peyron et al., 2000).

1.5.2.2 Secondary Somatosensory Cortex

The secondary somatosensory cortex (S2) is locate11d in the parietal operculum on the ceiling of the lateral sulcus, and comprises the BAs 40 and 43. It is generally thought that S2 is not involved in sensory discrimination because the neurons constituting this area respond to a broad range of sensory stimuli, such as tactile, vibratory, thermal, olfactory and gustatory, beyond noxious stimulation. Despite the narrow number of nociceptive-specific neurons in S2, activation of this region is one of the most common finding in pain studies. The activity in S2 usually occurs in parallel to the activity in S1 and insular cortex (Peyron et al., 2000; Schnitzler & Ploner, 2000). Moreover, it increases in function of the intensity of the stimulus and is reliably augmented when stimuli are perceived as painful (Peyron et al., 2000). S2 has been revealed as the principal source generator of EEG and MEG signals, occurring between 100 and 200 ms, shaping the N2 amplitude (Apkarian et al., 2005).

1.5.2.3 The Insular Cortex

The secondary somatosensory and the operculo-insular (S2 and IC, respectively) are considered to constitute the core network of thermosensory and pain processing, as well as of the so-called pain matrix. S2 and IC represent the most consistent regions activated irrespectively of stimulus modality (e.g., thermal, electrical, mechanical), as revealed by fMRI and PET studies.

The IC is folded in a deep cortical region within the lateral sulcus, in between the frontal and the temporal lobes. The cytoarchitecture of the IC is characterized by a smaller granular posterior region and a larger agranular anterior region. Structurally, the posterior region is connected to S2, whereas the anterior regions of IC projects to the amygdala and ACC. Since both S2 and IC activity increases in function of thermal stimulus intensity, the functional role of these regions is thought to consist in thermal discrimination. Both anterior and posterior insular regions are considered involved in the sensory-discriminative pain processing (Peyron et al., 2000). However, the anterior part is thought to play a crucial role also in visceral, autonomic and affective processing, as well as interoception and body awareness (Craig, 2003; Critchley, Wiens, Rotshtein, Öhman, & Dolan, 2004). Recently, the dorsal posterior insula has been suggested to represent the primary sensory cortex for temperature and pain in humans (Craig, Chen, Bandy, & Reiman, 2000; Garcia-Larrea et al., 2010; Mazzola, Isnard, Peyron, & Mauguière, 2012), so that this area may be labeled S3 (third somatosensory cortex; Garcia-Larrea, oral communication), N1 (primary nociceptive cortex; Iannetti, oral communication), or even P1 (primary cortical pain area; Mazzola et al., 2012).

More than 60 years later, Mazzola and collaborators (2012) reinvestigated the same issue addressed by Penfield and collaborators (1937; 1954; 1955; 1963), taking into account the possibility that the deep parietal operculum and posterior insular cortex might be involved in primary pain processing. Through stereotactic implantations of intracerebral electrodes, the authors documented operculo-insular pain responses, but with a low frequency rate: 12.8% for parietal operculum and 10.4% for insula. However, in a greater percentage, pain was elicited by the stimulation of the posterior insula compared with more anterior insular areas. Consistent with previous findings, pain could not be induced by any other cortical stimulation, including in areas typical of the pain matrix. The authors speculated that Penfield and collaborators failed to report these pain responses, because the electrical stimulation adopted in their studies was

limited by the lack of access to the deep insula and neighboring parietal opercular cortex. However, the pain sensations were so rare, that the authors concluded, "focal cortical stimulation of the operculo-insular region is per se insufficient to consistently reproduce the global 'experience' of pain, but can only initiate the aversive sensation qualified as 'pain' in some privileged circumstances".

Cortical lesion of the posterior IC can lead to a clinical condition called "pain asymbolia" in which pain is not experienced as aversive or unpleasant (Berthier, Starkstein, & Leiguarda, 2004). Asymbolic patients do not report any pain, nor unpleasant feelings neither motivation to avoid and to stop the stimulation. On the contrary, they laugh or giggle when a noxious stimulus is administered. However, these patients can discriminate sensory features of the stimulus, such as location and intensity, and are able to describe the noxious stimulation in terms of qualitative properties, such as burning or stinging. This clinical condition is supposed to depend on a disconnection between sensory and limbic regions.

Noteworthy, several studies identified increased neural activity in anterior IC, beyond other prefrontal regions, in patients with chronic pain diseases irrespectively of their specific condition (Bingel & Tracey, 2008). Moreover, anterior IC activity is also associated to anxiety, depression, irritable syndrome, chronic fatigue, somatization, and fear.

1.5.2.4 The Anterior Cingulate Cortex

The ACC corresponds to the frontal part of the cingulate cortex, which is located immediately above the corpus callosum, in the medial cortical regions (Bas 24, 25, 32, 33, Fig. 4b). ACC has been functionally divided into an anterior region, called 'rostral' ACC (rACC), comprising the pregenual and the subgenual ACC (pgACC and sgACC, Fig. 4c), and a central region, namely 'dorsal' ACC or midcingulate cortex (MCC), divided into anterior and posterior divisions (aMCC and pMCC, Fig. 4c).

ACC activity shows high consistency between several neuroimaging studies. In fMRI and PET studies is the commonest activated area after S2 and IC, and is reported by 100% of EEG studies, according to Apkarian and coauthors (2005). ACC and, in particular MCC, consists of the principal source generator of late EEG and MEG signals occurring after 200 ms, such as the P2 and late positive potentials (Apkarian et al., 2005; Legrain, Van Damme, et al., 2009).

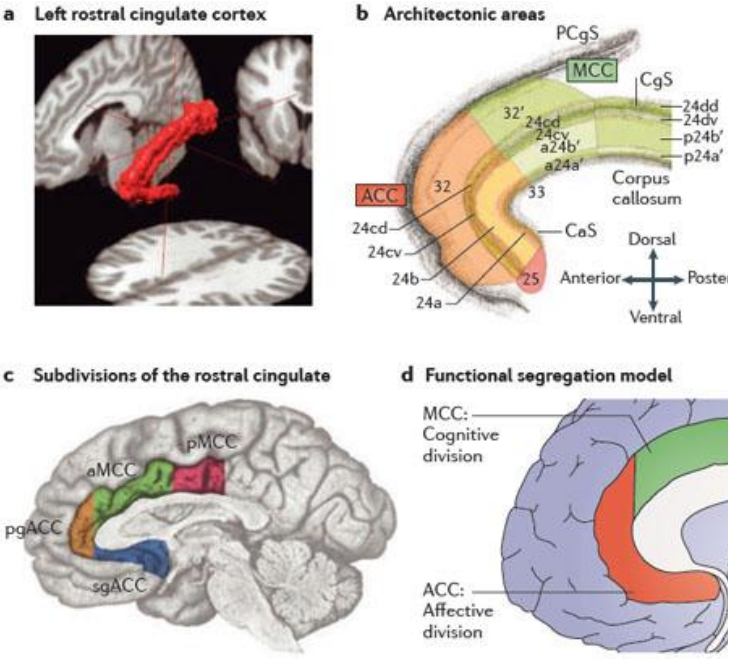

Fig. 4. Divisions of the human rostral cingulate cortex:
(a) 3-D rendering of the left rostral cingulate cortex of a single subject;
(b) Architectonic areas of the ACC, defined on the basis of differences in neuronal structure and neurochemistry;
(c) The four major subdivisions of the cingulate cortex (sgACC, pgACC, aMCC, pMCC);
(d) The functional segregation of ACC (red) and MCC (green) according to the model of Bush, Luu, and Posner (2000)
PCgS, paracingulate sulcus
CaS, callosal sulcus
CgS, cingulate sulcus

(Shackman et al., 2011)

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Generally, ACC is considered to have an important role in emotional regulation, attention-demanding processes as problem-solving, monitoring of performance, error recognition, adaptive response to changing conditions and conflict resolution, as in the case of the Stroop task (Allman, Hakeem, Erwin, Nimchinsky, & Hof, 2001). Classic imaging studies implicated that the rostral and dorsal ACC consists of two functionally segregated regions subserving affective and cognitive processes, respectively (the functional segregation model of Bush et al., Fig. 4d).

In the context of pain processing, functional experimental and clinical studies suggest that different regions of the rACC can be implicated in the processing of subjective pain sensations, affective reaction, cognitive and attentional responses to pain, as well as motor and anticipatory responses (Peyron et al., 2000). According to the segregationist model proposed by Bush et al. (2000), some neuroimaging pain studies reported the implication of ACC in affective responses and of MCC in cognitive, attentional and motor responses to pain (Peyron et al., 2000).

For instance, rACC is considered an antinociceptive brain area, which exerts top-down influences on brainstem structures involved in pain control. Indeed, increased activity in this area has been found in placebo analgesia (Petrovic, Kalso, Petersson, & Ingvar, 2002), opioid analgesia (Adler et al., 1997; Petrovic et al., 2002) and during habituation to pain. In addition, ACC is one of the targets of Deep Brain Stimulation (DBS) to reduce pain sensations in patients affected by chronic conditions (Mohseni et al., 2012).

Activity in aMCC (BA 32) was found to reflect sustained attention during the task for both painful and non-painful stimuli, while the activity in pMCC (BA 24) has been associated to phasic orienting of attention to salient stimuli (Peyron et al., 1999). However, the dichotomy rostral-affective vs. middle-cognitive may be an oversimplification that has not been consistently replicated across studies (Adler et al., 1997; Rainville, Duncan, Price, Carrier, & Bushnell, 1997; Tölle et al., 2001). Moreover, there is evidence pointing to a large overlap between ACC activations, suggesting that the same cingulate region may be involved in multiple functional networks (Peyron et al., 2000).

Interestingly, a recent meta-analysis, by using a coordinate-based approach and the activation likelihood estimate algorithm (Shackman et al., 2011), defied the classic segregation model and revealed that negative affect, pain and cognitive control activate an overlapping region within aMCC, approximately corresponding to architectonic areas 32' and a24b'/c' (Fig. 4b). However, studies on both cognitive control and pain were more frequently to activate MCC than ACC. Thus, this meta-analysis provide evidence against a strictly segregation of cognitive and affective functions into differentiated divisions within the rostral ACC. Conversely, it suggests that aMCC may represent a hub linked to motor centres that serves emotional facial expressions and aversively motivated instrumental behaviors.

Finally, lesions in the ACC can alter the affective and motivational responses to pain, but do not generate alterations in sensory and discriminative pain processing. Indeed, lesions here can lead to "loss of spontaneity in emotion, thought and activity" (Papez, 1937). For instance, Damasio and Van Hoesen (1983) described a clinical case of left anterior cingulate lesion, due to a stroke. One month after the stroke, the patient was described as "remarkably recovered. She had considerable insight into the acute period of the illness and was able to give precious testimony as to her experiences then.

Asked if she had ever experienced anguish for being apparently unable to communicate she answered negatively. She didn't talk because she had nothing to say. Her mind was empty. She apparently was able to follow our conversations even during the early period of the illness, but felt no will to reply to our questions".

1.5.2.5 Summary

In summary, the history of pain studies tells the story of the unsuccessful hunt for unique pain centers in the thalamus (Head & Holmes, 1911) and in the primary somatosensory cortex (Penfield & Boldrey, 1937). Melzack and Casey (1968), postulating pain as a multidimensional experience, which include sensory-discriminative and affective-motivational aspects, inspired the view of different brain structures subserving different aspects of pain. Accordingly, anatomical and physiological studies suggested the notion that sensory aspects are processed by the "lateral pain system", composed by lateral thalamic nuclei and somatosensory cortices; whereas the emotional are processed by the "medial pain system", which is constituted by medial thalamic nuclei and limbic regions. In addition, the advent of neuroimaging techniques, in particular fMRI, led to the definition of the pain matrix, where the sensory network includes primary, secondary somatosensory cortices, and posterior insula, whereas the affective network includes cingulate, anterior insular and prefrontal cortices. However, unique and reliable markers of the different domains have not be found or replicated, pointing against a strict segregation of sensory and emotional processing in specific brain areas.

The next chapter delineates an experimental framework for pain studies, and discusses several mechanisms of pain modulation, organized in sensory-motor, attentional, emotional, placebo and cognitive domains. For each domain, a brief introduction is followed by a review of the functional and electrophysiological correlates.

CHAPTER II

MODULATION OF PAIN EXPERIENCE AND PAIN-RELATED CORTICAL RESPONSES

2.1 EXPERIMENTAL PAIN

Thanks to the introduction and advances of non-invasive neuroimaging techniques, brain responses to pain have been largely investigated by EEG, MEG, PET and fMRI studies. EEG and MEG techniques directly measure electrical and magnetic signals elicited by noxious stimulation, whereas the activation PET and fMRI studies estimate the indirect functional correlates of pain processing by measuring increases in the regional cerebral blood flow (rCBF) and in the blood oxygen level (BOLD). The noxious stimulation administered in the laboratory can be either electrical, thermal, laser, or less frequently mechanical. The neuroimaging techniques documented electrophysiological and hemodynamical correlates of several aspects of pain processing, including sensory-motor (Par. 2.2), attentional (Par. 2.3), emotional aspects (Par. 2.4), expectations of analgesia (placebo; Par. 2.5) and other cognitive strategies (Par 2.6). In the following sessions, the main neuroimaging findings regarding the contribution of these aspects in pain processing are reviewed. First, however, to better understand some critical issues when interpreting the results of pain research, are introduced the principal characteristics of stimuli typically administered to provoke pain in the laboratory, common dependent variables, and the main features of each neuroimaging technique.

2.1.1 PAIN ASSESSMENT

Pain is a highly complex and subjective experience, of which the description, measure and objective assessment has largely defied researchers and clinicians. Only indirect measures are possible, through subjective verbal reports or more objective physiological or behavioral indexes. The subjective pain assessment refers to verbal evaluations through numerical or visuo-analogue scales. Instead, the so-called objective indexes refer to those measures which do not involve an intermediary subjective filtering, such as reflexes, functional, and electrophysiological correlates.

In the laboratory, a pain assessment requires pain stimulation (Par. 2.1.1.1) and the concomitant evaluation of the elicited sensations or processing (Par. 2.1.1.2, 2.1.1.4, 2.1.1.6). Subjective evaluations occur by asking the participants to (1) self-evaluate their perceived pain (intensity and unpleasantness), (2) discriminate several intensities to determine the point a sensation starts to be perceived as pain (pain threshold,), (3) endure prolonged pain sensations (pain tolerance). Instead, objective measures refer to (1) functional correlates, which correspond to the neural activity revealed by rCBF and BOLD signals in response, for instance, to electrical, thermal or mechanical stimulation, (2) electrophysiological correlates, which consist of ERPs or ERFs elicited by either electrical or laser stimulation (somatosensory or laser evoked potentials, respectively).

2.1.1.1 Pain Stimulation

Experimental studies used several techniques and procedures to provoke pain. The techniques include electrical, thermal and mechanical stimulation, while the procedures can differ, depending on the site of application, temporal parameters of the stimulation, intensity and the quality of the elicited sensation. Electrical stimuli are administered by an apparatus which deliver low electrical intensity for brief periods of time and to specific sites of the body. The elicited sensation is usually similar to a pinprick. Thermal stimuli are produced by either a contact thermo-electrode or a laser device. The contact thermo-electrode conducts heat to the area of the skin in contact with the surface of the thermo-electrode. Instead, the laser stimulator emanates CO_2 that is completely absorbed by the superficial layers of the skin and selectively activates nociceptors (Bromm & Lorenz, 1998). Contact thermal stimulation elicits longer and less spatial definite sensations, whereas laser stimuli are usually perceived as brief and well-localized. Both thermal stimuli produce a “dual pain”, consisting of an initial stinging sensation (primary pain) followed by a burning pain (secondary pain). Mechanical stimuli are administered exerting a constant pressure on a specific body part.

According to Bromm and Lorenz (1998), experimental painful stimulation needs to respect the following criteria: (1) reproducible, without causing tissue damage, (2) short duration that allow a precise identification of pain onset, (3) when the experimental paradigm implicates repetitive measures, stimulation must be administered in different parts of the body in a counterbalanced order, (4) Inter Stimulus Intervals

(ISIs) with random duration, (5) brief blocks of pain stimulation with maximum duration of 15 minutes, (6) at least two different intensities within the same block. Varying the stimulus intensity and the ISI duration may guarantee constant vigilance and attentional levels, so that participants anticipate each stimulus as potentially painful.

2.1.1.2 Subjective Evaluations, pain threshold and tolerance

Pain can be measured through subjective indexes including verbal scales, subjective ratings of pain intensity and unpleasantness, estimation of pain threshold and tolerance. Verbal scales make use of lists of adjectives that describe the intensity or the quality of pain sensations. Participants or patients are required to choose the adjectives that match their perception. Numerical verbal scales require participants to provide a number that reflects the intensity or the unpleasantness of their pain sensation, comprised from a minimum (usually 0, corresponding to 'no pain sensation') and a maximum (usually 10 or 100, corresponding to the 'worst imaginable pain'). Visuo-analogue scales consist of vertical or horizontal lines, usually of 10 cm length, which represent a continuum from no pain sensations and the worst imaginable pain. Participants are required to sign the point along the continuum that corresponds to their pain perception in terms of either intensity or unpleasantness. The point is then transformed in a meaningful number, from 0 to 10 or from 0 to 100. Pain threshold is defined as the intensity identified by the subject as a starting painful sensation, namely the level of intensity that the participant reports to switch from bother to pain. Instead, pain tolerance refers to the temporal extent that a subject can sustain pain sensations of certain intensity, without breaking down, either physically or emotionally.

2.1.1.3 PET and fMRI techniques

Functional investigations, such as PET and fMRI studies, provide an indirect estimation of the neural activity by measuring hemodynamic correlates of brain processing. These correlates consist of changes in rCBF for PET and in BOLD signals for fMRI studies. The rCBF is measured by analyzing the distribution of radioactivity related to molecule carrying a positron-emitting isotope, previously injected. Instead, the BOLD (Blood Oxygenation Level Dependent) signal makes relies on the endogenous contrast agent deoxyhemoglobin. However, despite the great advances in

such techniques, how changes in rCBF and BOLD signals relate to the neural activity is not fully comprehended.

PET presents a low temporal resolution of around 1 minute, a middle spatial resolution and is considered invasive, since requires injections of a radioactive tracer in the blood stream of participants or patients. On the other hand, fMRI has a better temporal resolution than PET, around 6 seconds, ma still lower of those offered by electrophysiological techniques (Par. 2.1.1.5). In addition, fMRI presents an excellent spatial resolution and no need for injections. However, conversely to PET, fMRI studies have to deal with pulsation artifacts that alter the signal from deep sources like the brainstem and the thalamus.

The activation PET[4] and fMRI studies, which investigate hemodynamical changes in response to a sensory stimulation or a cognitive task, are based on the comparison between an experimental condition and a control condition. In many pain studies, the comparison refers to painful vs. non-painful stimulation. The results emerge from the subtraction of the activity related to the experimental minus the control condition, so that changes in either rCBF or BOLD signal depend on unique differences across the chosen conditions. Thus, the subtraction results do not necessarily provide a complete image of the processing underlying the phenomenon in question. For instance, differences between painful and non-painful conditions in the activity of a certain brain region do not implicate that the region is sufficient for the pain experience, as well as the lack of differences between painful and non-painful conditions in the activity of another brain region, cannot exclude the participation of that region in pain processing. Moreover, the activity of a region can serve different functional roles, according to which other brain regions are either co-activated or de-activated. Finally, changes in rCBF and BOLD signal may reflect either activating or inhibiting processes, rendering controversial the interpretation of the results in terms of activation of underpinning brain regions (Apkarian et al., 2005; Peyron et al., 2000).

2.1.1.4 Functional correlates

PET and fMRI studies revealed pain-related activations in somatosensory insular and cingulate cortices (S1, S2, IC, ACC), and within other frontal, parietal and

[4] PET offers other possibilities than the 'activation' studies. Other PET studies, thanks to the use of suitable ligands, can map in vivo the distribution of neurotransmitter or receptors.

subcortical regions (see Par. 1.5.2). Somatosensory and posterior insular cortices are thought to constitute the cortical part of the "lateral pain" network, underpinning sensory and discriminative processing. Instead, anterior insular and cingulate cortex are supposed to constitute the cortical part of the "medial pain" network, which underlie affective and cognitive processes related to pain (Apkarian et al., 2005). Collectively, this common pattern of activation, initially interpreted as pain-specific and referred to as "pain matrix", has been recently suggested to constitute a non-specific network subserving the processing of salient and attentional-demanding stimuli, irrespectively of the stimulus modality (Iannetti & Mouraux, 2010).

2.1.1.5 EEG and MEG techniques

Electrophysiological investigations, such as EEG and MEG, measure directly the neural activity elicited by sensory stimulation or cognitive tasks, quantifying voltage changes supposedly generated by synchronized postsynaptic activity within cortical pyramidal cells (Cacioppo, Tassinary, & Berntson, 2007).

Overall, EEG and MEG present an unsurpassed temporal resolution, but their spatial resolution can be considered undetermined. The EEG and MEG spatial irresolution is related to the fact that the brain sources of the signal cannot be directly inferred from scalp potential recording, since electric and magnetic signals within the brain, when reach the scalp level, are smoothed and distorted by the meninges, the cerebrospinal fluid, the skull and the skin. This issue has been referred to as the "inverse problem", which states that there are infinite rather than an optimal unique solution of brain sources matching the superficial electric potentials. However, recently, many authors considered the inverse problem as ill-posed, since it can be addressed if we establish physiological and anatomical assumptions about putative EEG sources and we implement mathematical models of electro-dynamical laws. To solve the EEG inverse problem, were developed two main categories of methods, consisting of parametric and non-parametric solutions. Parametric methods, such as BESA dipole simulation, require a-priori definition of number, direction and strength of the underlying dipoles (Schimpf, Ramon, & Haueisen, 2002). Instead, non-parametric methods, such as sLORETA, require no a-priori assumptions about the distribution of the underpinning sources (Pascual-Marqui, 2002). However, non-parametric solutions identify one main cortical

generator, not excluding the possibility that additional sources may contribute to the processing in question.

Noteworthy, EEG and MEG present a different sensitivity in determining the sources of electrical and magnetic signals. MEG is able to capture neural activity generated by cortical columns oriented perpendicular to the scalp and thus is well suited for detecting activity within S1 and S2, but not ACC. On the other hand, EEG detects any signal, irrespectively of the orientation of the underlying dipole, including neural activity originating from ACC. However, the sensitivity of both techniques decreases with increasing the distance from the scalp, rendering them not suitable for the investigations of deep sources, such as thalamus.

2.1.1.6 Electrophysiological correlates

Electrophysiological pain correlates are related to spontaneous electrical activity and time-locked event-related potentials. Spontaneous pain-related EEG activity consists of decreased alpha (8-12 Hz) and increased beta (15-30 Hz) rhythms. This pattern is typically observed in response to any sensory stimulation, contributing very little to the specific understanding of pain processing (Bromm and Lorenz, 1998). Instead, pain-related potentials and fields refer to EEG and MEG deflections elicited by discrete events consisting of either electrical or laser stimulation. These potentials result from procedure of filtering and averaging, based on the assumption that the electrical activity related to the stimulus processing remains constant across trials, whereas the random differences associated with the background noise are nullified by the averaging.

In EEG and MEG studies, painful stimuli can be administered through either an electrical or a laser stimulator. Electrical stimulation activates both Aβ and Aδ fibers which convey tactile and nociceptive information, respectively, and elicits the so-called Somatosensory Evoked Potentials or Fields (SEPs, SEFs). The elicited potentials include positive and negative deflection from around 50 ms, consisting of the P1 (40-50 ms), N1 (80-90 ms), N2 (100-200 ms), P2 (200-400 ms) and late positive potentials (400-1000 ms). P1 and N1 components are usually observed in the contralateral sites respect to the upcoming stimulation, whereas later components show a bilateral distribution, with peaks over central sites (i.e., Fz or Cz). On the other hand, the laser induces a thermal stimulation considered nociceptive-specific, since it selectively activates Aδ and C fibers. The laser stimuli are absorbed by the skin and directly

activate thermal nociceptors, likely through capsaicin-sensitive ionic channels (Bromm & Lorenz, 1998), and elicit the so-called Laser Evoked Potentials or Fields (LEPs, LEFs). The elicited potentials include N1 (100-200 ms), N2 (200-400 ms), P2 (400-1000 ms) and ultra-late components (> 1 s). This stimulation reproduces the dual pain sensation, consisting of a first acute and stinging sensation, associated to Aδ fibers activity, and a second burning pain, associated to C fibers activity. The activity of Aδ fibers is reflected in the N1, N2, and P2 potentials, whereas the activity of C fibers is thought to correspond to ultra-late potentials occurring after 1 second. The longest latency observed for potentials elicited by laser compared with electrical stimuli, is coherent with the conduction speed associated to the activated fibers by the two types of stimulation, Aβ and Aδ fibers on one hand, and Aδ and C fibers, on the other hand. When laser stimuli are administered to the dorsum of the hand, N1 deflections occur in the contralateral temporal sites (T3/T4 when the reference is Fz), whereas N2 and P2 present a typical bilateral distribution with the largest peak over the vertex (Cz).

Importantly, electrical devices do not elicit pure pain stimulation, but consist of a more ecological situation. Indeed, in everyday life, the selective activation of only nociceptive fibers is uncommon, and in most of the cases, painful sensations arise from the activation of different types of fibers. Furthermore, electrical stimulation is easy to reproduce in laboratory and does not require a sophisticate and expensive equipment such as the laser device (Bromm & Lorenz, 1998).

Irrespectively of the type of stimulation, early pain-related components, such as P1 and N1, are considered to reflect the processing of physical features of the stimuli and activity within somatosensory cortices (Kakigi et al., 2000). Instead, later components, such as N2, P2 and other late potentials, reflect the integration of sensory, cognitive and affective pain-related information and depend on the level of arousal, vigilance, attention, distraction of the individual. MEG studies which made use of laser stimulation identified that the earlier negative components N1 and N2 reflect neural activity originating from operculo-insula and S2 region, whereas later component such as the P2 reflect signals from MCC (Garcia-Larrea, Frot, & Valeriani, 2003).

In the past three decades, electroencephalographic measures were applied to investigate cortical processing involved in pain perception, with the principal aim of isolating specific components of pain experience that can be considered objective biomarkers. A first generation of electrophysiological studies identified the N1, N2, and

P2 ERPs elicited by noxious stimulation as objective indexes of pain perception (Bromm & Lorenz, 1998). These deflections were thought to reflect pain-specific cortical responses as their amplitudes correlated with the perceived pain intensity reported by the subject. However, Iannetti et al. (2008) pointed out that this correlation may be explained by non-specific bottom-up attentional mechanisms, due to the close relationship between pain intensity and salience. In their studies, the authors manipulated the stimulus salience to disclose the contribution of this aspect in pain perception and cortical processing represented by N1, N2, and P2 deflections (Iannetti et al., 2008). They showed that stimulus salience, manipulated by the presentation of three identical stimuli at constant and short inter-stimulus intervals (ISI), explained the supposedly pain-related amplitudes of the ERP components. Indeed, amplitudes associated to salient stimuli (first stimulus of each triplet) were greater compared to those associated with less salient stimuli (second and third stimuli of each triplet). Furthermore, the correlations between ERP amplitudes and pain ratings were significant only when the stimuli were salient. Intriguingly, the pain perception was not affected by the salience manipulation, showing a clear dissociation between perceived pain and the ERPs previously thought to represent an objective neural pain correlate.

In addition, the same authors (Mouraux & Iannetti, 2009) provided compelling evidence in support of this notion, by applying a blind source localization technique, called probabilistic independent component analysis (Beckmann & Smith, 2004) to disentangle the specific neural activity associated to nociceptive, somatosensory, auditory and visual ERPs. The results showed that nociceptive activity was entirely explained by multimodal neural patterns, namely the overlapping activity elicited independently of the sensory modality. Conversely, the other sensory modalities taken into consideration (i.e., somatosensory, auditory and visual) were explained by a combination of both specific and multimodal neural activity. In line with these findings, the authors proposed to frame the functional interpretation of the N1, N2, and P2 ERPs as bottom-up salience mechanisms unspecific for pain.

2.1.1.7 Limitations in pain assessment

Both subjective and objective approaches present intrinsic limitations. When participants and patients are required to rate the intensity or the unpleasantness of pain sensations, they provide an evaluation that can be biased depending on several factors.

Pain measures can be influenced, for instance, by previous experiences that have shaped the individual's "metric" of pain, levels of attention and arousal, affective and cognitive contextual variables such as emotions and expectations, cultural and social factors that may influence the tendency to under- or over-estimate the pain sensations, as well as interfering memory processes when retrospective ratings are required (Turk & Melzack, 2010).

To overcome these limitations, many studies have considered alleged objective measures (e.g., reflexes, ERP-EEG indexes), that have the clear advantage to set a common and a comparable metric across participants. However, many researchers agree that even physiological measures cannot be considered to be a pure objective index of pain experience and are amenable of contextual cognitive and affective processes. In addition, the crucial problem of objective indices refers to capability to measure pain for real. It is doubtless possible to measure a pain correlate, but in most cases, the relationship between the measured index and the pain experience is far from being understood.

In summary, there is no objective pain that individuals can refer to as a common metric, and no objective marker of pain has been reliably individuated. Thus, pain assessment inevitably requires subjective pain evaluations, since only the participant can provide information of certain reliability about the quality and the quantity of his or her pain experience. A solution that can at least partially deal with the intrinsic limitations of subjective reports refers to within-subjects studies, in which different conditions, when adequately chosen, are comparable in terms of uncontrollable variables such as an individual's metric, past experience, affective, attentional, and cognitive aspects, as well as socio-cultural background. During the PhD, my awareness of these limitations emerged gradually and influenced decisions regarding the experimental design of the last experiment of the present thesis. Indeed, the first three experiments consist of between-subjects studies, whereas the last experiment implicated a within-subjects design.

2.2 SENSORY-MOTOR PAIN MODULAITON

Sensory-motor modulation refers to the contextual influence of multisensory perceptual aspects in modulating pain. For example, visually induced analgesia refers to the analgesic effects produced by viewing one's own body, revealed by increased heat

pain thresholds (Mancini, Longo, Kammers, & Haggard, 2011) and decreased pain ratings (Longo, Betti, Aglioti, & Haggard, 2009; Longo, Iannetti, Mancini, Driver, & Haggard, 2012; Mancini, Bolognini, Haggard, & Vallar, 2012). Interestingly, visual induced analgesia emerges only when looking at one's own body part, since no modulation in pain perception and cortical pain processing was found when looking at the hand of another person (Longo et al., 2009). The effect may depend upon cross-modal visual-somatosensory interactions and changes in the connectivity between visual areas underpinning the body representation, and other areas involved in pain perception (Longo et al., 2012). A recent tDCS study demonstrated that the activity within visual extrastriate areas might directly modulate the somatosensory pain processing. Indeed, tDCS over occipital sites did not influence the overall levels of perceived pain, but specifically modulated the analgesic effects of viewing the body (Mancini et al., 2012).

Furthermore, visually induced analgesia is influenced by specific features of the visual content, since the analgesic effects depends upon the spatial scale at which the body is seen. For instance, when the size of a hand appeared either reduced or enlarged, the pain heat threshold is either decreased or amplified, respectively. Thus, visual enlargement increases analgesia, whereas visual reduction decreases it. However, these effects were not found when an object appeared visually reduced or enlarged (Mancini et al., 2011).

Another example of sensory-motor pain modulation refers to the position of the arms in the space. For instance, crossing the arms and the hands over the body midline reduces both tactile and pain processing (Adler et al., 1997; Gallace, Torta, Moseley, & Iannetti, 2011; Yamamoto & Kitazawa, 2001). Indeed, if two sequential tactile stimuli are administered one on each hand, the ability to determine which hand was stimulated first is decreased when participants cross the arms (Yamamoto & Kitazawa, 2001). In addition, participants reported diminished intensity ratings of both electrical non-noxious and laser noxious stimuli when their arms were crossed, irrespectively of the intensity and the quality of the stimulation (Gallace et al., 2011). It is likely that when the processing mediating the stimulus localization is less accurate, the awareness of the stimulus is limited.

Moreover, the body position plays an important role in pain modulation. An extreme condition is Head Down Bed Rest (HDBR), in which the body is tilted down by 6 degrees and the gravity force is orthogonal to the cephalic-caudal axis. HDBR is

conceived of a model to simulate weightless space condition and is used to investigate the consequences of altered gravity states on physiological, perceptual and cognitive functioning (Trappe et al., 2006). A recent investigation reported reduces subjective pain perception as a consequence of the HDBR position maintained for 2 hours (Spironelli & Angrilli, 2011).

2.2.1 Functional correlates of sensory-motor pain modulation

A recent fMRI investigation showed that visually induced analgesia depends upon effective connectivity between visual and cortical areas involved in nociception (Longo et al., 2012). Indeed, an increased functional coupling between the visual extrastriate area and key areas of the pain matrix was found while participants were looking at their own hand. The visual area in question corresponded to the extrastriate body area (EBA) in the lateral occipital cortex, whereas the pain areas included S1 and operculoinsular cortex. Noteworthy, no modulation of ACC activity was found, suggesting that viewing the body alters the connectivity between visual areas and restricted regions within the pain network. Collectively, the results suggest that pain modulation may depend upon the interplay between key nodes of the so-called pain matrix and other brain networks, and are in line with the notion that pain is an emergent property of simultaneous activity within several pain regions.

2.2.2 Electrophysiological correlates of sensory-motor pain modulation

Compared with viewing a neutral object, looking at one's own hand reduces both pain perception and the N2-P2 complex elicited by laser stimulation (Longo et al., 2009). Similarly, crossing the arms over the midline reduced the amplitude of the N2-P2 wave, regardless the stimulus modality that was either electrical nonnoxious or laser noxious. Collectively, these findings suggest that the visual representation of the body contributes to pain processing and that this modulatory effect may depend upon interactions between visual and pain cortical areas. At a cortical level, both the analgesic effects of seeing the body and crossing the arms were associated with reduced N2-P2 complex, but not with modulation of the N1 amplitude. Furthermore, the N2-P2 magnitude was reduced regardless the non-noxious or the noxious stimulus modality. Thus, these effects support the notion that pain-related ERPs mostly reflect multimodal processing of somatosensory stimuli, rather than pain-specific processing.

Interestingly, the HDBR position inhibited pain-related somatosensory processing as documented by a lack of significant P45 component contralaterally to the stimulation side and a lack of modulation of late potentials (N1, N2, P2) across different levels of painful and tactile intensity, in HDBR compared with sitting controls. Moreover, as revealed by a sLORETA analysis, the HDBR group displayed a significant delay in the activation of pain-related areas (Spironelli & Angrilli, 2011).

2.3 ATTENTIONAL AND COGNITIVE PAIN MODULATION

Attention is the most studied psychological variable in pain modulation. Attentional states have been classified in the following theoretical categories, which are partially overlapping: (1) distraction from pain vs. attention to pain, (2) bottom-up vs. top-down attentional processes (Legrain, Van Damme, et al., 2009), (3) attentional processing when pain is irrelevant vs. goal-relevant (Van Damme, Legrain, Vogt, & Crombez, 2010). These classifications highlight different facets of attentional processes involved in pain.

2.3.1 Distraction and Bottom-up Attention

The continuum distraction-attention refers to the focus of the attentional selection. Distraction refers to conditions in which the individual's attention is focused on another sensory modality or cognitive task, during pain stimulation. Reduced attentional resources available for pain processing are invoked to explain the distraction hypoalgesic effects (Legrain, Van Damme, et al., 2009; Van Damme et al., 2010). Instead, attention to pain implicates that the current focus is on upcoming pain stimulation or pain-related information. Attention to pain is conceived to lead to hyperalgesic states, enhancing pain sensations. However, experimental and clinical studies reported contradictory findings, suggesting that the effects of distraction and attention are not as simple and unidirectional as they have been previously defined (Seminowicz & Davis, 2007). The continuum bottom up-top down attention refers to the intentionality of the attentional processes. Bottom-up attention refers to unintentional selection related to salience and novelty detection. Even if unintentional, the bottom-up selection is not purely automatic, since it may be influenced by top-down processes (Legrain, Van Damme, et al., 2009). Top-down attention consists of intentional goal-related selection, which operate by facilitating the neural activity associated to relevant stimuli, and by inhibiting the neural activity associated to

irrelevant stimuli (Desimone & Duncan, 1995). Top-down attentional processes are supposed to act through attentional load and attentional set. Attentional load corresponds to the amount of attention engaged in the task, while attentional set refers to the set of features or criteria used by participants to detect task-relevant stimuli. This cognitive model of attention to pain proposed by Legrain, Van Damme, et al. (2009) has the advantage to provide coherent explanations of attentional pain effects, based on the interaction between co-activated bottom-up and top-down attentional processes.

The continuum pain as task irrelevant-relevant refers to the role exerted by pain stimulation in the experimental task. Pain is an irrelevant stimulus when participants pay attention to another stimulus modality, whereas is goal-relevant when the task performance relies on either pain detection, or discrimination, or evaluation.

The effects of distraction and bottom-up capture of attention on pain have been studied through paradigm in which pain is task-irrelevant. The classic procedure, called "primary task paradigm" (Crombez, Baeyens, & Eelen, 1994), consists of requiring the participant to attend a different sensory modality (e.g., visual, auditory, tactile) or perform a cognitive task, and to ignore the pain stimulation. Distraction effects are inferred by the reduced pain ratings reported by participants when pain occurs and a non-nociceptive stimulus was attended (e.g., auditory stimulus), compared to when the pain occurrence was attended. In line with the limited-capacity models of human cognition, which propose attention as a necessary resource to select and process sensory signals, the analgesic effects of distraction may emerge because the amount of attention available for the upcoming pain is reduced, and further processing of the signal is limited (Legrain, Van Damme, et al., 2009). However, when pain is intense, novel and threatening (Eccleston & Crombez, 1999), the pain stimulation is highly salient, attention-demanding and resistant to distraction. This involuntary bottom-up capture of attention by pain can be indexed by the reduced performance at the primary task, in terms of speed and accuracy, in trials with irrelevant pain compared with trials without pain.

2.3.2 Functional correlates of distraction and bottom-up attention

Findings from functional MRI studies, during the primary task paradigm, reported decreased BOLD signals in the midcingulate region of ACC (Bantick et al., 2002), somatosensory areas (Bushnell et al., 1999), suggesting the idea that reduced

bottom-up processing is associated to inhibited sensory and affective analysis of nociceptive stimuli. Moreover, Tracey et al. (2002) showed that, compared with attention, distraction was associated to activation in PAG, and the subjective analgesic effect of distraction was predicted by the level of PAG activity. These results suggested that distraction may activate descending antinociceptive pathways, resulting in a net inhibition of pain processing.

2.3.3 Electrophysiological correlates of distraction and bottom-up attention

ERP studies suggest that the bottom-up attentional capture by pain may be related to P2 responses elicited by laser-evoked potentials, since greater P2 amplitudes are elicited by salient and novel stimuli, despite attention is directed to another stimulus modality or to another part of the body. However, decreased pain-related P2 amplitudes are observed when the primary task is highly demanding (Legrain, Bruyer, Guérit, & Plaghki, 2005) and increased P2 amplitudes are elicited by pain stimuli, which consistently reduce the performance at the primary task (Legrain, Perchet, & García-Larrea, 2009). Interestingly, the main generator of the P2 has been identified in the midcingulate region of the Anterior Cingulate Cortex (Garcia-Larrea et al., 2003). MCC represents a key structure of the salience network, involved in orienting of attention (Downar et al., 2000), conflict monitoring (Bush et al., 2000), motivation and motor control (Garcia-Larrea et al., 2003).

2.3.4 Limits of the studies on distraction and bottom-up attention

Noteworthy, the interpretation of the results concerning the above studies aimed to investigate attentional processing of task-irrelevant pain or distraction are limited by the fact that the attentional selection in question may not be purely driven by bottom-up or distraction processes, since the effects may be influenced by top-down regulation (Adler et al., 1997; Van Damme et al., 2010). Indeed, every experiment creates a context that influence participants' appraisal and individuals may engage uncontrolled top-down expectations related to the task and their own performance.

2.3.5 Attention and Cognitive Top-Down Mechanisms

On the other hand, the effects of attention to pain and top-down goal-related mechanisms have been studied through paradigm in which pain is task-relevant. The

role of attention in enhancing pain has been studied by asking patients to rate the feeling of a post-surgical pain more or less often, as well as by requiring participants to attend the stimulation and to focus the attention on pain (Tracey et al., 2002). Counter-intuitively, focusing attention on pain could lead to pain reduction (Seminowicz & Davis, 2007).

An important cognitive top-down mechanism consists of reappraisal, which is a form of emotion regulation aimed at changing the interpretation or the meaning of a stimulus. Reappraisal involves the use of selective attention focused on the stimulus that is re-appraised. Usually, reappraisal is conceived of a cognitive strategy applied to change the meaning of negative events, in order to render them less unpleasant and more acceptable. However, negative reappraisal includes situations when the re-interpretation of the event leads to exacerbate negative sensations, unpleasant emotions and distress.

2.3.6 Functional correlates of attention and top-down mechanisms

Since reappraisal requires to attend and to process the affective meaning of the stimulus to be regulated, the typical functional activity observed in such contexts is spread within several brain regions, including areas involved in sensory, cognitive and emotional processing. Thus, activity within somatosensory, insular and cingulate cortices are found in combination with activity within regions involved in emotional and cognitive regulation, such as amygdala, ventrolateral and dorsolateral pre-frontal cortex (McRae et al., 2010).

2.3.7 Electrophysiological correlates of attention and top-down mechanisms

Attention to pain represents the opposite condition of distraction to pain. Usually, when a painful stimulus is attended, we observe increased pain perception associate with an enhancement of the N2-P2 complex. Indeed, both N2 and P2 potentials increase with attention and vigilance (Legrain, Van Damme, et al., 2009). However, a previous study reported that a reappraisal strategy based on mental imagery induced greater N2 potentials associated with greater analgesic effect compared to a neutral condition, suggesting that the N2 may reflect different processes when top-down reappraisal mechanisms are involved (De Pascalis, Magurano, & Bellusci, 1999).

2.4 EMOTIONAL PAIN MODULATION

2.4.1 Emotions, mood and pain

The individual's mood and emotional state are other psychological variables that alter the subjective pain experience. Emotions consist of relatively brief episodes of synchronized responses involving multiple components from physiology to cognition and behaviors, associated with perceived subjective feeling. By contrast, mood is a more diffused motivational state, which comprises feelings of low intensity but longer duration, lasting from hours to days.

The multidimensionality of pain implicates differentiated sensory and cognitive-affective aspects. Indeed, the sensory stimulation is commonly associated with negative affect, revealing a tight relationship between pain sensations, emotions and mood. Interestingly, sensory and affective dimensions were revealed to be dissociable under hypnosis. Sensory but not affective changes induced by hypnotic suggestion were related to activity within S1 (Hofbauer, Rainville, Duncan, & Bushnell, 2001). Conversely, affective but not sensory changes were correlated with ACC activation (Rainville et al., 1997). Additionally, chronic pain can cause a considerable emotional distress. Long-lasting pain conditions can interfere with patients' life, limiting their daily activities, as well as their ability to endure unpleasant sensations and to cope with future implications. These emotional aspects are often referred to as "secondary pain" (Price, 2000) and may contribute to establish a vicious circle, maintaining chronic conditions and aggravating pain-related symptoms and disabilities.

The interpretation of the emotional pain modulation is particularly challenging because the strict relationship between emotion and attention. Indeed, different emotions can reflect different attentional states, rendering uncertain whether the specific pain modulation depends upon changes in either emotions or attention or an interaction between the two processes. For instance, a positive affect may implicate a greater distraction than a neutral affect, rendering ambiguous whether pain inhibition driven by positive emotions depends upon the positive emotion or the increased distraction. Moreover, negative affects related to fear of pain implicate an attentional bias to pain and pain-related information, creating a vicious cycle that increases both the attentional level and the negative feelings (Keogh, Ellery, Hunt, & Hannent, 2001). As a consequence, the strong interdependence between emotion and attention complicates the understanding of the separate contribution of the two processes.

2.4.2 The Motivational Priming Theory

Lang (1995) proposed a two-factorial motivational model of emotions, consisting of an appetitive and aversive motivational system. Each system is defined depending on a dimension of affective valence (continuum from attraction to aversion) and a dimension of activation (continuum from calm to aroused). Thus, appetitive (e.g., sex, food) and aversive stimuli (e.g., threat, pain) elicit positive and negative emotions, respectively, and a motivational drive aimed to either approach to or withdraw from the stimulus. Lang (1995) theorized that when a certain emotion is primed or activated, the responses of the system subserving the emotion in question will be facilitated, whereas the responses of the opposed system will be inhibited. In accordance with this theory, several studies found that positive and negative emotional manipulations determine clear-cut effects on pain perception: positive emotions decrease pain, whereas negative emotions increase it. However, the manipulation of the positive affect lead to reliable pain reduction (Godinho, Magnin, Frot, Perchet, & Garcia-Larrea, 2006; Kenntner-Mabiala, Andreatta, Wieser, Muhlberger, & Pauli, 2008; Villemure, Slotnick, & Bushnell, 2003), whereas results from studies aimed to induce negative emotions have reported more controversial effects (Rhudy & Meagher, 2000, 2003).

Additionally, Lang's group produced a set of emotional pictures, referred to as the "International Affective Pictures System" – IAPS (Lang, Bradley, & Cuthbert, 2005), which includes numerous pictures associated with different emotional contents (families, babies, erotic couples, opposed sex erotica, adventure, sports, food, nature, household objects, pollution, loss, illness, accidents, mutilation, animal and human attack) and normative ratings of valence and arousal for each picture. This set has been largely used in studies aimed to investigate affective and emotional processing. Other procedures to induce positive, neutral and negative affect include exposure to film scenes, reading emotional statements, listening to music, smelling pleasant and unpleasant odors.

Overall, these studies determined that the perceived pleasantness and unpleasantness induced by picture viewing influences valence ratings, heart rate, facial muscle activity, early cortical potentials, and primarily affects the directionality of the modulation accordingly with the primed appetitive or defensive system. Instead, the arousal induced by the emotional context affects arousal ratings, viewing times, reaction times, skin conductance, cortical late potentials, which provide indexes about the intensity of the

current motivational state. The late ERPs, such as the P300 elicited by either startle (H. Schupp et al., 2004) or picture stimuli (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000) and the P2 elicited by a noxious stimulation (Kenntner-Mabiala et al., 2008; Kenntner-Mabiala & Pauli, 2005) are reduced by arousing compared with non-arousing emotional states. These data suggest that the processing of arousing images require a greater amount of attentional resources, regardless of the picture valence. However, this pattern of results is not observed when pictures elicit high levels of distress or fear. For instance, individuals with a specific phobia showed reduced viewing times and reduced P300 amplitudes elicited by pictures representing the object of their specific phobia, consistently with an avoidant behavior pattern.

2.4.3 Functional correlates of emotional pain modulation

Recently, the functional correlates of emotional pain modulation have been investigated in two fMRI studies, which induced emotional states by using either olfactory (Villemure & Bushnell, 2009) or visual stimuli (Roy, Piché, Chen, Peretz, & Rainville, 2009). Villemure and Bushnell (2009) found that, compared with pleasant, unpleasant odors increased both pain perception and activations in the ACC, medial thalamus, S1 and S2. However, the interpretation of these results are limited by the fact that odors and painful stimulation were simultaneously applied, rendering not possible to distinguish pain-related from odor-related activations. Indeed, regions such as the ACC are commonly activated by both stimuli. Roy et al. (2009) showed that emotions induced by pleasant or unpleasant pictures modulated the responses to painful electrical stimuli both at the spinal and the supraspinal level. At the spinal level, the nociceptive processing was indirectly measured by the nociceptive flexion reflex (RIII-reflex). At the supraspinal level, the neural activity associated with the interaction between emotion and pain was identified within the right insula, paracentral lobule, parahippocampal gyrus, thalamus, and amygdala. The effects of emotion on pain ratings correlated with activity in the right anterior insula, consistent with a key role of this structure in the integration of pain signals with the ongoing emotion and in the representation of the subjective feeling of pain (Craig et al., 2000). In contrast, the effects of emotions on the RIII reflex correlated with activity in the left medial thalamus, bilateral amygdalae, left pons, subgenual cingulate gyrus, ventromedial, and medial prefrontal cortices (VMPFC and MPFC). Finally, the connectivity PPI analyses suggested the involvement of

prefrontal (especially OFC), parahippocampal, and brainstem structures in the emotional modulation of pain.

2.4.4 Electrophysiological correlates of emotional pain modulation

Two important electrophysiological studies were conducted on emotional pain modulation (Kenntner-Mabiala et al., 2008; Kenntner-Mabiala & Pauli, 2005). Both studies applied the classic picture-viewing paradigm to induce either positive or negative emotional states and delivered electrical painful and non-painful stimulation in specific time windows during the primary picture-viewing task. Pain perception and pain processing were reduced while participants were involved in the task, compared to passive periods (i.e., intervals or post-picture time windows), suggesting that the extent to which pain is perceived depends upon the overall attentional resources available for the sensory processing. However, within the primary task, pain perception and pain-related potentials were influenced by the emotional backgrounds, with increased perceived pain and N2 potentials during the viewing of unpleasant pictures, whereas decreased pain and N2 potentials during the viewing of pleasant pictures. Instead, P2 potentials reflect the level of arousal elicited by the pictures, with increased amplitudes associated with non-emotional (neutral) pictures and decreased amplitudes related to emotional, either positive or negative pictures.

2.4.5 Gender differences in pain and emotions

Gender differences are extremely widespread at all levels of brain function, from the molecular to the cognitive and behavioral domains. However, they have been largely ignored by many neuroscientists (Mogil, 2012). For instance, it's not uncommon that researches on human disorders have used male animals to model disorders that mainly affect women and that the male animal model cannot even be generalized to a female animal model, rendering not predictable whether the findings will be equally legitimate for understanding brain processes of both sexes. The mechanisms concerning sex differences in pain have been related to sexual dimorphisms, as well as to quantity and quality sex-related differences (Mogil, 2012). A sexual dimorphism is a structure or a behavior present in only one sex or in different forms in both sexes. A quantity differences emerge when males and females differ along a continuum. Instead, quality differences implicate that the same behavior, showed by males and females, is mediate

by different underlying neural mechanisms (Mogil, 2012). Thus, studying gender differences in neural activation and network activity underlying pain mechanisms may have relevant implications for understanding cortical pain processing and for developing effective gender-specific and, in particular female-specific, pain treatments.

Epidemiological data and experimental findings clearly indicate striking sex differences in pain, showing that women suffer of chronic pain syndromes with a highly prevalence compared to men. This net predominance have been shown for common diseases concerning both sexes, including headache, migraine, low back pain, neck pain, knee pain, chronic fatigue syndrome, fibromyalgia, and temporomandibular disorder. In addition, female-specific diseases, such as endometriosis, vulvodynia and menstrual pain, are more prevalent than male-specific diseases, such as chronic prostatitis (Mogil, 2012). Even the abundant evidence indicating that chronic pain is more prevalent in women, the underlying mechanisms of gender biases are not clear.

Sex differences in chronic pain prevalence might be explained considering psychological and biological variables. On one hand, sex differences may be related to gender roles and expectations. On the other hand, sex differences can be linked to hormonal influences, genetic and epigenetic control mechanisms. All these variables are known to alter the ascending pain transmission and/or the descending pain modulation, implicating different susceptibilities to pain syndromes and sensitivity to pain. As a matter of fact, women show higher tendency to use health care services and to report higher pain levels within chronic pain syndromes. However, it's not straightforward to disentangle the contribution of psychosocial and biological susceptibility/sensitivity factors on such attitude. Mogil (2012) reported that gender differences in the prevalence of common and specific pain diseases can be explained by a combination of the following mechanisms: (1) the "social hypothesis" which refers to the greater willingness of women to report symptoms and ask for help; (2) the "susceptibility hypothesis" which claims a greater biological susceptibility of women in developing pain conditions; (3) the "sensitivity hypothesis" which predicts lower pain thresholds and lower pain tolerance for women compared to men. The sensitivity hypothesis has been directly tested in laboratory, but not all the studies reported clear-cut evidence for gender differences. However, the studies showing reliable difference point to women as greatly sensitive to pain, due to the observation of lower threshold and tolerance to pain in female participants. The lack of straightforward results might depend upon confounds

related to biased criteria in participant selection, verbal suggestions about gender differences included in the instruction given to the participant, and the unavoidable social and psychological context which implicitly shape the expectations of the individual, and in turn influence the individual's performance (Racine et al., 2012). A remarkable example is the gender of experimenter, which refers to the evidence that male participants rate pain as less intense in presence of a female rather than a male experimenter. However, a reliable reverse pattern has not been found for women, which tend to evaluate pain with similar ratings, irrespectively of the gender of the experimenter (F. M. Levine & De Simone, 1991). Interestingly, if a female vs. male experimenter is present, male participants showed decreased pain rating, but similar autonomic activation, suggesting the key role of gender roles and expectations in mediating the present effect (Aslaksen, Myrbakk, Høifødt, & Flaten, 2007).

2.5 PLACEBO PAIN MODULATION

The placebo effect denotes "the beneficial effects of a treatment that cannot be ascribed to the physical action of the treatment itself" (Wager, 2005). The phenomenon has been largely studied in pain analgesia, but occurs in a broad range of conditions, such as physical fatigue, hypertension, depression, anxiety and sexual disorders, as well as Parkinson's disease.

2.5.1 Placebo, Expectations and Conditioning

In pain contexts, the placebo effect refers to manipulation of social and contextual factors that produce significant and even radical changes in pain physiology, subjective experience and behavior, mimicking the action of an effective pharmacological treatment. Physiological changes at various levels of the pain processing indicate that the phenomenon is not limited to a response bias or compliance with experimenter's expectation, but shape the pain experience itself (Wager, 2005). The mechanisms of placebo effects have been related to expectations, appraisal, motivation, and classical conditioning procedures. Expectations, appraisal and motivation interact at an explicit level and correspond to a conscious experience, whereas conditioning operates implicitly and the individual is not necessarily aware of the conditioned responses. However, conditioning contributes to the formation of

specific expectations, attesting the presence of dynamical interplays between explicit and implicit processing (Kirsch, 1985).

Expectations can be conceived as "moment-by-moment prediction of the nature and emotional value of upcoming events", elicited by cues that inform an imminent pain (Wager, 2005). Appraisal consists of the evaluation of the event. Instead, motivation refers to subjective goals, such as desire for pain relief.

Placebo-induced expectations of analgesia change the individual's appraisal and motivation, influencing the sensory and emotional processing of the upcoming stimulation. Thus, positive expectations, by influencing the way the stimulation is attended and appraised, lead to reduced perceived pain. In experimental setting, positive expectancy can be induced by verbal suggestions for pain relief in combination with an overt treatment (e.g., administration of an inert pill, injection of a saline solution, sham acupuncture). Verbal suggestions that induce certain expectations of analgesia (i.e., deceptive paradigm) are associated to greater placebo responses, compared to verbal suggestions that induced uncertain expectations; i.e., double blind paradigm (Pollo et al., 2001). On the other hand, placebo-induced conditioning of analgesia refers to repeated associations between a conditioned stimulus (e.g., shape and color of a pill) and an unconditioned stimulus (pharmacological effect of the pill). Thus, conditioning is a learning phenomenon that leads to the acquisition of specific responses eliciting pain relief. In clinical practice, the social context and the patients-practitioner relationship may serve as conditioned stimulus. In experimental setting, conditioning can be obtained by reducing surreptitiously the intensity of pain stimulation so that the participant perceives the treatment as effective (Colloca, Sigaudo, & Benedetti, 2008). The commonest conditioning paradigm consists of three phases: a first phase (baseline) in which the stimulation present certain intensity; a second phase (conditioning) in which the intensity is surreptitiously reduced, and finally a third phase (test) in which stimulation presents the same intensity previously used in the baseline. Conditioning placebo effects are observed whether the intensity ratings in the test phase are significantly reduced compared with the baseline phase.

In clinical settings, prior experience with an active drug increases the placebo effects associated with that drug. However, the response to a specific placebo shows greater consistency with the individual's expectations rather than with the

pharmacological effects of the active drug, suggesting that placebo effects depend upon the interaction between conditioning and expectations (Kirsch, 1985).

Interestingly, the investigations of placebo effects may have relevant repercussion for understanding the mechanisms by which a pharmacological therapy exert its effects, separating the specific effect of a treatment from the effect of expectations in regulating physiological states of the body. Moreover, a better comprehension of placebo effects can have remarkable consequences for reducing the cost of clinical trials, maximizing drug-expectation interactions and thus enhancing the specific effect of a treatment (Benedetti, Mayberg, Wager, Stohler, & Zubieta, 2005).

2.5.2 Functional correlates of placebo pain modulation

Recent evidence from functional studies on placebo with expectation of analgesia showed an increased activity in prefrontal cortex (Petrovic et al., 2002; Wager et al., 2004). Petrovic and coauthors (2002) showed that both the μ-opioid receptor agonist Remifentanil and the placebo were associated to an increased regional cerebral blood flow (CBF) in the rostral anterior cingulate cortex (rACC). Interestingly, only the placebo condition induced the activation of the orbitofrontal cortex (OFC). Moreover, the results revealed a covariation in the activity between the rACC and the rostral ventral medulla (RVM), as well as between the rACC and the periaqueductal gray (PAG), suggesting that activity in rACC may trigger a descending modulation through the RVM and PAG. Wager and coauthors (2004) found an increased activity in OFC, DLPFC, superior parietal cortex and other frontal regions, occurring in anticipation to pain stimulation associated to later reduced feeling of pain. In the pain stimulation phase, the placebo treatment was associated to decreased activity in the thalamus, anterior insula and caudal rACC.

2.5.3 Electrophysiological correlates of placebo pain modulation

Recent ERP studies documented that verbal suggestion and conditioning induce changes in LEP magnitude. Compared to a natural history condition, placebo suggestions reduced the P2 amplitude without subjective perception of pain decrease, whereas conditioning implicated reduced N2 and P2 amplitudes in combination with a dampened pain perception (Colloca et al., 2008). Moreover, the modulation of the LEP magnitude was greater for the conditioning compared with the verbal suggestion alone.

Thus, the results indicate that learning is a crucial aspect in subjective placebo effects (i.e., experience of pain relief) and verbal suggestions without a previous conditioning may exert weaker effects. Indeed, placebo responses are graded according to past experience and learning is crucial for long-lasting and robust effects (Colloca & Benedetti, 2006; Colloca et al., 2008).

2.5.4 Pharmacological studies on placebo pain modulation

A series of studies from Benedetti's group indicated that placebo analgesia results from a balance between endogenous opioids and endogenous CKK (Benedetti et al., 2005). Both opioid effects and placebo analgesia can be blocked by the opioid antagonist naloxone, as initially revealed by J. D. Levine, Gordon, and Fields (1978). Neuropharmacological and PET studies clearly established that at least some types of placebo analgesia is mediated by the opioidergic system (Petrovic et al., 2002; Zubieta et al., 2005). In addition, the endogenous opioid system and placebo analgesia can be counteract by cholecystokinin (CKK), and facilitate by the CKK antagonist proglumide (Benedetti, 1996; Benedetti, Amanzio, & Maggi, 1995). However, other studies revealed non-opioids mechanisms underlying placebo effects (Amanzio & Benedetti, 1999; Benedetti, Amanzio, & Thoen, 2011; Vase, Robinson, Verne, & Price, 2005). For instance, Amanzio and Benedetti (1999) found that a prior conditioning with a non-opioid drug (e.g., ketorolac) was naloxone-insensitive, and speculated that the involvement of either opioids or non-opioids mechanisms in placebo responses contextually depends upon the type of drug used in the preconditioning.

The placebo-activated endogenous opioid system has been implicated in conditions of strong expectation of analgesia and its effects are somatotopic-specific, since differentiated analgesic responses can be induced in different parts of the body (Benedetti, Arduino, & Amanzio, 1999). Interestingly, placebo opioid responses mimic also "side-effects" of effective opioids drug in the respiratory and cardiovascular systems, such as respiratory depression (Benedetti, Amanzio, Baldi, Casadio, & Maggi, 1999), reduced heart rate and β-adrenergic response (Pollo, Vighetti, Rainero, & Benedetti, 2003). These side-effects are naloxone-reversible, clearly indicating an overlap between opioids and placebo analgesia.

2.5.5 The placebo effect in alternative medicine

Kaptchuk (2002) proposed to consider the placebo effect of unconventional alternative medicine as a distinct entity. Indeed, "two interventions may have different effects on patient outcome even though both [are] equivalent to placebo in clinical trials" (Vickers & de Craen, 2000). Thus, labeling the non-specific effects of treatments as "just a placebo", prevent the understanding of important therapeutic implications from being accessed. A better comprehension of non-specific effects implicates the possibility to isolate the "authentic" specific-effects of a treatment, as well as to clarify how treatment specific and placebo non-specific effects interact in influencing the brain activity, and in enhancing the effectiveness of the therapy.

The outcome of placebo effects in both conventional and unconventional medicine depends upon the interaction of several components including (1) patient characteristics, (2) practitioner characteristics, (3) patient-practitioner interaction, (4) the nature of the illness and (5) treatment and settings (Kaptchuk, 2002). Patient characteristics refer, for instance, to individual's expectations, preferences for one type of treatment, and adherence. Practitioner characteristics include optimistic and enthusiastic vs. neutral and skeptic attitudes. Patient-practitioner interaction can influence either positive or negative patients' expectations and favor a therapeutic alliance dominated by reciprocal trust. In unconventional settings, practitioners never discuss the presence or absence of 'reliable' diseases and the diagnosis is personalized, matching the patient's beliefs. The nature of the illness is another important element, since some conditions have been shown to benefit from both placebos and unconventional medicine more than others. These conditions include symptoms lacking of pathological or physiological markers, fluctuating chronic syndromes and affective disorders. Finally, the contextual scenario may contain elements that reinforce the patients' expectations, help to create rituals that may serve as conditioned placebo responses (Kaptchuk, 2002).

CHAPTER III

Experimental investigations of pain modulations: The effects of body position, emotional contexts, placebo and cognitive reappraisal.

The work, upon which the present dissertation is based, was aimed at elucidating subjective and cortical responses to electrical pain stimulation, under non-pharmacological modulation of pain. In particular, embodied sensory-motor, emotional, placebo and cognitive manipulations are considered. In a series of studies, the high temporal resolution allowed by EEG and pain-related somatosensory potentials (i.e., pain-related ERPs) was used to disclose cortical dynamics elicited by the different forms of pain modulation.

The present chapter consists of a collection of four experiments: (1) *Horizontal body position reduces late cortical pain-related processing;* (2) *Gender differences in pain responses under emotional stimulation;* (3) *Placebo effect in participants with high and low confidence in homeopathy*; (4) *Reappraisal of pain and Mental Imagery induce hypoalgesic and allodynic effects*. For each experiment, the method, the results and a discussion are reported. The data collection of the first three experiments took place in the "Psychophysiology Laboratory" at University of Padova, using the same facilities and instruments, including EEG amplifiers and recording system, 38-channel montage, electrical stimulator for pain and no-pain stimulation, and applying similar procedures, consisting of pain threshold assessment, computation of under-threshold and over-threshold intensities, EEG-ERP preprocessing and data analysis procedures. Thus, there will be overlaps in the method and data recording paragraphs (Par. 3.1.3, 3.1.4, 3.2.3, 3.2.4, 3.3.3, and 3.3.4). The last experiment was conducted at the Center for Functionally Integrative Neuroscience (CFIN) at Aarhus University in Denmark, during my period abroad.

STUDY 1: HORIZONTAL BODY POSITION REDUCES LATE CORTICAL PAIN-RELATED PROCESSING

3.1.1 Introduction

The present study investigated the role of an embodied sensory factor, the horizontal body position, on pain perception and cortical pain processing in young and healthy women.

Among the conditions involved in pain modulation, body position plays an important role, but has received little attention so far compared with cognitive and emotional variables. Convergent evidence suggests that simple tasks, such as arm crossing (Gallace et al., 2011) or looking at the stimulated hand versus another object during pain stimulation (Longo et al., 2012), have considerable analgesic effects. An interesting effective manipulation of embodied pain alteration is Head Down Bed Rest (HDBR) in which the body is tilted down by 6 degrees. This condition is also termed "simulated microgravity" as it mimics the perceptual and physiological effects of weightless experienced by astronauts during spaceflight. HDBR has been shown to inhibit cortical activity through an increase of the slow frequency EEG delta and theta bands (Vaitl & Gruppe, 1992; Vaitl, Gruppe, Stark, & Pössel, 1996). In addition, HDBR was associated with both impaired brain plasticity as measured by startle reflex habituation (Messerotti Benvenuti, Bianchin, & Angrilli, 2011), and reduced pain perception and cortical pain responses elicited by electrical stimulation (Spironelli & Angrilli, 2011). In particular, both early Somatosensory Evoked Potentials (P1), reflecting stimulus physical features, and late potentials (N1 and P2), associated with multimodal integration of sensory, cognitive, and affective pain-related information, were altered in young subjects submitted to HDBR (Spironelli & Angrilli, 2011).

A similar, but less extreme, condition is the horizontal Bed Rest (BR) which corresponds to the supine position. The present study was aimed at investigating the effects of BR on pain-related responses elicited by electrical tactile stimulation. We aimed at establishing to what extent pain inhibition induced by HDBR position occurs also in the BR position, by analyzing self-evaluations and somatosensory ERPs collected in two groups of participants (i.e., BR group and Sitting Controls). In addition, we aimed to clarify the functional meaning of the observed electrophysiological effects through correlations between subjective and cortical responses and the localization of

the main cortical generators by sLORETA. In line with our previous study on HDBR (Spironelli & Angrilli, 2011), we expected decreased pain sensitivity and cortical processing in BR participants compared with controls.

It is important to underline that horizontal BR represents a more ecological condition, equivalent to that held for long times by bedridden hospitalized patients. Establishing the influence of this body position on pain might be relevant for the clinical practice, for instance in medical diagnosis based on pain-related symptoms which, if delayed, could have fatal consequences for the patient (e.g., in case of medical complications such as an internal hemorrhagic lesion).

3.1.2 Participants

A total of 32 healthy female volunteers were recruited from the University of Padova and randomly assigned to the experimental (i.e., Bed Rest, BR) or control condition (i.e., Sitting Control, SC). Inclusion criteria required that participants did not suffer from chronic pain diseases or other important medical pathologies, and had not consumed drugs or alcohol within three days of the experiment. Every subject received a course credit for participating in the experiment. Four participants, two from each group, were excluded because they systematically underestimated or overestimated pain thresholds and consequently, during the experimental task, they had the tendency to evaluate each stimulus as painless or painful, respectively. Thus, the final sample consisted of 28 participants, randomly assigned to either the experimental BR (n = 14) or the control SC (n = 14) condition. Groups had similar age ($t(1,26) = -1.37$, n.s.), state-anxiety ($t(1,26) = -0.09$, n.s.), and trait-anxiety levels ($t(1,26) = 0.47$, n.s.). Mean and Standard Deviation (SD) of these variables are reported in Tab.1.1.

Tab.1.1

	GROUP 1 (n=14) Bed Rest		GROUP 2 (n=14) Sitting Control	
	Mean	SD	Mean	SD
Age	23.14	1.83	22.43	0.65
Trait-Anx	36.36	4.84	37.29	5.61
State-Anx	37.71	11.27	37.36	8.63

Tab. 1.1 Mean and Standard Deviation (SD) of age, trait and state anxiety levels, separately for the experimental bed rest (BR) and the sitting control (SC) group.

Participants were on average 90% right-handed, according to the Edinburgh Handedness Inventory (Oldfield, 1971); had normal or corrected to normal vision and were naïve about the purpose of the experiment. In accordance with the Declaration of Helsinki, every participant gave her written informed consent to the study, which was approved by the Ethics Committee of the Faculty of Psychology, University of Padova (Italy).

3.1.3 Stimuli, Task and Procedure

After arrival at the laboratory, participants were randomly assigned to the BR or SC condition, completed the Trait and State Anxiety Inventory STAI-Y2 and STAI-Y1 (Spielberger et al., 1968), the Edinburgh Handedness Inventory (Oldfield, 1971) and were prepared for EEG recording. Throughout the experiment, students laid on a mattress parallel to the floor (experimental BR position) or sat on a soft chair (control SC position). A PC laptop screen was firmly placed 50 cm in front of subject's eyes, to collect pain evaluations. After 90 minutes of rest, during which participants received experimental instructions and performed filler tasks, the pain session started with the assessment of participant's pain threshold. This phase was guided by a LabVIEW (National Instruments, TX) *ad hoc* program, which controlled electrical stimulation through a parallel port. Electrical stimuli were administered to the left forearm by two surface 10 mm gold electrodes and were delivered by a battery powered optoisolated constant current stimulator controlled by PC through the parallel port. Every electrical pulse lasted 10 ms and the session started with a weak fixed intensity (39 microAmperes, μA), typically undetected by participants. Next, stimulus intensity progressively increased with current increments randomly ranging between 39 and 234 μA. Participants had to evaluate each electric pulse using a visuo-analogue scale (range = 0-10) representing different levels of pain intensities: the critical subjective level to be determined was 5, corresponding to "I start to feel pain". The interval between the end of one evaluation and the beginning of the next one randomly varied between 3 and 4 seconds. The procedure stopped as soon as the critical subjective pain threshold was reached, namely when the mean evaluation of three consecutive electric pulses surpassed the level of 5. After the last pain evaluation, the program computed an on-line regression coefficient between the last seven electrical currents and the corresponding

subjective evaluations, and interpolated the exact current intensity (in μA) corresponding to the subjective pain threshold, a-priori set to 5.

After pain threshold assessment, participants began the experimental task consisting of EEG recording plus subjective pain evaluation during the administration of a series of 180 electrical stimuli. Starting from subjects' individual pain thresholds, three different levels of electrical intensities were administered,. The program generated, randomly interspersed: (1) sixty under-threshold electrical pulses, corresponding to -40% pain electrical threshold level, (2) sixty electrical pulses at pain threshold level, and (3) sixty over-threshold electrical pulses, corresponding to +40% pain electrical threshold level. Soon after the delivery of each stimulus subjects evaluated the perceived pain level, furthermore they were not made aware that stimuli were of three different intensities. As for pain threshold assessment, each electrical pulse lasted 10 ms and the inter-trial interval randomly varied between 3 and 4 seconds.

3.1.4 Data recording and analysis

EEG cortical activity was recorded by means of 38 tin electrodes, 31 placed on an elastic cap (Electrocap) according to the International 10-20 system (Oostenveld & Praamstra, 2001), and the remaining 7 electrodes applied below each eye (Io1, Io2), on the two external canthi (F9, F10), nasion (Nz) and mastoids (M1, M2). Cz was used as an on-line recording reference for all channels. Amplitude resolution was 0.1 μV; bandwidth ranged from DC to 100 Hz (6 dB/octave). Sampling rate was set at 500 Hz and impedance was kept below 5 KΩ. EEG was continuously recorded in DC mode and stored for following analysis using the acquisition software NeuroScan version 4.1. Data were off-line re-referenced to the average reference and epoched into 1.2-s intervals, divided into 200 ms before and 1 s after stimulus onset. A 100-ms baseline preceding electric pulse was subtracted from the whole trial epoch. Single trials were corrected for eye movement artifacts, i.e., vertical, horizontal movements and blinking. BESA software (Brain Electrical Source Analysis, 5.1 version) was used to compute ocular correction coefficients, according to Berg and Scherg (1991; 1994). Each trial was then visually inspected in order to reject any residual artifacts: overall, 12.4% of trails were rejected.

After visual inspection of grand-average waveforms, EEG data analysis was carried out on a late component (i.e., the LPP), between 300 and 600 ms. Electrodes

were clustered into four regions of interest to perform statistical analysis with two spatial factors of two levels each: Caudality and Laterality. Clusters comprised the average activity of four electrodes and were labeled Anterior Left (AL: IO1, FP1, F7, F9), Anterior Right (AR: IO2, FP2, F8, F10), Posterior Left (PL: CP3, P3, P7, O1), Posterior Right (PR: CP4, P4, P8, O2).

Subjective pain judgments and electrophysiological components were analyzed by means of analysis of variance (ANOVA), including a between-subjects Group factor (two levels: BR vs. SC position) and a within-subjects Intensity factor (three levels: under-threshold vs. threshold vs. over-threshold). Furthermore, for electrophysiological analyses only, two within-subjects factors were added: Caudality (two levels: anterior vs. posterior) and Laterality (two levels: left vs. right). The Huynh–Feldt correction was applied when sphericity assumptions were violated (Huynh & Feldt, 1970). In these cases, the uncorrected degrees of freedom, epsilon HF and the adjusted p-values were reported. Post-hoc comparisons were computed using the Newman-Keuls test, and statistical significance was expressed at the $p < 0.05$ level.

To clarify the functional meaning of cortical activity occurring in the time window from 300 to 600 ms, Pearson's correlation analyses were carried out between mean self-evaluation and mean ERP amplitudes, separately for under-threshold, threshold and over-threshold intensities.

As a final step, source localization was performed by means of standardized Low-Resolution Brain Electromagnetic Tomography – sLORETA (Pascual-Marqui, 2002) to identify the neural generators of cortical activity measured in the time interval of interest (i.e., 300-600 ms). Since sLORETA computes the smoothest possible 3D-distributed current source density solution constrained to grey matter, this approach is particularly suited for our analysis since, due to the smoothness constraint, it does not need an a priori number of known sources. As a counterpart, sLORETA statistically localizes only the main generator of the maximum EEG/ERP component within a specific interval. This does not exclude the co-existence of other generators (which, in experiments like this are typically many), but the tool highlights only the main source among the many activated in a specific interval. Thus, the regions with largest cerebral activation were analyzed in SC compared with BR participants by performing separated two-tailed t-tests between ERP responses corresponding to each pain intensity (under-threshold, threshold and over-threshold). A positive t value points to a significantly

greater activation of SC participants with respect to BR group, whereas a negative t value indicates a significantly greater activation of BR vs. SC students. All source location results are expressed in Talairach coordinates (Talairach & Tournoux, 1988).

3.1.5 Subjective results

The behavioral analysis was aimed at identifying whether pain perception (pain threshold and subjective pain ratings) is influenced by body position. In the present paragraph, behavioral results concerning electrical thresholds, as well as pain ratings of electrical non-painful (under-threshold) and painful (threshold, over-threshold) intensities are reported.

Pain Threshold. A Student's *t* test was carried out to evaluate whether the two groups differed in the electrical pain threshold: according to the main hypothesis, greater current levels could be expected in the BR group. Analysis revealed no between-group differences ($t(1,26) = -0.73$, n.s.), as BR and SC participants revealed similar electrical thresholds (3.51 mA ± 2.19 and 2.89 mA ± 2.34, respectively).

Pain Ratings. ANOVA computed on subjective pain evaluation collected during the EEG recording task revealed a main effect of the Intensity factor (F (2,52) = 143.89, HF ε = 0.60, $p < 0.001$) and a significant Group by Intensity interaction (F (2,52) = 6.01, HF ε = 0.60, $p < 0.01$; Fig. 1). Both groups reported low pain ratings during the under-threshold condition, moderate ratings to threshold, and high ratings to over-threshold conditions (all $p < 0.001$). However, compared with controls, BR participants evaluated under-threshold stimuli as less intense ($p = 0.01$). No between-group differences in subjective evaluations were found in threshold and over-threshold conditions (Fig. 1.1).

Fig. 1.1

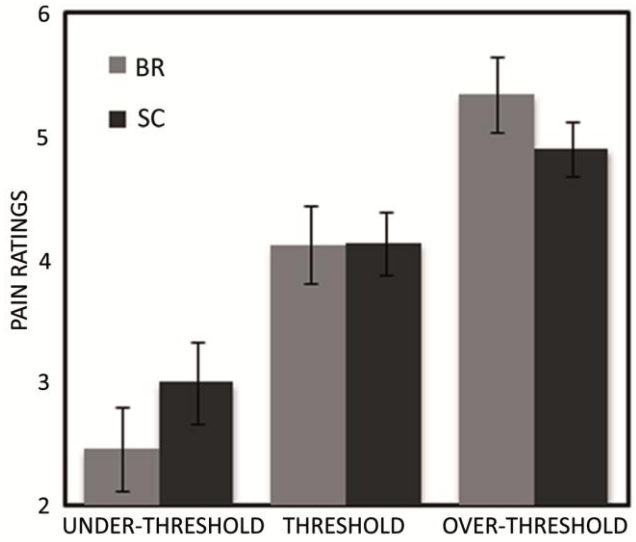

Fig. 1.1 Analysis of subjective pain ratings for the experimental Bed Rest (BR; light grey bars) and the sitting control group (SC; dark gray bars).

3.1.6 Electrophysiological results

ANOVA carried out on ERPs in the 300- to 600-ms time window yielded a significant main effect of Intensity ($F_{(2,52)} = 3.51$, HF $\varepsilon = 1.00$, $p < 0.05$): over-threshold stimuli elicited significant greater negativity in comparison with the other two intensities ($p < 0.001$). Significant Caudality ($F_{(1,26)} = 60.12$, $p < 0.001$) and Laterality main effects ($F_{(1,26)} = 40.37$, $p < 0.001$) were observed in both groups, which showed greater positivity in posterior with respect to anterior sites ($p < 0.001$), and greater positivity in right with respect to left sites ($p < 0.001$). The significant three-way Group by Caudality by Laterality interaction ($F_{(1,26)} = 6.52$, $p < 0.05$) indicated that there were systematic between-group differences in both anterior and posterior regions (Fig. 1.2 and 1.3). Late potentials evoked in the BR group showed smaller negativity at anterior sites and smaller positivity at posterior sites with respect to the SC group ($p < 0.001$), regardless of stimulus intensity. Furthermore, at anterior sites, controls exhibited a bilateral activation, whereas BR participants showed greater negativity on left vs. right locations ($p < 0.01$). Concerning posterior clusters, greater positivity was found in right compared with left electrodes in both groups (all p-values < 0.001; Fig. 1.2 and 1.3).

Fig. 1.2

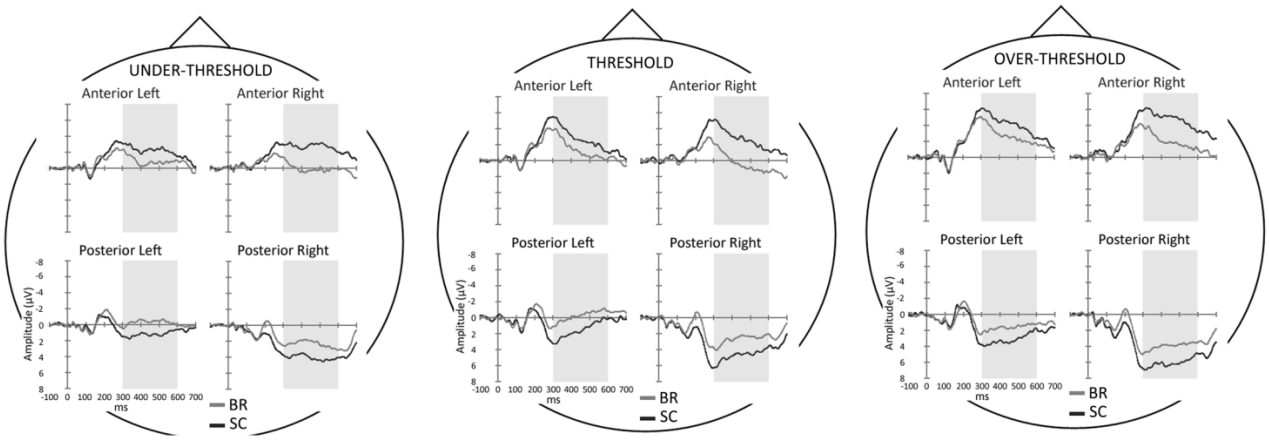

Fig. 1.2 ERP waveforms from the four clusters of electrodes, including the experimental BR (grey line) and the SC control group (black line), in the under-threshold, threshold and over-threshold conditions (from left to right). Time-scale is from -100 to 700 ms. Negativity is displayed upward.

Fig. 1.3

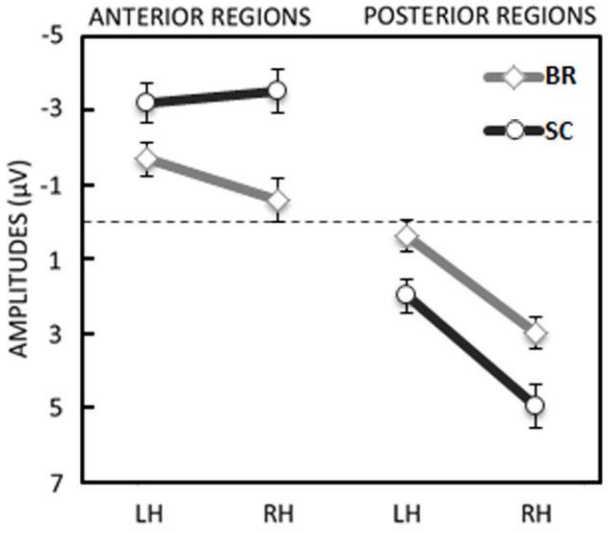

Fig. 1.3 Analysis of the late potentials measured in the 300- to 600-ms epoch elicited by electrical stimuli, for the experimental BR (light grey lines) and the SC control group (dark gray lines). The graph displays the three-way Group (BR vs. SC) by Caudality (Anterior vs Posterior regions) by Laterality (Left vs. Right regions) interaction.

3.1.7 Correlational results

Pearson's correlation analyses were carried out between mean subjective evaluation and mean slow wave amplitude (300-600 ms) for the three levels of stimulation. In SC control group, no significant correlation was found. In contrast, in the BR group, for the conditions threshold and over-threshold, a negative correlation was found at both left and right anterior clusters (threshold: r (12) = - 0.54, $p < 0.05$ and r (12) = - 0.53, $p < 0.05$, respectively; over-threshold: : r (12) = - 0.54, $p < 0.05$ and r (12) = - 0.51, $p = 0.06$, respectively): the higher the pain ratings, the greater was the negativity in anterior left and right locations (Fig. 1.4).

Fig. 1.4

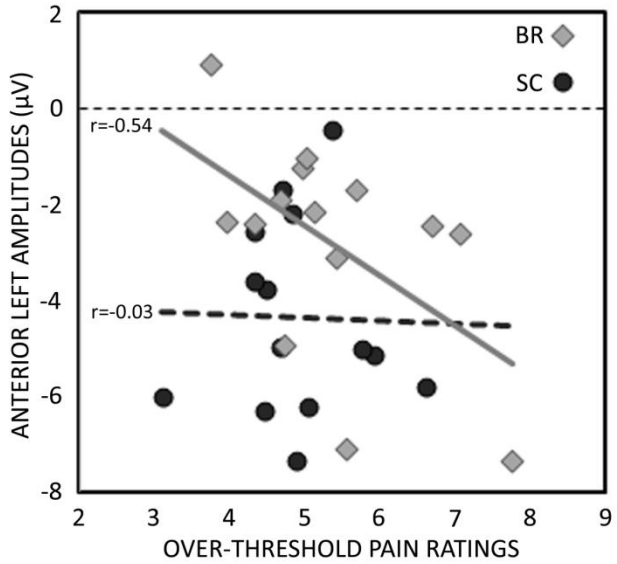

Fig. 1.4 Pearson's correlations, for the over-threshold condition, between subjective pain ratings and the amplitude of the late potentials (300-600-ms epoch) at left anterior sites in the two groups BR and SC.

3.1.8 Source Localization results

sLORETA analyses carried out on the comparison BR vs. SC groups revealed a significant different activity in the 300-600 ms interval after threshold and over-threshold electrical stimulation ($t_{(26)} = 1.82$ and t (26) = 1.63, respectively; all $p < 0.05$), but not after under-threshold pulses ($t_{(26)} = 1.37$, n.s.). Both source analyses located the cortical generator of the 300-600 ms component in the (right) Superior Frontal Gyrus/Cingulate Gyrus (Brodmann Areas (BAs) 6, 24; threshold, coordinates: 10, -5, 58; over-threshold, coordinates: 5, -5, 63; Fig. 1.5). Although the between-group difference was not significant for the under-threshold intensity, the location of the main

generator was similar to that found for the other two intensities (i.e., right Superior Frontal Gyrus/Cingulate Gyrus; BAs 6, 24; coordinates: 10, -11, 54).

Fig. 1.5

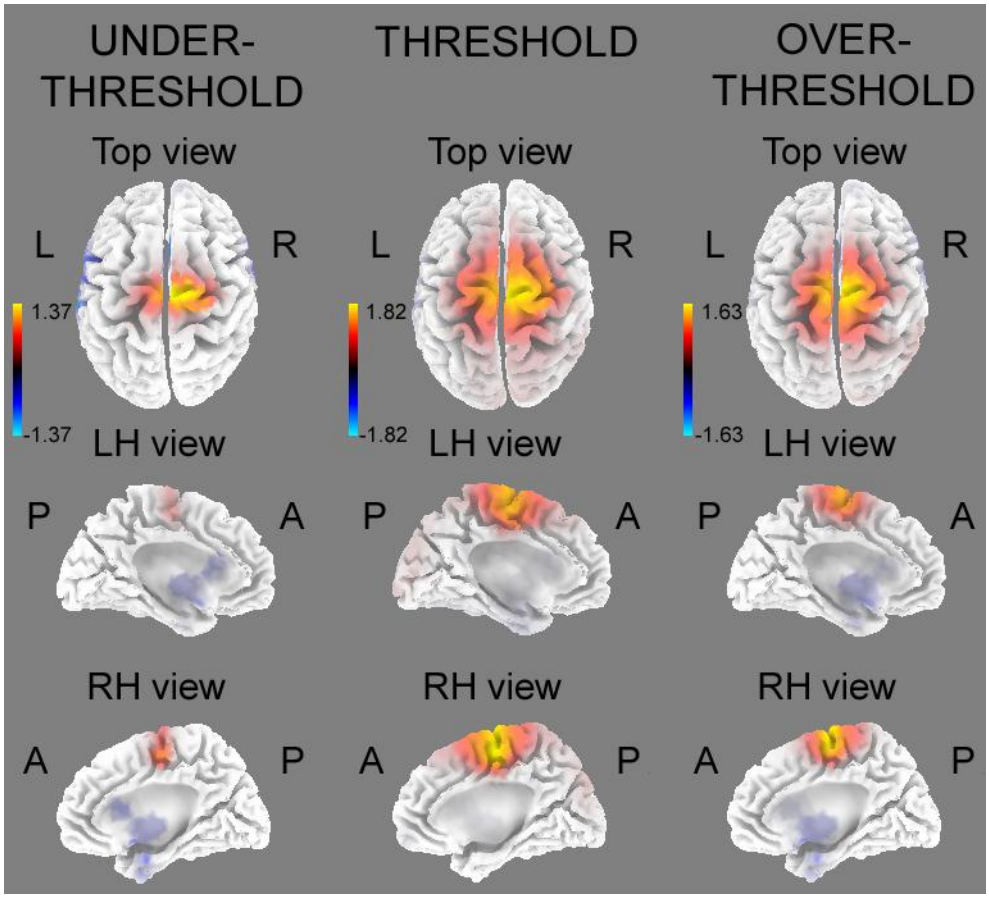

Fig. 1.5 Source localization computed with sLORETA in the 300- to 600-ms epoch: BR group, compared with SC, showed decreased activations of right Superior Frontal Gyrus/ACC.

3.1.9 Discussion

The present study aimed at investigating the effects of horizontal body position (i.e., Bed Rest - BR) on pain evaluation and ERP responses elicited by electrical stimulation in young and healthy women. Mean electrical current corresponding to participants' electrical pain threshold was comparable in the two groups, but the subjective pain evaluation collected during the ERP recording revealed reduced sensitivity to painless electrical stimuli in the BR group. In contrast, painful intensities (corresponding to threshold and over-threshold stimuli) were evaluated similarly by the two groups (Fig. 1.1). In a previous study on the effects of Head Down Bed Rest (HDBR), between-group differences were found for subjective pain evaluations at

threshold and over-threshold conditions (Spironelli & Angrilli, 2011). A simple interpretation of this effect is that horizontal BR could be less effective in modulating subjective pain/perceptual evaluations, compared to a more extreme condition such as HDBR.

Concerning electrophysiological data, the typical components elicited by electrical stimulation are early-evoked potentials with peak latencies ranging between 40 and 80 ms (P1 and N1), followed by late cortical potentials with latencies from 80-100 to 700 ms (Bromm & Lorenz, 1998). In particular, late potentials consist of three components, i.e., a negative peak (N2), a positive peak (P2) and a long-latency positive wave ranging between 300 and 700 ms with the amplitude maximum on the vertex.

According to Bromm and Lorenz (1998), early-evoked potentials reflect the sensory and discriminative analysis of electrical stimulation, In contrast, late components are supposed to reflect the integration of sensory features with emotional and cognitive aspects of pain processing. Indeed, a long latency posterior positivity has been found when the experimental task requires to discriminate and to evaluate unpredictable pain stimuli of different intensities (Becker, Haley, Ureña, & Yingling, 2000). Interestingly, in different experimental contexts, the late positive component is modulated by greater processing of biologically relevant emotional stimuli, particularly with negative contents (Palomba, Angrilli, & Mini, 1997), in women more than in men (Bianchin & Angrilli, 2011), and by anxiety levels (MacNamara & Hajcak, 2009).

Direct intracranial recordings suggest that the cortical generators of very early components are located in the somatosensory associative areas, parietal operculum and insula (Dowman, Darcey, Barkan, Thadani, & Roberts, 2007). Sources of the late N2 component were identified in the medial prefrontal and primary somatosensory cortices; whereas the generators of the late positive potentials (i.e., P2 and P3a) have been found in the anterior cingulate cortex, but also within frontal, temporal, and parietal associative areas (Dowman et al., 2007).

In the present experiment, in comparison to controls, BR participants exhibited reduced late amplitudes from 300 to 600 ms, in posterior and anterior regions of interested, regardless of stimulus intensity (Fig. 2 and 3). This is in line with previous reports of inhibition of P2 and late potentials induced by the HDBR position (Spironelli & Angrilli, 2011). Notwithstanding the decreased amplitude exhibited by BR participants, the pattern of posterior activation was similar in both groups, since greater

positivity was found in right vs. left posterior locations. This finding is in agreement with that found in past studies using Somatosensory Evoked Potentials, in which authors argued that a long latency posterior positivity is found when the experimental task requires to evaluate stimuli with different painful intensity (Becker et al., 2000), or in complex situations such as painful stimulation during background affective processing (Mini, Rau, Montoya, Palomba, & Birbaumer, 1995) and suggested that the late positive component reflects cognitive evaluation and emotional processes. In the present study, the greater right vs. left amplitude was related to the site of stimulation which was contralateral (left forearm) and corresponded to the cortical projection of the analyzed site. Considering anterior sites, SC group exhibited a bilateral activation, whereas the experimental BR group revealed greater negativity on left vs. right locations. However, the greatest between-group difference was found in BR anterior right region of interest. Taken together, electrophysiological results suggest that BR participants had a poor representation of painful stimuli due to an overall inhibition of the fronto-parietal network, especially at the level of right frontal areas.

Correlation analyses carried out between subjective and cortical responses showed significant results for Threshold and Over-threshold conditions on BR group's anterior regions only. In BR group, self-evaluations of both stimulus intensities were negatively correlated with the mean ERP amplitude on anterior clusters, both on right and left sites: the higher the pain ratings, the greater the negativity in anterior left and right locations (Tab. 1.2). Thus, anterior negativity reflected pain-related cortical activation, and the high variability of BR cortical amplitude suggests different effects, across BR participants, of horizontal position on the engagement of frontal areas for pain evaluation. Some participants showed reduced pain sensitivity and decreased anterior negative amplitudes, whereas others, who evaluated painful stimulus with higher ratings, exhibited negative amplitudes which were comparable to those measured in sitting participants.

Analyses carried out with sLORETA revealed between-group differences in the 300-600 ms interval for Threshold and Over Threshold intensities. In both cases, controls exhibited greater activation of (right) Superior Frontal Gyrus/Cingulate Gyrus. The main generator of Under-threshold condition was found in this same location, but no significant between-group difference was found. The Superior Frontal Gyrus (BA6) is a cortical structure which includes the premotor cortex and the supplementary motor

area (SMA), the two main structures involved in movement selection and planning (Nachev, Kennard, & Husain, 2008). The activation of these regions could represent the mechanism underlying protective behaviors, such as the automatic tendency to trigger a physical avoidance reaction with motor involvement of the pain-stimulated arm. The Anterior Cingulate Cortex (BA 24) is involved in several cognitive functions, such as attention orienting, cognitive control and motor inhibition (Carter, Botvinick, & Cohen, 1999; Devinsky, Morrell, & Vogt, 1995; Vogt, Derbyshire, & Jones, 1996). In pain contexts, this structure is one of the main generators of the late positive potentials (Apkarian et al., 2005). In addition, neuroimaging studies found that different subregions of the ACC were related to subjective pain perception and affective-emotional responses (Vogt, 2005), as well as sustained attention and phasic orienting to painful stimuli (Garcia-Larrea et al., 2003; Peyron et al., 1999; Tölle et al., 2001). These results suggest that the ACC activation may have a key role for the emotional processing of pain, by orienting attention towards painful stimuli and by planning adequate motor reaction/inhibition.

The greater right vs. left activation revealed by source analyses, as well as by the statistics showing greater right posterior amplitudes, could reflect enhanced attention to painful stimuli delivered to the left side of the body. However, results are also consistent with past studies which found a right-hemisphere dominance in pain perception, regardless the side of stimulation (Adler et al., 1997; López-Solà et al., 2010; Peyron et al., 1999; Symonds, Gordon, Bixby, & Mande, 2006; Wiech et al., 2006). In line with our results, Symonds et al. (2006) identified five active cortical regions in right hemisphere involved in electrical pain stimulation, i.e., middle frontal gurus, anterior cingulate, inferior frontal gyrus, medial superior frontal gyri, and inferior parietal lobe. In addition, a general bias towards the right hemisphere has been found in both attentive responses to salient sensory stimuli (Corbetta & Shulman, 2002) and negative emotion processing (Palomba et al., 1997). According to these studies, the right hemisphere has a key role in pain modulation, in particular through the activation of the right-lateralized attention system, which automatically orients the cognitive resources to the stimulated body area, but it might involve also the cognitive and emotional processing of aversive stimulation. Thus, the decreased activation of right Superior Frontal Gyrus/ACC observed in the experimental BR group confirms the

inhibitory effects induced by the horizontal position, suggesting that cognitive and emotional resources are reduced and less available for pain processing and coping.

Taken together, our data indicate that the altered cortical processing found for different levels of painful electrical stimulations in BR women was the direct consequence of our experimental manipulation (i.e., the horizontal position). Indeed, correlation results provided evidence in BR subjects of an association between different individual responses to pain Threshold and Over-threshold stimuli in women's anterior areas and the subjective evaluations of painful conditions. Therefore, in horizontal bed rest, orbitofrontal negativity represents an important correlated index of pain processing, and the horizontal position altered the neurophysiologic functioning of this neural circuit. It should be noticed that our sample included healthy and young women, and that results of our experimental manipulation were achieved after just 90 minutes after participants' positioning to horizontal position. Further research should be addressed to study the impact of horizontal position on cognitive functioning in elderly adults, with particular attention to pain processing. Indeed, the analysis of the conditions which alter pain is particularly critical for bedridden hospitalized patients, for whom a decreased pain sensitivity might lead to delayed diagnosis of fatal medical complications. Bedridden patients usually lie for long time on the bed and they are often elderly with age-related cognitive decay: thus, future investigations aimed at clarifying bedridden patients' pain processing could improve their medical treatment and, at the same time, it might also prevent their rapid mental and physical degradation.

In conclusion, compared with sitting controls, healthy BR participants showed an overall inhibition of the fronto-parietal network underlying late phases of pain processing, as revealed by reduced anterior and posterior slow wave amplitude. In addition, the experimental group exhibited a selective inhibition of right frontal structures (the right superior frontal gyrus/ACC), which have an important role in cognitive, affective and motor aspects of pain processing. Results highlight the effects of short-term horizontal position – the inhibition of cortical responses to painful stimulation in young and healthy women – and suggest important implications for the clinical treatment and diagnosis of medical complications arising in bedridden patients.

STUDY 2: GENDER DIFFERENCES IN PAIN RESPONSES UNDER EMOTIONAL STIMULATION

3.2.1 Introduction

The present study investigated the effects of different appetite pleasant and aversive unpleasant backgrounds on pain processing and highlighted the roles of emotional and attentional modulation of pain on subjective and cortical pain responses. Past studies showed gender differences both in pain (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009) and in emotional processing (Bradley, Codispoti, Sabatinelli, & Lang, 2001), suggesting that women are more sensitive to pain and more reactive to unpleasant events than men. However, it is not well-understood how emotions and pain interact and whether this interaction is subserved by sex-specific neural circuits.

According to the motivational priming hypothesis (Lang, 1995), responses to valenced stimuli depend upon the individual's emotional and motivational state. Aversive stimuli trigger responses that are facilitated in the context of a negative emotional state and inhibited in the context of a positive emotional state. Evidence supporting this theory comes from studies that used emotional pictures for affect induction (IAPS; Lang, 1995) and measured acoustic startle reflex, pain threshold, pain tolerance assessed with the cold pressor test, pain ratings, nociceptive flexion reflex, skin conductance response, heart rate acceleration, and event related potentials elicited by either startle stimuli or painful electrical stimuli.

Interestingly, H. T. Schupp, Cuthbert, Bradley, Birbaumer, and Lang (2007) reported that the startle blink reflex and the P300 were differentially modulated by the emotional context. The startle reflex varied with the picture valence, since it was reduced in co-occurrence with a positive emotional state, but increased when a negative emotional state was induced. Instead, the amplitude of the ERP P300 varied with the picture arousal, since an arousing either negative or positive context elicited reduced P300 responses, compared with a non-arousing context. Consistently, Kenntner-Mabiala and Pauli (2005) reported that pain ratings and the N150 (or N2) amplitudes elicited by painful stimulation, but not non-painful ones, were linearly modulated by the valence dimension. Conversely, the P260 (or P2) amplitudes were modulated by the arousal dimension, suggesting that the reduced late component, elicited by both painful and

non-painful stimulation in the context of arousing stimuli, reflect an enhanced allocation of attentional resources.

To better elucidate pain and emotion interaction mechanisms and gender differences in the emotional modulation of pain at both the subjective and cortical levels, we applied the classic picture-viewing paradigm, in combination with electrical painful stimulation, during EEG recording. Emotional states were induced through pictures varying in the content (erotic couples, sport, household object, fear/threat, and mutilation), valence (positive vs. neutral vs. negative) and arousal (low vs. moderate). Moreover, painful electrical stimuli of fixed intensity were delivered during picture-viewing. Participants were required to rate the perceived pain intensity, as well as the perceived pleasantness and arousal associated with the emotional background. Although the pain stimulation was fixed throughout the experiment, we predicted a modulation of pain subjective ratings according to the emotional context, namely pain reduction during the viewing of pleasant pictures and pain enhancement during the viewing of unpleasant contents, compared with neutral events. Concerning ERPs, we also expected a modulation of N2 and P2 amplitudes according to the emotional context, since such components are thought to reflect multimodal neural activity underlying the integration of sensory features with other sensory, cognitive, and emotional aspects of pain processing (Iannetti et al., 2008). Moreover, according to previous findings (Kenntner-Mabiala et al., 2008; Kenntner-Mabiala & Pauli, 2005), we expected reduced pain-related N2 amplitudes for pleasant vs. neutral vs. unpleasant contents (i.e., modulation that mimics the valence associated to the categories), whereas reduced-pain related P2 amplitudes for moderate activating vs. low activating vs. neutral contents (i.e., modulation that mimics the arousal associated to the categories).

3.2.2 Participants

Thirty-eight healthy students (19 males and 19 females) from University of Padova participated in the study. Inclusion criteria required that participants did not suffer from chronic pain diseases or other important medical pathologies, presented no blood phobia, and had not consumed drugs or alcohol within three days of the experiment. Every subject received a course credit for participating in the experiment. Four participants were excluded, two males because their pain threshold was more than two SDs higher than the sample mean and two females because technical failures. Thus, the final sample consisted in 34 participants. Male (n = 17) and female (n = 17) students

were similar for age ($t_{(1,32)}$ = -1.59, n.s.). *t* tests carried out on STAI-Y1 and STAI-Y2 ratings pointed to gender differences in trait ($t_{(1,32)}$ = 2.66, p = 0.01, Tab. 2.1) and state-anxiety rated before the experiment started ($t_{(1,32)}$ = 3.02, p < 0.01, Tab. 2.1). Female participants revealed a greater trait as well as state anxiety in comparison with males. However, state-anxiety assessed by participants after experiment revealed no between-group differences ($t_{(1,32)}$ = 0.08, p = n. s., Tab. 2.1.).

Tab. 2.1

	GROUP 1 (n=17) MALES		GROUP 2 (n=17) FEMALES	
	Mean	SD	Mean	SD
Age	23.41	1.69	22.70	0.68
Trait-Anx	33.00	5.44	39.70	8.82
State-Anx1	31.64	5.30	38.88	8.31
State-Anx2	33.23	8.35	38.71	9.56

Tab. 2.1. Mean and Standard Deviation (SD) of age, trait and state anxiety levels, separately for male and female participants. State-Anx1 and State-Anx2 refer to the compilation of STAI-Y2 before and after the experiment, respectively.

Participants were on average 92.35% right-handed, according to the Edinburgh Handedness Inventory (Oldfield, 1971) and had normal or corrected to normal vision. According to the Declaration of Helsinki, every participant gave the informed consent to the study, which was approved by the Ethics Committee of the Faculty of Psychology, University of Padova (Italy).

3.2.3 Stimuli, Task and Procedure

To examine the relationship between pain and emotion, we administered painful electrical stimuli during the vision of affective pictures of five different contents (erotic, sport, neutral, fear/threat and mutilation). The visual stimuli, consisting of 90 pictures (19 pictures for each condition), were taken from the International Affective Picture System (IAPS; NIMH Center for the Study of Emotion and Attention; Lang et al., 2005). Erotic pictures depicting erotic couples, and sport pictures consisting in sport scenes, were selected as pleasant contents. Instead, fear/threat pictures showing aimed guns, animal or human attack, and mutilation pictures depicting injury, were selected as

unpleasant contents. The pictures[5] were chosen according to the normative ratings (means and standard deviations) on the dimensions of affective valence and arousal (Lang et al., 2005). Criteria for the choice of the pictures included: (1) similar valence and arousal ratings for erotic and sport/adventure pictures, as well as for fear/threat and mutilation pictures; (2) similar valence and arousal ratings for males and females[6]. Unexpectedly, erotic and mutilation pictures induced greater arousal than sport/adventure and fear/threat pictures, differently from our prediction based on the normative ratings (see Par. 3.2.5 and Fig. 2.2, and 2.3).

Painful stimuli consisted of fixed-intensity electrical pulses of 10 ms duration, which intensity was customized for each participant and corresponded to the 40% increment of the subjective pain threshold. The stimuli were administered on the left forearm by means of two surface Ag/AgCl electrodes and delivered by a battery powered constant current stimulator controlled by PC through the parallel port (for further details on the instrument, see Par. 2.1.2). Each picture was presented for 4 seconds. 5 pictures (1 for each category) served as practice trials, whereas the experimental task included 90 pictorial stimuli. While participants were viewing 75 up to 90 pictures (15 for each category), electrical stimuli were administered with equal probability for each content, in a time window after 2.5-3.5 s from the picture onset. Images were displayed with two fixed and pseudo-randomized orders (direct and reverse). The pseudo-randomization set that pictorial stimuli within the same category

[5] The slide numbers were as follows: Erotic (practice trials or trials without pain stimulation): 4607, 4608, 4681, and 4800; Erotic (trials with pain stimulation): 4643, 4645, 4650, 4653, 4658, 4659, 4660, 4670, 4672, 4676, 4677, 4680, 4687, 4694, and 4695. Sport/adventure (practice trials or trials without pain stimulation): 5621, 8117, 8200, and 8370; Sport/adventure (trials with pain stimulation): 5626, 8030, 8080, 8178, 8179, 8180, 8185, 8186, 8190, 8191, 8193, 8250, 8311, 8340, and 8400. Neutral (practice trials or trials without pain stimulation): 7002, 7010, 7190, and 7224; Neutral (trials with pain stimulation): 7000, 7004, 7009, 7025, 7035, 7050, 7080, 7090, 7100, 7110, 7150, 7175, 7233, 7235, and 7950. Fear/threat (practice trials or trials without pain stimulation): 3500, 6190, 6211, and 6350; Fear/threat (trials with pain stimulation): 1050, 1120, 1300, 1301, 3530, 6230, 6243, 6250, 6260, 6313, 6315, 6360, 6510, 6571, and 6821. Mutilation (practice trials or trials without pain stimulation): 3016, 3102, 3130, and 3181; Mutilation (trials with pain stimulation): 3010, 3017, 3030, 3051, 3060, 3071, 3101, 3110, 3120, 3150, 3215, 3225, 3400, 3550, and 9405.

[6] Males and females had comparable normative valence ratings for erotic (mean M = 7.19, mean F = 6.50), sport/adventure (mean M = 7.09, mean F = 6.71), neutral (mean M = 4.87, mean F = 5.03), fear/threat (mean M = 3.09, mean F = 2.30), mutilation (mean M = 2.30, mean F = 1.76); as well as normative arousal ratings for erotic (mean M = 6.48, mean F = 6.10), sport/adventure (mean M = 6.40, mean F = 6.16), neutral (mean M = 2.38, mean F = 2.67), fear/threat (mean M = 6.39, mean F = 6.75), mutilation (mean M = 5.99, mean F = 6.67). Here, are reported the mean ratings of the pictures associated with the pain stimulation, since practice trials and trials without pain stimulation were not included in the statistical analysis.

or more than two pictures of the same affective valence (pleasant: erotic and sport; unpleasant: threat and mutilation) never occurred consecutively.

After arrival at the laboratory, participants completed a trait and a pre-experiment state anxiety inventory, i.e., STAI-Y1 and STAI-Y2 (Spielberger, Gorsuch, & Lushene, 1970); a handedness inventory (Oldfield, 1971); and a 17-item reduced form of the Fear-Survey Schedule, i.e., FSS-III (Wolpe & Lang, 1964). Subjects who reported specific fears to stimuli used in the study were not included. The experimental session started with the electrical threshold assessment (see Par 2.1.2, for further details on the procedure), to identify the electrical current level needed to elicit a pain percept and to compute the intensity used in the experimental task that corresponded to the increment of 40% of the subjective threshold. Therefore, participants were instructed that a series of pictures were shown and that they had to carefully attend and watch each one for the entire duration it was presented on the screen. To guarantee that participants paid attention to each picture, they were told that a free-recall task was required at the end of the session. Additional instructions informed the participants that during the viewing of most of the pictures, they would receive a painful stimulus that they had to ignore. Participants were not informed that the same intensity was presented for each picture, but they were told that the intensity varied in a range above and below the pain threshold intensity previously identified. After 4-6 seconds from the picture offset, two visuo-analogue scales were presented and participants were invited to think back to the painful stimulus and the emotional context elicited by the picture and to rate the perceived pain intensity (first visuo-analogue scale) and the pleasantness/unpleasantness they experienced watching the picture (second visuo-analogue scale).

After the experimental task, the Self-Assessment Manikin, i.e., SAM (Bradley & Lang, 1994); and a state (post-experiment) anxiety survey, i.e. STAI-Y1 (Spielberger et al., 1970) were administered. The SAM consists of "a non-verbal pictorial assessment technique that directly measures the pleasure, arousal, and dominance associated with a person's affective reaction to a wide variety of stimuli" (Bradley & Lang, 1994). In the present experiment participants were required to rate the perceived pleasantness (valence) and activation (arousal), but not the dominance associated to their affective reaction. The state-anxiety survey was exactly identical to the one administered prior to the beginning of the experimental session. The entire duration of the experimental procedure was around 2 hours.

3.2.4 Data recording and analysis

The same settings and EEG parameters were used in data collection of the first, second and third experiment (see Par. 2.1.3). Thus, EEG cortical activity was recorded by means of 38 tin electrodes, 31 placed on an elastic cap (Electrocap) according to the International 10-20 system (Oostenveld & Praamstra, 2001), and the remaining 7 electrodes applied below each eye (Io1, Io2), on the two external canthi (F9, F10), nasion (Nz) and mastoids (M1, M2). Cz was used as on-line recording reference for all channels. Amplitude resolution was 0.1 μV; bandwidth ranged from DC to 100 Hz (6 dB/octave). Sampling rate was set at 500 Hz and impedance was kept below 5 KΩ. EEG was continuously recorded in DC mode and stored for following analysis using the acquire software NeuroScan 4.1 version. Data were off-line re-referenced to the average reference and epoched into 1.2-s intervals, divided into 200 ms before and 1 s after stimulus onset. A 100-ms baseline preceding every electric pulse was subtracted from the whole trial epoch. Single trials were corrected for eye movement artifacts, i.e., vertical and horizontal movements, and blinking. BESA software (Brain Electrical Source Analysis, 5.1 version) was used to compute ocular correction coefficients, according to Berg and Scherg (1991; 1994). Each trial was then visually inspected for any residual artifacts: overall, 11.65 % of trails were rejected. After visual inspection of grand-average waveforms, EEG data analysis was carried out on two components, in the intervals between 100-150 ms and 260-320 ms, corresponding to the N2 and P2 amplitudes. Three electrodes along the midline, namely FCz, Cz and CPz, in which both components showed the maximum amplitude, were considered.

Subjective pain ratings and electrophysiological components were analyzed by means of analysis of variance (ANOVA), including the between-subject Group (two levels: males vs. females) and the within-subject Picture Content (five levels: erotic vs. sport/adventure vs. neutral vs. fear/threat vs. mutilation) factors. Furthermore, to compare the average amplitude of the three electrodes, the within-subject factor Electrode (three levels: Fcz vs. Cz vs. CPz) was added to the ANOVA carried out on electrophysiological components. The Huynh–Feldt correction was applied where sphericity assumptions were violated (Huynh & Feldt, 1970). In these cases, the uncorrected degrees of freedom, epsilon HF values and the adjusted p-values were reported. Post-hoc comparisons were computed using the Newman-Keuls test, and statistical significance was expressed at the $p < 0.05$ level.

3.2.5 Subjective results

The subjective analysis was aimed at identifying whether pain perception, indexed by pain threshold and subjective pain ratings, is influenced by emotional picture viewing, and whether gender differences occur in emotional pain modulation. In the present paragraph, subjective results concerning pain threshold, pain ratings, emotional valence and arousal ratings, and picture viewing times are reported.

Pain Threshold. t test carried out on electrical levels corresponding to pain threshold revealed no gender differences ($t_{(1,32)}$ = 0.37, n.s.), since male and female participants had similar current intensity threshold (4.43 ± 1.77 mA and 4.73 ± 2.73 mA, respectively).

Pain ratings. Analysis of subjective ratings to painful stimuli pointed to a main effect of picture content ($F_{(4,128)}$ = 15.11, HF ε = 0.71, $p < 0.001$, Fig. 2.1). Pain evaluations were lower during vision of erotic pictures compared to all other conditions ($p < 0.001$), and during vision of sport compared to mutilation pictures ($p = 0.01$). Differences on pain ratings between sport and neutral pictures, as well as sport and fear/threat pictures failed to reach the significance ($p = 0.06$ and $p = 0.056$, respectively). No significant difference concerned the evaluations of perceived pain during the presentation of neutral, threat and mutilation pictures; and no interaction between picture content and gender was found.

Fig. 2.1

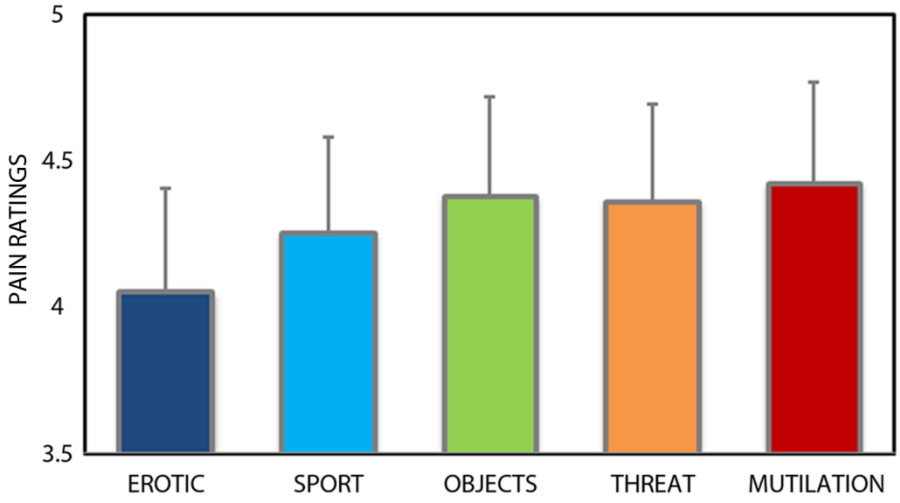

Fig. 2.1 Analysis of subjective pain ratings. Pain stimuli were administered during the vision of emotional pictures, which was classified in five categories depending on the emotional content (erotic vs. sport/adventure vs. objects vs. fear/threat vs. mutilation/injuries). Significant differnces concern pain ratings associated with erotic pictures vs. pain ratings associated with all other contents.

Picture Ratings. Three separate one-way ANOVAs were carried out on valence, arousal and viewing times. All the analysis included the factors Gender (males vs. females) and Picture Content (erotic vs. sport vs. neutral vs. fear/threat vs. mutilation). In addition, since two evaluations of valence picture were required during the experiment, the ANOVA performed on valence ratings involved the additional factor Time (first vs. second evaluation).

(1) Valence. Analysis of valence ratings showed a main effect of Gender ($F_{(1,32)}$ = 7.86, $p < 0.05$) and Picture Content ($F_{(4,128)}$ = 278.68, HF ε = 0.53, $p < 0.001$). Overall, female participants rated pictures with lower ratings compared with males during both the first and the second evaluation. In addition, erotic and sport pictures were evaluated as more pleasant than neutral pictures, and erotic were rated as more pleasant than sport ones. Conversely, attack and mutilation pictures were evaluated as more unpleasant than neutral pictures, and mutilation were rated as more unpleasant than attack ones (All p-values < .001). Finally, the interaction Picture Content by Time was significant ($F_{(4,128)}$ = 6.76, HF ε = 0.57, $p < 0.001$). The second time, participants rated erotic pictures as more pleasant, whereas fear/threat and mutilation pictures as more unpleasant (All p-values < 0.05), regardless the gender of the participant.

Fig. 2.2

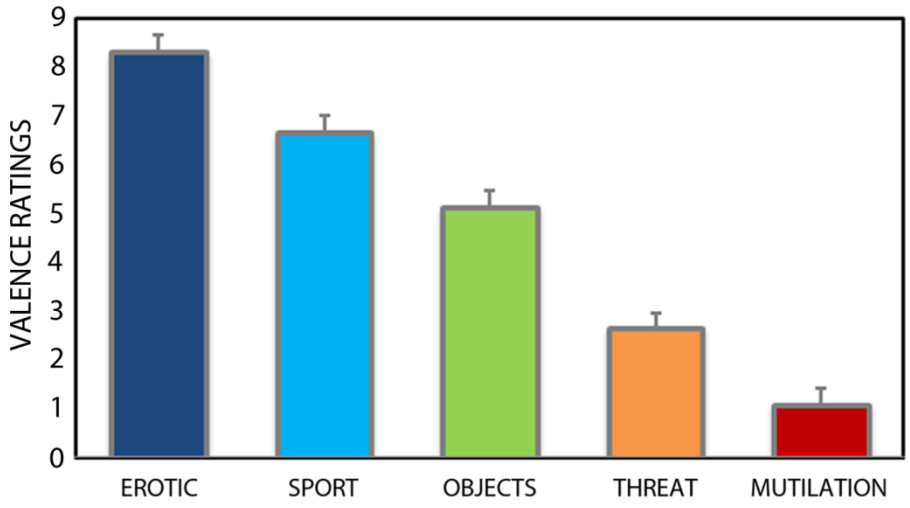

Fig. 2.2 Analysis of subjective valence ratings (erotic vs. sport/adventure vs. objects vs. fear/threat vs. mutilation/injuries). Significant differences between all categories were found.

(2) Arousal. Analysis of arousal ratings revealed a main effect of Picture Content ($F_{(4,128)}$ = 95.55, HF ε = 0.88, p < 0.001, Fig. 2.3). Erotic and mutilation pictures were evaluated as more arousing than sport, fear/threat and neutral ones (p < 0.001), and sport and threat pictures were rated as more arousing than neutral ones (p < 0.001). Erotic and mutilation pictures, as well as sport and threat ones were rated as equally arousing by participants.

Fig. 2.3

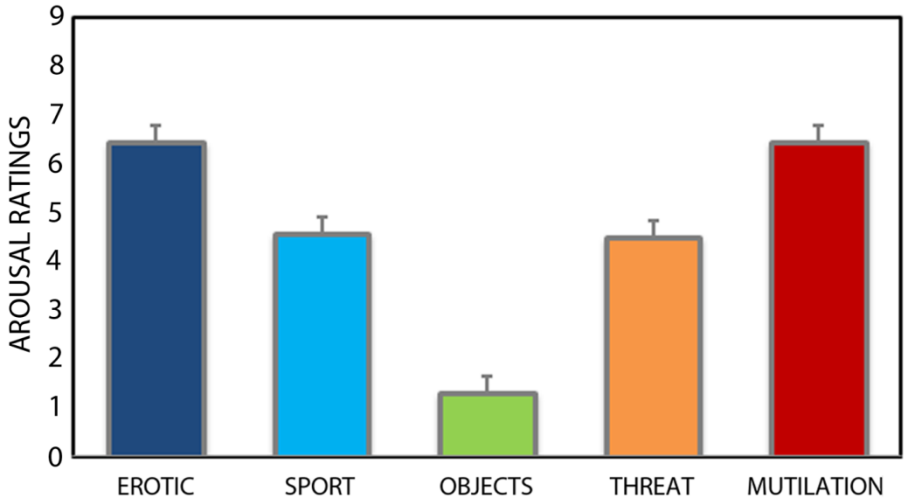

Fig. 2.3 Analysis of subjective arousal ratings (erotic vs. sport/adventure vs. objects vs. fear/threat vs. mutilation/injuries). Significant differences concern arousal ratings associated with erotic and mutilation vs. sport/adventure and fear/threat vs. neutral/objects.

(3) Viewing Times. Analysis of viewing times, showed a main effect of Picture Content ($F_{(4,128)}$ = 9.99, HF ε = 0.74, p = 0.001, Fig. 2.4). Both positive and negative emotional pictures were viewed longer than the neutral (All p-values < 0.05). Participants watched for a longer time the positive pictures (no difference between erotic and sport) compared with the negative pictures (no difference between fear/threat and mutilation). No interaction between picture content and gender was found for valence and arousal ratings, as well as viewing times.

Fig. 2.4

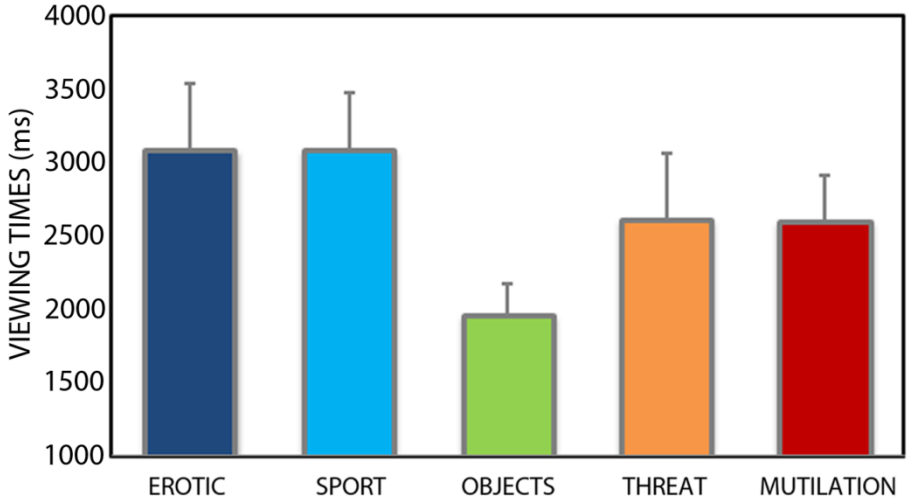

Fig. 2.4 Analysis of viewing times (erotic vs. sport/adventure vs. objects vs. fear/threat vs. mutilation/injuries). Significant differences concern viewing times associated with erotic and sport/adventure vs. fear/threat and mutilation vs. neutral/objects.

3.2.6 Electrophysiological results

The waveforms resulting from the averaging of the epochs time-locked with the pain stimulation are depicted separately for males and females in Fig. 2.5. The figure includes the three electrodes chosen for the statistical analysis (FCz, Cz and CPz), in the time interval from -200 to 800 ms. The gender differences in the emotional pain modulation is indicated by the differentiated pain responses elicited during the vision of pleasant (erotic and sport/adventure), unpleasant (fear/threat and mutilation), and neutral (household objects) emotional contents in males and females.

Two ANOVAs were carried out on ERPs in the 100- to 150-ms and on the 260- to 320-ms time windows (Fig. 2.6). The analysis of the first component, which is labeled N2 or N150, yielded the significant main effects of Picture Content ($F_{(4,128)}$ = 5.69, HF ε = 0.95, $p < 0.001$) and Electrode ($F(2,64) = 24.05$, HF ε = 0.68, $p < 0.001$). Collectively, pain stimuli elicited a reduced N2 during the vision of erotic pictures compared with all other contents ($p < 0.005$). No differences in pain processing during the vision of other emotional contents were found. The N2 amplitudes were greater in the central electrode Cz compared with FCz ($p = 0.01$), and in FCz compared with CPz ($p < 0.001$). However, the two-way interaction Gender by Electrode ($F(2,64) = 5.41$, HF ε = 0.68, $p < 0.01$) revealed that males elicited greater amplitude in Cz compared with FCz and CPz, but no difference between FCz and CPz were found.

Fig. 2.5

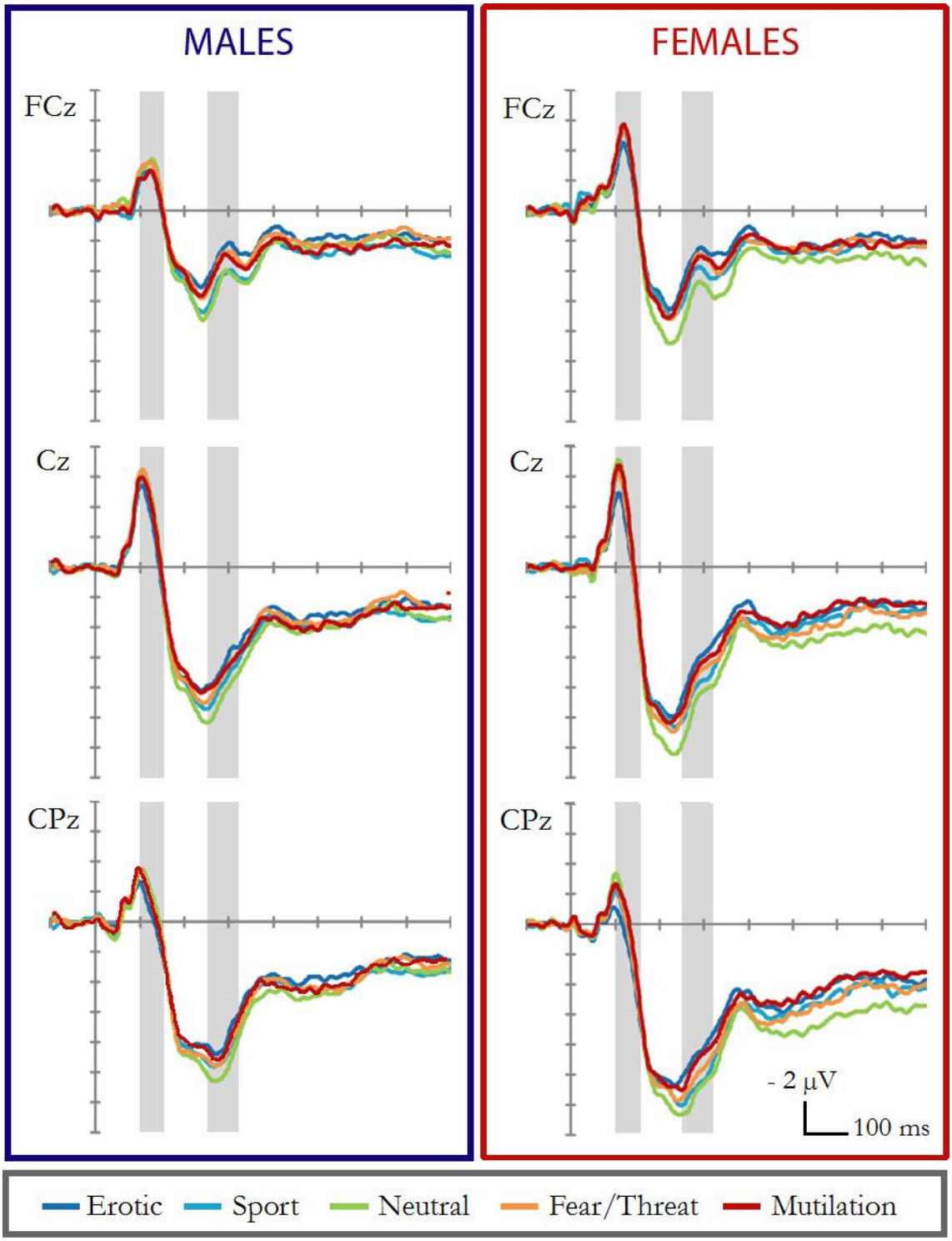

Fig. 3.5 ERP waveforms, from three electrodes (FCz, Cz, CPz), elicited by painful stimulation during the vision of emotional pictures, consisting of erotic (blue line), sport (light blue line), neutral (green line), threat (orange line), mutilitation (red line) pictures. The ERP are depicted separately for male (left panel) and female (right panel) participants. Time-scale is from -100 to 800 ms. Negativity is displayed upward. The two grey areas depict the time windows considered in the statistical analysis: 100-150 ms and 260-320 ms.

Instead, females elicited similar and increased amplitudes in fronto-central electrodes (FCz and Cz) compared with the more posterior electrode (CPz). No between-group differences were found at the level of Cz electrode, indicating that greater gender differences emerged in more frontal and more parietal electrodes (i.e., FCz and CPz). The significant three-way Gender by Electrode by Picture Content interaction ($F_{(8,256)}$ = 2.08, HFε= 0.86, $p < 0.05$) pointed to systematic between-group differences, especially in CPz (Fig. 2.5 and 2.6).

Fig. 2.6

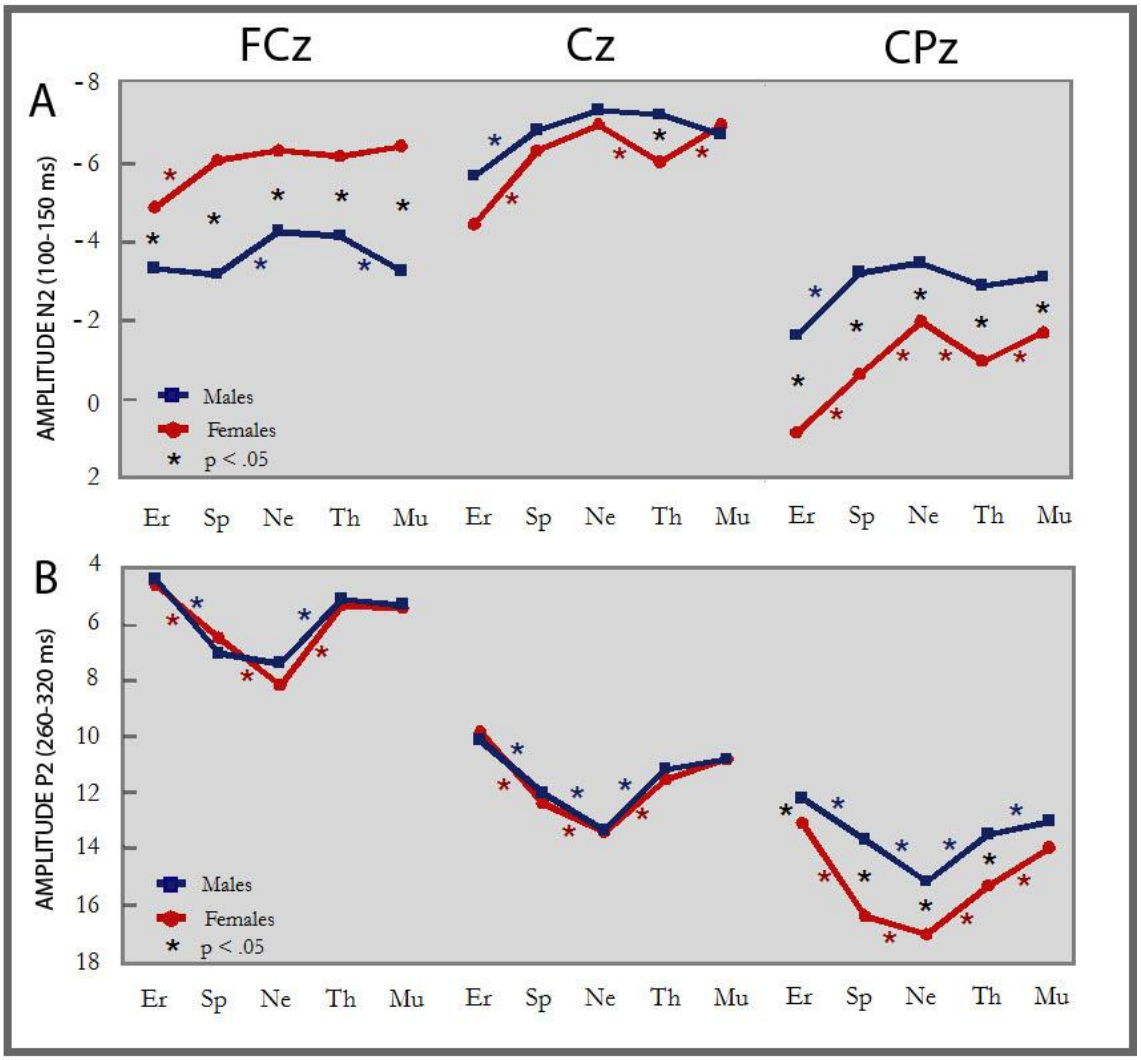

Fig. 3.6 Analysis of the late potentials **(A)** N2 and **(B)** P2 measured in the 100-150 ms and 260-320 ms from the onset of the pain stimulation, for male (blue lines) and female (red lines). The graphs display the four-way Group (male vs. female) by Emotion (erotic vs. sport vs. objects vs. threat vs. mutilation) by Electrode (FCz vs. Cz vs. CPz) interaction.

Compared with males, females displayed a more differentiated pattern of cortical pain responses with an increasingly diversification of cortical pain modulation in the posterior electrode (CPz). Indeed, females showed reduced N2 amplitudes for the erotic emotional content in the three electrodes (i.e., FCz, Cz, CPz). However, in Cz, an additional cortical modulation was found in association with fear/threat images, which displayed reduced negative potentials compared with both neutral and mutilation backgrounds, similar negative potentials with sport pictures, but increased negative amplitudes in comparison with the erotic content. No difference between sport, neutral and mutilation backgrounds was revealed. Finally, CPz displayed the more differentiated cortical responses to pain since an N2 reduction was also found for sport/adventure pictures compared with neutral ones. In summary, the pattern showed by females indicated inhibited cortical pain processing with the greatest modulation for erotic pictures compared with sport/adventure compared with neutral. Fear/threat picture-viewing was associated with similar cortical modulation elicited by sport/adventure backgrounds, whereas mutilation presented similar cortical modulation elicited by neutral images (Fig. 2.6, read line). On the other hand, males showed reduced N2 for erotic, sport and mutilation emotional contents compared with neutral and threat pictures in the frontal electrode (Fz), but only reduced N2 for the erotic pictures compared with all the other pictures in Cz and CPz electrodes (Fig. 2.6, blue line).

The analysis of the second component, which is labeled P2 or P260, yielded also the significant main effects of Picture Content ($F_{(4,128)} = 16.80$, HF $\varepsilon = 1.00$, $p < 0.001$) and Electrode ($F_{(2,64)} = 174.39$, HF $\varepsilon = 0.78$, $p < 0.001$). Collectively, pain stimuli elicited an enhanced pain-related P2 during the vision of neutral pictures in comparison with all other contents ($p < 0.01$), whereas reduced P2 was found during the vision of erotic and mutilation pictures ($p < 0.001$). The P2 amplitudes were greater in the posterior electrode CPz compared with Cz ($p < 0.001$), and in Cz compared with FCz ($p < 0.001$). Finally, the significant three-way Gender by Electrode by Picture Content interaction ($F_{(8,256)} = 2.18$, HF $\varepsilon = 0.62$, $p = 0.05$) indicated gender between-group differences, especially in CPz (Fig. 2.5 and 2.6). In CPz, females displayed increased cortical pain responses than males, regardless the emotional content. Both females (Fig. 2.6, read line) and males (Fig. 2.6, blue lines) showed enhanced P2 for neutral pictures, compared with other emotional contents. Moreover, reduced P2 amplitudes were

significant for erotic and mutilation pictures in all electrodes. However, in the CPz electrode, females showed increased positive amplitudes for each category, with increasing differences for pictures eliciting reduced arousal.

3.2.7 Discussion

The present study aimed at investigating gender differences in the emotional modulation of pain. Several studies described the effects of emotional processing on both subjective pain and pain-related physiological responses, such as nociceptive flexion reflex, skin conductance response, heart rate acceleration and event-related potentials (e.g., Kenntner-Mabiala & Pauli, 2005; Kenntner-Mabiala et al., 2008). These studies indicated that pain-related processing was reduced during pleasant and enhanced during unpleasant pictures relative to neutral ones, as predicted by the motivational priming theory (Lang., 1995). However, Rhudy, Williams, McCabe, Russell, and Maynard (2008) reported that nociceptive spinally-mediated responses (i.e., nociceptive flexion reflex) elicited during erotic and threat pictures, but not during food and loss contents, were differently modulated as compared with neutral pictures. The authors suggested that the magnitude of pain-related responses may depend upon the valence-by-arousal interaction with the greatest modulation for high-arousing emotions. However, it is not clear whether gender differences in emotion processing contribute to gender differences in pain modulation. Consistent gender-related effects are well documented in both the emotional and the pain domains, with striking differences at a disadvantage to women in the prevalence of affective and chronic pain disorders (e.g., Bianchin & Angrilli, 2012; Mogil, 2012).

To clarify the relationship between gender differences in the pain and emotion domains, we analyzed subjective pain ratings and ERP responses elicited by electrical stimulation in young and healthy women and men, while they were viewing pictures depicting an emotional context. The five categories of images were chosen from the IAPS (Lang et al., 2005) and included erotic, sport/adventure, neutral, fear/threat, and mutilation contents. On the basis of normative ratings, we chose pictures that are similarly rated by men and women. As such, by limiting emotion-related gender biases, we might isolate the presence of distinctive gender differences in emotional pain modulation. Analysis of the subjective ratings of affective valence, arousal and viewing

times provided by the participants of the study confirmed the absence of gender-related differences in picture processing (see Fig. 2.2, 2.3, 2.4).

At the pain subjective level, the mean electrical current corresponding to participants' electrical pain threshold was comparable in male and female participants, suggesting that the two groups were similarly sensitive to pain. Although clinical investigations clearly report that women experience chronic pain states with higher prevalence and greater severity, gender differences in experimental pain are less consistent: some studies found gender differences in pain threshold and tolerance, but some others did not. The studies reporting gender-related differences indicate that women experience pain with lower stimulation (lower pain thresholds) and endure pain less than men (lower pain tolerance), especially when the noxious stimulation induce a pronounced affective reaction (Rhudy & Williams, 2005). However, some investigators argue against a straightforward presence of gender differences in experimental pain. For instance, Mogil (2012) considers that some studies may be confounded by gender role expectations (Par. 2.3.6). Importantly, to avoid this confound in the present study, a male and a female experimenter collected the data of male and female participants, respectively.

Concerning subjective data, pain ratings collected during the ERP recording revealed a differentiated pain perception accordingly with the emotional background. Despite the stimuli had identical physical intensity throughout the task, reduced perceived pain was reported during the vision of erotic pictures compared with the other contents, and during the vision of sport/adventure pictures compared with mutilation, regardless the gender of the participant. Trends to significance was observed for the perceived pain intensity associated with the vision of sport/adventure pictures compared with neutral, and fear/threat ones (Fig. 2.1). Noteworthy, no gender differences emerged at the subjective level, suggesting a lack of gender-related quantitative differences in the emotional pain modulation in this sample. However, qualitative differences emerged at the cortical level. The evidence of comparable subjective or behavioral responses in men and woman, but different gender-related neural activity has been previously reported in several studies investigating gender differences in emotional processing (Wrase et al., 2003) and regulation (Domes et al., 2010; McRae, Ochsner, Mauss, Gabrieli, & Gross, 2008).

Concerning electrophysiological data, we focused on the late potentials N2 and P2, which are supposed to reflect the integration of sensory features with emotional and cognitive aspects of pain processing (Fig. 2.5). Compared with men, women showed greater N2 amplitudes in FCz, whereas lower negative amplitudes in CPz (Fig. 2.6). Moreover, in Cz and CPz electrodes, women revealed differential pain-related effects depending upon the background content. Pain stimuli elicited the lowest N2 amplitudes during the vision of erotic pictures, whereas greater N2 amplitudes were elicited during the vision of mutilation and neutral pictures compared with sport and fear/threat ones. In contrast, in Cz and CPz electrodes, men showed reduced N2 amplitudes for only erotic pictures compared with all other emotional events. Furthermore, both for men and women, P2 amplitudes were greater during the vision of neutral pictures compared with sport, fear/threat and mutilation ones, whereas the lowest amplitudes were elicited during vision of erotic pictures (Fig. 2.7). Gender differences were revealed in CPz electrode, where women elicited greater positive amplitudes than men for all contents. Such differences were larger for low-arousing pictures.

Accordingly with previous studies, both subjective pain ratings and N2 modulation mirrored the affective valence, with the lowest N2 amplitudes elicited in the context of erotic pictures and the highest N2 amplitudes in the context of mutilation scenes (Kenntner-Mabiala & Pauli, 2005; Kenntner-Mabiala et al., 2008). Instead, the P2 modulation mirrored the picture arousal with the lowest amplitudes with high-arousing pictures (erotic and mutilation), intermediated amplitudes with moderate-arousing pictures (sport/adventure and fear/threat) and the highest amplitudes with low-arousing pictures (images of neutral objects). However, no differences were revealed between the processing of neutral and negative contents at both the subjective and N2 modulation, with the only exception of the N2 pain-related modulation in the context of threat images in women. Indeed, in Cz and CPz, women displayed reduced pain-related cortical processing during the vision of fear/threat images compared with neutral and mutilation pictures.

According to the motivational priming hypothesis (Lang, 1995), pain responses are inhibited by positive emotional contexts, whereas are facilitated by negative emotions, suggesting that the emotional valence determines the directionality of the modulation. Moreover, emotional modulation may depend on the degree of the system activation (arousal), with greater changes during highly arousing emotional contexts

(Rhudy & Williams, 2005; Rhudy et al., 2008). However, this theory fails to predict the pain outcome associated with moderate-to-high arousing negative emotions reported in the present study both in the subjective and ERP responses. We speculate that the lack of pain facilitation exerted by unpleasant contexts may be related to the parallel co-activations of emotional and attentional processes. Indeed, cues with intrinsic motivational relevance engage a widespread activation, which is functional to sustain emergency reactions in dangerous situations for the individual's survival (Lang et al., 1993). Thus, the attentional effect may contrast the affective facilitation triggered by the aversive background, leading to a net null result. However, the N2 potential suggested reduced pain-related cortical processing during the vision of fear/threat pictures in women, but not in men. The results are consistent with previous studies on emotional processing which show that, compared with men, women exhibit a defensive system that is more attuned to threatening stimuli (Bradley et al., 2001). Indeed, fear/threat stimuli lead to higher arousal in women. This high level of activation could contrast the pain facilitation induced by the negative valenced background, leading to relative hypoalgesic effects. Thus, the same negative stimulus could lead to a different outcome in men, because fear/threat stimuli induce lower arousal (Rhudy and Williams, 2005).

In summary, participants revealed pain inhibition during the vision of pleasant pictures. In particular, erotic stimuli exerted the greatest effect on pain modulation in both females and males, revealed by the reduced pain ratings and the decreased N2-P2 amplitudes. The influence of sport pictures was less pronounced as compared with erotic, suggesting the specific effect of arousal and stimulus content within the same valence category. Results showed no reliable pain facilitation both for subjective ratings and ERP amplitudes, since the unpleasant and neutral conditions similarly modulated pain perception and pain-related cortical processing. Gender differences concerned the N2 and P2 modulation, with the maximal effects in the CPz electrode. Compared with men, women revealed a more differentiated pain modulation according to different emotional contexts (modulation of the N2 amplitudes) and an overall greater late cortical processing (modulation of the P2 amplitudes). Interestingly, the results revealed no straightforward pain facilitatory effect by unpleasant emotions compared with neutral pictures. Both subjective pain ratings and N2 modulation revealed no systematic differences in pain modulation driven by neutral, fear/threat and mutilation emotional contexts, suggesting that pain facilitatory effects may be counteracted by increased

attention levels allocated during the viewing of arousing vs. non-arousing pictures. However, only women displayed reduced cortical processing in the context of fear/threat pictures compared with neutral and mutilation scenes in the electrodes Cz and CPz. This result suggests that qualitative gender differences in emotional processing may lead to a differentiated pain modulation in women and men.

Collectively, both pain ratings and N2 amplitudes varied with picture valence and may represent the net pain modulation emerging from the interaction of affective and attentional processes. Instead, P2 amplitudes varied with picture arousal and may reflect contextual attentional processes. Altogether, these findings indicate qualitative gender differences in pain processing and suggest that emotional pain modulation depends upon complex interactions between pain-specific, emotional and attentional processing.

STUDY 3: PLACEBO EFFECTS IN PARTICIPANTS WITH HIGH AND LOW CONFIDENCE IN HOMEOPATHY

3.3.1 Introduction

The present study investigated the placebo effects associated with the individual confidence in a homeopathic or traditional analgesic treatment. Several studies showed that pain experience can be strongly modulated by beliefs and expectancy concerning the efficacy of the taken drug, so that even inert substances can produce pain relief, i.e., placebo effect (Price, Finniss, & Benedetti, 2008; Wager, 2005). Interestingly, objectively active analgesic pain treatments may induce differential outcome depending on the patient's awareness of taking an analgesic (open vs. hidden administration), verbal suggestions (reinforcement of the efficacy of the drug) and past experience (previous conditioning). Recently, the conceptualization of the phenomenon has been moved from "placebo" as the "effect" of an inert substance, to "placebo effects" as a product of the simulation of an active therapy within a psychosocial context (Price et al., 2008).

Alternative medicines, such as homeopathy, have not been extensively explored using placebo procedure. Most of placebo studies are conducted in clinical practice and refers to traditional medicine. Moreover, researches aimed to assess clinical trials (irrespectively of the type of the specific treatment), rarely consider individual's belief as an important variable in influencing analgesic outcome, thus ignoring a remarkable methodological confound. However, the few placebo investigations on alternative medicine suggested that this treatment may potentiate placebo effects (Kaptchuck, 2002).

To date, it has not been fully clarified whether the increased placebo effects in alternative contexts may be related to the beliefs associated to the treatment or to other therapeutic elements (e.g., doctor-patient relationship). As a first step in this field, the present work was conducted to clarify the role of personal beliefs on traditional and alternative medicine in the placebo modulation of pain, by comparing different groups of participants with equally strong belief in either the traditional or alternative medicine. The experimental procedure consisted in a deceptive paradigm, in which a supposedly effective traditional or homeopathic treatment was administered to each participant at the beginning of the experimental session. Thus, both experimenters and participants

were not aware that the administered treatment was inert. Participants were assigned to three groups, according to an ad-hoc questionnaire build to evaluate to which extent an individual trusts traditional and homeopathic treatments. For two groups (first and third group), the treatment matched the individual's belief. Instead, for one group (second group), the treatment was incoherent with the individual's belief about the effectiveness of the treatment. In summary, the first group consisted of participants with high confidence in traditional pain treatments (allopathy), which took an inert pill of ibuprofen. The second group comprised participants with high confidence in traditional pain treatments (allopathy), which took three inert granules of Aconitum Napellus. Finally, the third group included participants with high confidence in homeopathic pain treatments, which took three inert granules of Aconitum Napellus. The virtual "firth group", consisting of participants with high beliefs in homeopathy, which took a traditional pain treatment, was missing since most individuals who make use of alternative medicine tend to refuse any traditional treatment.

Compared to a double-blind study, the deceptive procedure has the advantage to induce certainty about the administered drug, avoiding that participants think that they could have received an ineffective treatment (i.e., placebo). This procedure has been shown to induce greater expectation of pain relief (Price et al., 2008). To exclude the possibility of involuntary verbal suggestions, the experimenters were not informed about the ineffective nature of the treatments used in the present study.

We hypothesized greater placebo effects for those groups of participants to which the administered treatment was coherent with the personal beliefs, compared to the incoherent group. Moreover, we expected amplified effects for the group of participants which believed in the efficacy of homeopathy.

3.3.2 Participants

An initial sample of 244 students participated to the screening for the study. The participant selection occurred considering individual's beliefs regarding the efficacy of traditional and alternative treatments. The construct in question was measured by an ad-hoc questionnaire written by Prof. Alessandro Angrilli and myself. The questionnaire consisted of 50 items that assess the tendency to prefer a traditional or an alternative treatment, as well as the use and the knowledge of the therapeutic mechanisms associated to the preferred medicine. A total of 68 participants who reported a clear

preference for either the traditional (n= 46) or the alternative medicine (n= 22) were selected. The individuals who preferred the traditional medicine were randomly assigned either to the coherent (traditional treatment, n = 23) or to the incoherent group (alternative treatment, n = 23). However, the final sample included 57 up the 68 initial participants, because technical failures in data collection (n = 2), low quality of the EEG recording (n = 1) or inaccurate estimations of the pain threshold by participants (8 participants were discarded because in the experimental task they evaluated over-threshold stimuli with mean ratings below 3, when the pain threshold was set at 5 on a VAS scale from 0 to 10). In conclusion the sample was differentiated in the following manner:

1st Group = 19 participants with high confidence in traditional pain treatments (allopathy), which took a supposedly active pill of ibuprofen (200 mg)

2nd Group = 20 participants with high confidence in traditional pain treatments (allopathy), which took three supposedly active granules of Aconitum Napellum (5CH)

3th Group = 18 participants with high confidence in homeopathic pain treatments, which took three inert granules of Aconitum Napellum (5CH)

All participants did not suffer from chronic pain diseases or other important medical pathologies, and had not consumed drugs or alcohol within three days of the experiment. Groups were similar for age ($F_{(1,54)} = 0.03$; $p = 0.97$); trait-anxiety ($F_{(1,54)} = 0.30$; $p = 0.74$) and state-anxiety, measured before ($F_{(1,54)} = 0.96$; $p = 0.39$) and after ($F_{(1,54)} = 2.35$; $p = 0.10$) the experiment, by the State-Trait Anxiety Inventory (STAI-Y1 and STAI–Y2, REF). Mean and standard deviations of these variables for each group are reported in Tab. 3.1.

Tab. 3.1

	GROUP 1 (n=19) ALLOP_IBUPROFEN		GROUP 2 (n=20) ALLOP_ACONITUM		GROUP 3 (n=18) HOMEOP_ACONITUM	
	Mean	SD	Mean	SD	Mean	SD
Age	20.63	1.83	20.65	2.25	20.47	1.47
Trait-Anx	39.68	4.80	40.90	4.54	41.11	8.30
State-Anx1	37.53	7.65	40.75	6.81	40.83	10.43
State-Anx2	30.37	3.83	34.15	6.71	31.22	6.17

Tab. 3.1. Mean and Standard Deviation (SD) of age, trait and state anxiety levels, separately for the three groups. State-Anx1 and State-Anx2 refer to the compilation of STAI-Y1 before and after the experiment, respectively.

Participants were on average 89.61% right-handed, according to the Edinburgh Handedness Inventory (Oldfield, 1971), had normal or corrected to normal vision and were naïve about the purpose of the experiment. Every subject received a course credit for participating in the experiment. In accordance with the Declaration of Helsinki, every participant gave her written informed consent to the study, which was approved by the Ethics Committee of the Faculty of Psychology, University of Padova (Italy).

3.3.3 Stimuli, Task and Procedure

After arrival at the laboratory, participants completed the Trait and State (pre-experiment) Anxiety Inventory STAI-Y2 and STAI-Y1 (Spielberger et al., 1970), the Edinburgh Handedness Inventory (Oldfield, 1971) and were prepared for EEG recording. The experimental session started with the assessment of three pain thresholds: thermic, mechanic and electrical.

The thermic pain threshold is the temperature level (in Celsius centigrade, C°) needed to elicit a pain percept. To assess this threshold, participants were asked to rest the palm of their hand on a metal griddle, which can adjust the metal temperature (both heating and cooling) up to 45° C. This instrument was set at 35° C at the beginning of the assessment and the temperature was increased of about 1° C every 10 seconds. Participants were instructed to withdraw the hand as soon as the temperature started to feel painful. This procedure was applied on both hands and the order of the hands was counterbalanced between participants. The threshold value consisted of the mean of two measured (one for the left and one for the right hand) obtained for each participant. The mechanic pain threshold is the weight level (in Kilograms, Kg) needed to elicit a pain percept. To assess this threshold, participants were asked to rest the fingers of their hand, whereas a little shaft terminating in a Teflon hemisphere was gradually lowered to exert a certain pressure on the second phalanx of a finger. Participants were instructed to refer to the experimenter as soon as the pressure started to feel painful. This procedure was applied on the index, middle and ring fingers of each hand. The order of the hands was counterbalanced between participants. The threshold value consisted of the mean of the six measured (three for each hand) obtained for each participant. The electrical pain threshold is the electrical current level needed to elicit a pain percept and was assessed by using the same program and procedure applied in the first study (Par 2.1.2).

After the pain thresholds assessment, participants began the experimental task consisting of EEG recording plus subjective pain evaluation during the administration of a series of 162 electrical stimuli. Starting from subjects' individual electrical pain thresholds, three different levels of electrical intensities were administered. The program generated, pseudo-randomly interspersed: (1) fifty six under-threshold electrical pulses, corresponding to -40% pain electrical threshold level, (2) fifty six electrical pulses at pain threshold level, and (3) fifty six over-threshold electrical pulses, corresponding to +40% pain electrical threshold level. Soon after the delivery of each stimulus, subjects evaluated the perceived pain level. Noteworthy, they were not made aware that stimuli were of three different intensities. As for pain threshold assessment, each electrical pulse lasted 10 ms and the inter-trial interval randomly varied between 3 and 4 seconds.

3.3.4 Data recording and analysis

The same settings and EEG parameters were used in data collection of the first, second and third experiment (see Par. 2.1.3 and 2.2.3). Thus, EEG cortical activity was recorded by means of 38 tin electrodes, 31 placed on an elastic cap (Electrocap) according to the International 10-20 system (Oostenveld and Praamstra, 2001), and the remaining 7 electrodes applied below each eye (Io1, Io2), on the two external canthi (F9, F10), nasion (Nz) and mastoids (M1, M2). Cz was used as on-line recording reference for all channels. Amplitude resolution was 0.1 μV; bandwidth ranged from DC to 100 Hz (6 dB/octave). Sampling rate was set at 500 Hz and impedance was kept below 5 KΩ. EEG was continuously recorded in DC mode and stored for following analysis using the acquire software Curry 7 version. Data were off-line re-referenced to the average reference and epoched into 1.2-s intervals, divided into 200 ms before and 1 s after stimulus onset. A 100-ms baseline preceding every electric pulse was subtracted from the whole trial epoch. Single trials were corrected for eye movement artifacts, i.e., vertical and horizontal movements, and blinking. BESA software (Brain Electrical Source Analysis, 5.1 version) was used to compute ocular correction coefficients, according to Berg and Scherg (1991; 1994). Each trial was then visually inspected for any residual artifacts: overall, 7.1 % of trails were rejected.

After visual inspection of grand-average waveforms, EEG data analysis was carried out on two components, in the intervals between 100-120 ms and 200-250 ms,

corresponding to the N2 and P2 amplitudes. Four electrodes along the midline, namely Fz, FCz, Cz, and CPz in which both components showed the maximum amplitude, were considered.

Thermic, mechanic and electric pain thresholds, subjective pain judgments and electrophysiological components were analyzed by means of analysis of variance (ANOVA), including the between-group factor Group (three levels: group 1 vs. group 2 vs. group 3) and the within-group factor Intensity (three levels: under-threshold vs. threshold vs. over-threshold). Furthermore, to compare the average amplitude of the three electrodes, the within-group factor Electrode (two levels: mean Fz-FCz vs. mean Cz-CPz) was added to the ANOVA carried out on electrophysiological components. The Huynh–Feldt correction was applied where sphericity assumptions were violated (Huynh & Feldt, 1970). In these cases, the uncorrected degrees of freedom, epsilon HF values and the adjusted *p* values were reported. Post-hoc comparisons were computed using the Newman-Keuls test, and statistical significance was expressed at the $p < 0.05$ level.

3.3.5 Subjective results

The subjective analysis was aimed at identifying whether pain perception (thermic, mechanic and electrical thresholds, as well as pain ratings) is influenced by the personal confidence on the efficacy of the pain treatment supposedly received (i.e., placebo effect for a homeopathic or an allopathic treatment).

Pain Thresholds. ANOVAs carried out on thermal, mechanic and electrical levels corresponding to pain thresholds revealed no between-group differences: Thermic threshold ($F_{(2,54)} = 0.41$; $p = 0.67$); mechanic threshold ($F_{(2,54)} = 0.81$; $p= 0.45$); electrical threshold ($F_{(2,54)} = 0.55$; $p = 0.58$). Mean and standard deviations of the thresholds (expressed in Celsius centigrade, C°; kilogram, Kg; and microampere, μA; respectively) are reported in Tab. 3.2.

Pain Ratings. ANOVA computed on subjective pain evaluation collected during the EEG recording revealed a main effect of the factor Intensity ($F_{(2,108)} = 321.76$, HF $\varepsilon = 0.60$, $p < 0.001$; Fig. 1), but no significant Group by Intensity interaction ($F_{(4,108)} = 0.54$, p = n.s.). All groups reported low pain ratings for the under-threshold condition, moderate ratings for the threshold, and high ratings for the over-threshold condition (all $p < 0.001$, Fig.3.1).

Tab. 3.2

	GROUP 1 (n=19) ALLOP_IBUPROFEN		GROUP 2 (n=20) ALLOP_ACONITUM		GROUP 3 (n=18) HOMEOP_ACONITUM	
	Mean	SD	Mean	SD	Mean	SD
C°	41.89	1.50	41.50	1.87	41.39	2.00
Kg	1.47	0.37	1.51	0.37	1.62	0.77
µA	4102	2162	4615	3571	3631	2730

Tab. 3.2. Mean and Standard Deviation (SD) of thermic (C°), mechanic (Kg) and electric (µA) pain threshold in the three groups. 1st group, left = Allop_Ibuprofen; 2nd group, middle = Allop_Aconitum; 3rd group, right = Homeop_Aconitum).

Fig. 3.1

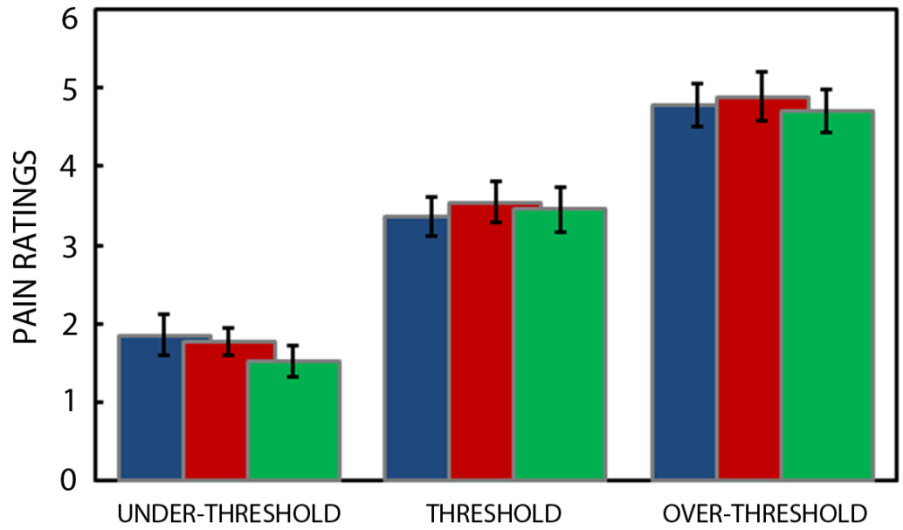

Fig. 3.1 Analysis of subjective pain ratings associated with electrical stimulation, for each Intensity (under-threshold, threshold and over-threshold) and each group (1st group, left/blue bar = Allop_Ibuprofen; 2nd group, middle/red bar = Allop_Aconitum; 3rd group, right/green bar = Homeop_Aconitum).

Personality Questionnaire (MPQ). The means and standard deviations associated with each group of participants and each subscale of the Multidimensional Personality Questionnaire are reported in Tab. 3.3.

ANOVA computed on each subscale revealed a main effect of the group for the constructs of Absorption ($F_{(2,54)} = 4.31$; $p = 0.02$, Fig. 3.2) and Unlikely virtues ($F_{(2,54)} = 3.15$; $p = 0.05$, Fig. 3.3). The Absorption scale refers to items that describe the individual predisposition to suggestionability. High scores on this scale reflect the perceived capability of being absorbed and influenced by experience, thoughts and images (Tellegen & Atkinson, 1974). The Unlikely virtues scale consists of items that

assert a highly desirable but improbable quality. High scores on this scale implicate that the individual is describing himself/herself in falsely favorable way to give a good impression according with the social desiderability standards (Patrick, Curtin, & Tellegen, 2002).

Tab. 3.3

	GROUP 1 (n=19) ALLOP_IBUPROFEN		GROUP 2 (n=20) ALLOP_ACONITUM		GROUP 3 (n=18) HOMEOP_ACONITUM	
	Mean	SD	Mean	SD	Mean	SD
Wellbeing	6.53	2.41	7.1	3.64	8.11	2.80
Social potency	8.00	3.26	6.15	3.22	7	3.11
Achievement	5.53	2.97	5.35	3.22	6.83	3.43
Social closeness	8.00	2.24	8.50	3.38	8.94	2.92
Stress reaction	6.42	2.99	6.20	3.62	6.39	3.01
Alienation	2.84	2.83	2.50	2.46	2	1.85
Aggression	3.84	2.24	3.10	1.62	2.67	1.61
Control	7.47	3.24	7.65	3.48	7.89	2.97
Harm avoidance	8.79	2.25	8.25	2.79	8.89	1.45
Traditionalism	6.16	2.09	6.10	2.17	5.33	2.83
Absorption*	6.95	2.44	6.95	2.58	9.05	2.53
Unlikely virtues*	1.74	1.19	2.45	1.39	3.33	2.87

Tab. 3.3 Mean and Standard Deviation (SD) of the ratings obtained at the Multidimensional Personality Questionnaire (MPQ), for each subscale (wellbeing, social potency, achievement, social closeness, stress reaction, alienation, aggression, control, harm avoidance, traditionalism, absorption, unlikely virtues) and group (1st group = Allop_Ibuprofen; 2nd group = Allop_Aconitum; 3rd group = Homeop_Aconitum). Between-group differences were reported for the sub-scales with the sign "*", i.e., absorption and unlikely virtues.

Between-group differences emerged for the participants who trusted homeopathy (3rd group), compared with the two groups that conversely trusted allopathy (1st and 2nd group). Thus, the participants believing in homeopathy reported higher levels of both absorption and unlikely virtues.

To address the possibility that participants' absorption scores might be influenced by social desiderability in the third group, we performed a correlation between the scores of the absorption and the unlikely virtues scales obtained within this group. However, no significant correlation was found ($r = -0.08$, $p = 0.74$).

Fig. 3.2

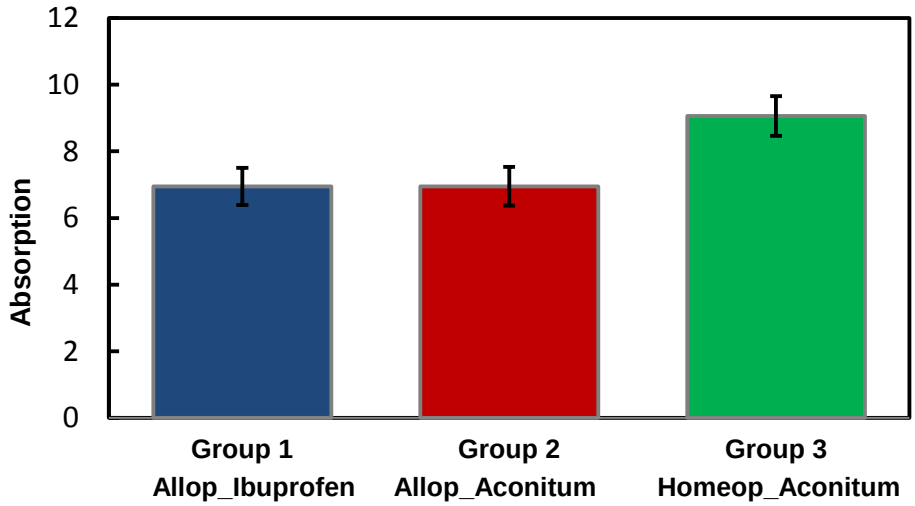

Fig. 3.2 Analysis of self-ratings associated with the subscale Absorption of the Multidimensional Personality Questionnaire. In the graph, are displayed the mean and the standard error associated with each group. The group trusting homeopathy exhibit significantly higher absorption scores than the two groups trusting traditional allopathic treatments.

Fig. 3.3

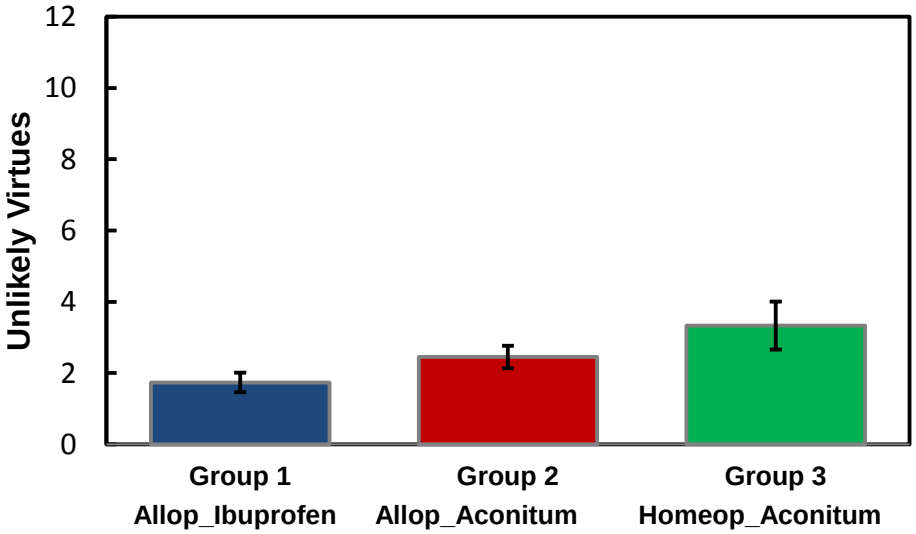

Fig. 3.3 Analysis of self-ratings associated with the subscale Unlikely Virtues of the Multidimensional Personality Questionnaire. In the graph, are displayed the mean and the standard error associated with each group. The group trusting homeopathy exhibit significantly higher unlikely virtues scores than the two groups trusting traditional allopathic treatments.

3.3.6 Electrophysiological results

The waveforms resulting from the averaging of the epochs time-locked with the pain stimulation are depicted separately for the three groups and three levels of intensity in Fig. 3.4. The figure includes one of the electrodes (Cz) chosen for the statistical analysis, in the time interval from -100 to 600 ms. The other electrodes included in the analysis (Fz, FCz, and CPz) displayed similar ERP patterns. The different modulation of pain-related ERP responses in the three groups points to diverse placebo effects, which may depend on the alleged effectiveness of the treatment believed by participants.

Fig. 3.4

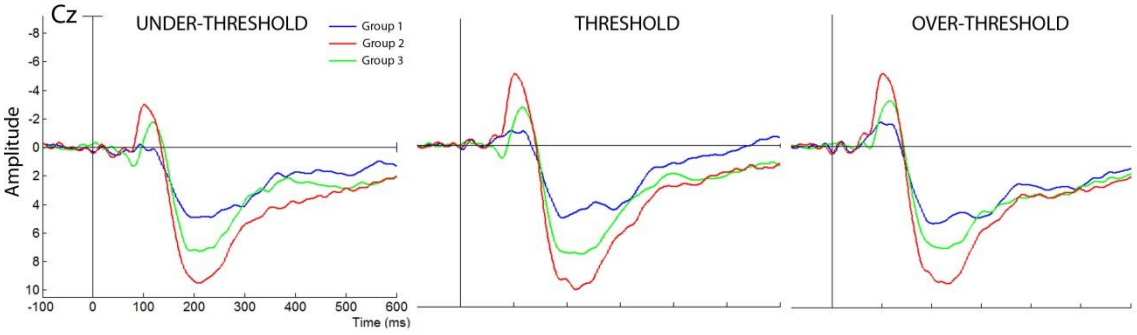

Fig. 3.4 ERP waveforms from the electrode Cz, elicited by non-painful under-threshold and painful threshold and over-threshold stimulation. The ERPs are depicted separately for each level of intensity (under-threshold, threshold, over-threshold) and each group: the first group "Allop_Ibuprofen" (blue line), the second group "Allop_Aconitum" (red line), and the third group "Homeop_Aconitum" (green line). Time-scale is from -100 to 600 ms. Negativity is displayed upward.

Two ANOVAs were carried out on ERPs in the 100- to 120-ms and on the 200- to 250-ms time windows (Fig. 3.5). The analysis of the first component, which is labeled N2 or N150, yielded the significant main effects of stimulus Intensity ($F_{(2,108)}$ = 7.32, HF ε = 0.62, p = 0.005). Under-threshold stimuli elicited the lowest N2 compared with threshold and over-threshold stimulation ($p < 0.05$). No differences emerged between the N2 amplitudes elicited by the two painful intensities (threshold and over-threshold). Both the main effect of the Group and the interaction Group by Intensity showed no statistical significance. Also the analysis of the second component, which is labeled P2 or P260, yielded the significant main effect of stimulus Intensity ($F_{(2,108)}$ = 7.32, HF ε = 0.62, p = 0.005). In comparison with threshold stimuli, under-threshold ones elicited lower positive amplitudes, whereas over-threshold stimuli elicited greater positive amplitudes (All $p < 0.01$). Moreover, the analysis showed the main effect Electrode ($F_{(1,54)}$ = 96.93, HF ε = 1.00, $p < 0.001$). The positive amplitudes were greater

in the more posterior electrodes Cz and CPz compared with the more anterior electrodes Fz and FCz ($p < 0.01$). Finally, the interaction Group by Intensity showed a significant effect ($F_{(4,108)} = 2.98$, HF $\varepsilon = 0.61$, $p = 0.05$, Fig. 3.5). The greatest P2 amplitudes were elicited by painless and painful stimulation in the group of participants who took a treatment incoherent with their beliefs compared with the other two groups which conversely were administered with a treatment coherent with the individuals' beliefs. No differences emerged between the two coherent groups (1st and 3rd group). This result indicated that the placebo effect is reduced in the participants that do not trust the administered treatment.

Fig. 3.5

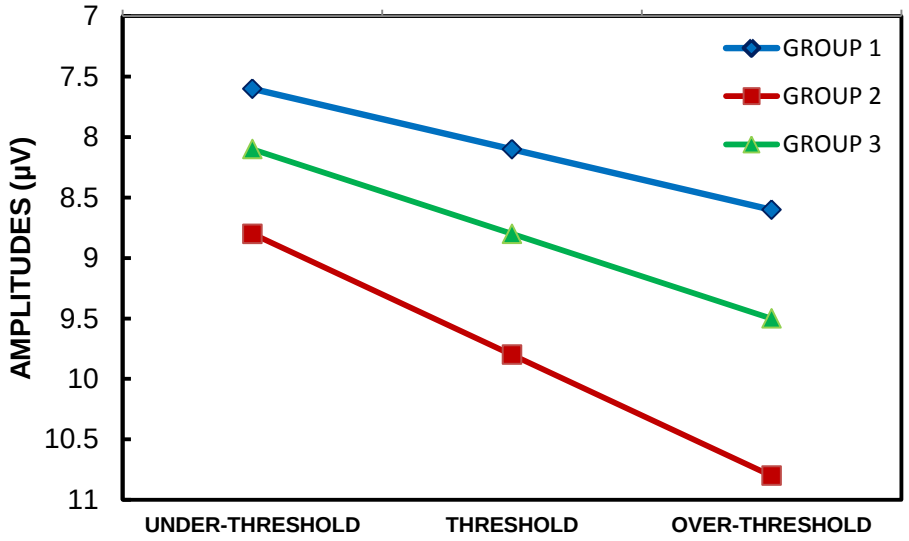

Fig. 3.5 Analysis of the late potentials P2 measured in the 200-250 ms from the onset of the pain stimulation, for the three groups: the first group "Allop_Ibuprofen" (blue line), the second group "Allop_Aconitum" (red line), and the third group "Homeop_Aconitum" (green line). The graphs display the two-way Group (1st vs. 2nd vs. 3rd group) by Intensity (under-threshold vs. threshold vs. over-threshold) interaction.

3.3.7 Discussion

Although medical treatments are often considered objectively effective or ineffective independently of personal beliefs about the expected outcome, a greater number of studies point to the active role of the patient in determining, or at least favoring, a successful cure. For instance, placebo is a specific contextual element that shapes both the evaluation of the incoming pain stimulation, as well as the pain experience itself (Wager, 2005; Par. 2.4). Placebo effects are supposed to operate

through positive expectations related to the incoming stimulation, such as prediction of lower intensity and greater pleasant valence, and prior classical conditioning, depending upon learnt associations, for instance between the color and shape of a pill and an analgesic effect.

The aim of the present study was to investigate the effects of perceived efficacy (i.e., placebo effects) of either a traditional (allopathic) or a homeopathic remedy, thought to be effective in pain reduction. The study considered young and healthy volunteers who trusted either the traditional or the alternative medicine. Two groups of participants received a treatment that they considered effective in pain reduction (Group 1: Allopathy-Ibuprofen, Group 3: Homeopathy-Aconitum), whereas a third group of participants was administered the homeopathic remedy, but those participants did not believe it could have any analgesic effect (Group 2: Allopathy-Aconitum). All the participants were not aware that the received treatment (one Ibuprofen pill or three granules of Aconitum Napellus, respectively) was an inert substance. The deceptive procedure guaranteed that the participants strongly believed in the efficacy of the taken treatment and that experimenters' instructions did not allude to involuntarily suggestions that the pills might be inert substances.

To investigate the effects of personal beliefs on the perception of effectiveness of the taken treatment, we compared subjective (pain thresholds and ratings) and ERP responses elicited by electrical stimulation in the three groups. Mean thermic (C°), mechanic (Kg) and electrical (μV) levels corresponding to participants' electrical pain threshold, as well as subjective pain evaluation collected during the ERP recording, were comparable in the three groups. However, positive late potentials (P2) were revealed to be differently modulated in the three groups. The two coherent groups showed decreased P2 amplitudes, compared to the incoherent group (Fig. 3.5). Thus, a placebo effect might be linked to the reduction of a late ERP component associated to pain processing. A previous study on the effects of expectation and conditioning in inducing a placebo response, reported that in comparison with natural history, verbal suggestions induced a P2 decrement without a reduction in pain intensity ratings (Colloca et al., 2009). Instead, a conditioning procedure lead to subjective perception of pain reduction, parallel to reduced N2 and P2 amplitudes.

The lack of behavioral between-group differences in the present study is line with other findings indicating that verbal suggestions alone are less robust in inducing a

pain reduction, compared to conditioning (Price et al., 2008). Noteworthy, the sample consisted of young and healthy students that even if they believed in the efficacy of traditional medicine or homeopathy, they had sparse and occasional experience especially with the alternative approach. Unpublished data suggested that the main users of alternative treatments are middle-age women with a high education, which choose alternative treatments to avoid side-effects of allopathic treatments that are perceived dangerous for their health. Albeit speculative, it is possible that the lack of prior exposure to prolonged homeopathic treatments might have lessened the placebo response in the homeopathic group.

Interestingly, the group of participants that believed in homeopathy showed greater ratings in the Absorption scale of the Multidimensional Personality Questionnaire (MPQ), compared to the two groups which trusted only the traditional medicine. The Absorption Scale quantified how much a person becomes absorbed in thoughts and mental imaginings, and is responsive to engaging or inductive stimuli (Tellegen & Atkinson, 1974). Moreover, a significant main effect emerged also for the Unlikely Virtues Scale: participants who believed in homeopathy tended to report higher scores in the present scale. The Unlikely Virtue Scale is related to social desiderability, namely response biases that distort ratings and subjective evaluations, since the respondent wishes to provide the answer that is most social acceptable. Both Absorption and Unlikely Virtue are related to suggestionability.

Absorption and other personality traits may exert a role in potentiating placebo effects, as well as favoring the active involvement and the compliance of patients in the therapy. Alternative medicines, in particular, put emphasis on patients' responsibility and personalized treatments; the doctor-patient relationship is usually positive and optimistic; the diagnosis fits the patient's expectations, the therapy aims to reach a holistic psycho-physical well-being that depends upon the interaction between somatic, psychological and contextual factors.

Importantly, even if the results suggest that the trustworthiness of a treatment has similar effects in traditional and alternative medicine, it is not excluded the possibility that enhanced placebo effects may occur for alternative medicine in clinical settings. The enhanced effects could depend for example on patients' characteristics and on the patient-physician relationship not considered in this experimental study. Indeed, the patients' active choice to use an alternative method, their hope and desire to get rid

of the disease, the attention and the intense monitoring from the practitioner are some elements that may remarkably facilitate placebo effects and influence the treatment outcome.

In conclusion, compared with expectation of non-effectiveness, expectations of effectiveness of a treatment are related to decreased cortical pain processing at a late stage (P2), independently of the supposedly effective traditional or alternative treatment. Results highlight the effects of the perception of effectiveness of a treatment, revealed by the inhibition of late positive cortical responses to painful stimulation in young and healthy volunteers.

STUDY 4: REAPPRAISAL OF PAIN AND MENTAL IMAGERY INDUCE HYPOALGESIC AND ALLODYNIC EFFECTS

3.4.1 Introduction

The present study investigated the role of reappraisal in pain experience and in cortical pain processing. Many psychological strategies including distraction, attention, expectations and reappraisal have been proven to reliably influence pain experience, causing either pain relief or pain exacerbation. Undeniably, pain is "an emergent, malleable experience rather than a single, static entity" (Tracey and Dickenson, 2012) and its "malleability" can be operationalized as dynamical interplays between bottom-up salience-related, individual differences, and top-down goal-related mechanisms. Pain gains priority over the flow of events in most circumstances, forcing a shift of attention from the individuals' activity to the eliciting stimulus, regardless the voluntary control (i.e., bottom-up capture of attention; see Legrain et al. 2009, 2012). The capture of attention by noxious stimuli forces a re-allocation of a certain amount of attentional resources to further elaborate dangerous situations, take decisions, and prompt opportune actions. At the same time, the amount of attentional resources captured by pain is influenced by higher cognitive functions (i.e., top-down regulatory mechanisms; see Legrain et al. 2009; 2012). Cognitive top-down effects depends upon "trait" variables (e.g., ability in cognitive control, hypervigilance, attentional bias), as well as context-dependent "state" strategies (e.g., distraction-attention, placebo-nocebo, reappraisal; for reviews, see Ochsner & Gross, 2005; Wiech et al., 2008). For pain and emotion regulation, cognitive reappraisal is shown to be a more effective strategy than distraction, relaxation or dissociative imagery in healthy participants (De Pascalis et al, 1999; MacRae et al., 2010; Totterdell and Parkinson, 1999). On one hand, it is well establish that directing attention on upcoming stimuli enhances perception regardless the sensory modality; on the other hand, when the attention is associated to a specific re-interpretation of the meaning of the stimulus, the perceptual processing may be either facilitated or inhibited.

With the present study, we investigated behavioral and ERPs correlates of inhibitory and facilitatory pain-related mechanisms, induced by focused attention and pain reappraisal, during electrical painful and non-painful stimulation of the right forearm. The attentional engagement was similar across all the conditions, whereas

reappraisal of the pain and no-pain experience, achieved through mental imagery, was expected to lead to hypoalgesic and anesthetic effects, by imaging a gloved forearm (Inhibition condition); or to hyperalgesic and allodynic effects, by imaging a wounded forearm (Facilitation condition). The two reappraisal conditions were compared to a non-reappraisal Baseline condition, in which the instruction suggested to imagine the skin of the forearm. In each block, participants were also asked to judge every stimulus as painful or non-painful and, after each block, to rate the perceived pain intensity and the unpleasantness of the worst pain, as well as their ability to reappraise the triggered responses according to the instructions. We expected that cognitive reappraisal either decreases or increases pain experience and cortical pain-related processing, according to the content of the suggested mental images. The principal aims were 1) to identify the relationship between inhibitory and facilitatory reappraisal effects on pain attenuation and amplification; 2) analyze the contribution of individual differences in reappraisal abilities and task performance; and finally 3) to elucidate the functional meaning of pain-related somatosensory potentials, by applying a false discovery approach across an array of 60 electrodes.

3.4.2 Participants

The 30 healthy volunteers who took part to the study were proficient Danish speakers, right-handed, with normal or corrected-to-normal vision. Nobody reported history of pain disorders, neurological or psychiatric illness, or daily use of analgesics. All participants received a reward of 200,00 DKK and signed a written informed consent before the participation. Data from six participants were not included in the analysis because of technical failures in data collection. Other six participants were excluded from statistical analysis because of excessive EEG artifacts. Thus, the final sample included 18 participants (8 females; mean age = 24.33; SD age = 2.06; range = 21:27). The study was approved by the Ethical Committee of Central Region Denmark and conducted in accordance with the Declaration of Helsinki.

3.4.3 Stimuli, Task and Procedure

Participants were asked to reappraise and identify painful and non-painful stimuli (Mental Imagery and Pain Judgment Task, respectively); and to evaluate their pain experience (Self-Ratings). In the Mental Imagery Task, reappraisal was induced by

instructions that suggested to use mental images to down-regulate (Inhibit or Facilitate) the triggered pain responses or to experience pain without modulation (Baseline). In the Inhibition and Facilitation conditions, instructions suggested to imagine a glove covering or a wound that was hurting the right forearm to attenuate or to exacerbate pain sensations, respectively. In the Baseline condition, instructions specified to simply imagine the skin of the right forearm. The instruction for Inhibition was chosen according to previous studies (De Pascalis et al., 2001, 2008), whereas the instruction for Facilitation was written to mirror the Inhibition condition, by changing the content of the image (wound instead of a glove) and the directionality of the modulation (amplification instead of attenuation). In the Baseline condition, in order to achieve a similar level of attentional engagement, the instruction suggested to imagine the skin of the forearm, without any pain modulation.

In the Pain Judgment Task, participants identified pain and no-pain stimuli by pressing two possible keyboard buttons, counterbalanced across participants. Finally, at the end of each block of stimulation, participants rated the worst pain intensity and unpleasantness felt in the last block and evaluated their ability to influence the triggered responses according to the given instruction.

In a single experimental session, participants performed a pain and an empathy task in a counterbalanced order. Here, only the pain task is reported. The pain session started with a calibration task aimed to identify the intensity of non-painful and painful stimulation suitable for each participant. To this aim, trains of increasing intensities were delivered by the electro-stimulator Digitimer through two electrodes placed on the right forearm over the medial nerve. Participants rated the intensity of each stimulus on a horizontal visuo-analogue scale (VAS; range = 0-10, where 0 equals ‘‘no pain sensation’’, 1 “just noticeable pain” and 10 ‘‘worst imaginable pain”). The calibration finished as soon as the participant rated intensities with a score corresponding to or greater than 8. Hence, intensities corresponding to 0.8 and 8 ratings on the VAS were chosen for the experimental task. The calibration task was followed by written and oral instructions of the experimental task and a brief training session consisting of three blocks, one for each condition (i.e., Inhibition, Baseline, and Facilitation). The training started always with the Baseline condition, whereas the second block could be either Inhibition or Facilitation in a counterbalanced order. All participants reported that the

three blocks were sufficient for understanding the task and they needed any additional practice.

The experimental task consisted of 24 blocks (8 for each condition). Each block started with an instruction (Inhibition or Baseline or Facilitation) presented for 15 s, followed by a 2 s inter-stimulus interval and a random set of 12 stimuli of two different and fixed intensities (painful or non-painful). The duration of each stimulus was 5 ms. Noteworthy, participants were not informed that only two electrical intensities were delivered and they were told that the stimuli could have any intensity corresponding to the range from 0.8 to 8 in the VAS scale, accordingly with the ratings they gave in the calibration task. Participants were asked to maintain the mental image throughout each block and, in the meantime, to perform a pain judgment task for each stimulus, by pressing a button with the middle or the index finger of the left hand. Buttons that identified pain and no-pain were counterbalanced across participants. Inter Stimulus Interval (ISI) between stimuli consisted of 1200-1800 ms after participants' response. At the end of each set of stimuli, participants were invited to think back to worst painful stimulus and to provide ratings on three VASs. On the first scale, participants were asked to rate the worst painful intensity they felt ("How much pain did you feel?"; 0 = no pain, 10 = the worst imaginable pain). On the second scale, they were asked to rate the worst unpleasant sensation they felt ("How unpleasant did you feel?"; 0 = no unpleasantness, 10 = the worst imaginable unpleasantness). Finally, in Inhibition and Facilitation blocks only, they were asked to rate the ability to reappraise their pain experience, ("How efficient were you in influencing your sensations?"; 0 = no control, 10 = perfect control). After each rating session, 5-s intervals separated contiguous blocks.

The blocks were presented with two possible pseudo-randomized orders (direct or reverse) to counterbalance order effects. The pseudo-randomization followed three main rules: (1) two blocks with the same instruction always occurred consecutively; (2) instructions changed every two blocks; (3) and were counterbalanced every six blocks.

3.4.4 Data recording and analysis

The E-Prime v.2.0 (PST, Inc.) software package was used for instructions, stimuli and visuo-analogue scales presentation. For stimulus presentation and its synchronization with markers needed for ERP time-locked analysis, the E-Prime

software sent outputs to two parallel ports; one connected to the Digitimer to control the electrical stimulation, the other connected to the amplifier to register the markers. The fixed delay of the output conveyed to the second parallel port was computed in the EEG preprocessing to reconstruct the exact timing of each stimulus.

EEG continuous data were recorded with an active 64-electrodes cap and amplified (BrainAmp MR plus amplifiers) by using the Brain Vision Recorder software (Brain Products, Munich, Germany). Two electrodes (i.e., PO9 and PO10) were removed from the cap and placed on the superior orbit and on the outer canthus of the right eye, to detect vertical and horizontal eyes movements. The EEG was referenced to the FCz electrode, grounded at AFz, and sampled at 1000 Hz. The impedance was kept below 20 kΩ. Offline, the continuous EEG was downsampled at 500 Hz, band-pass filtered (0.1-30 Hz) and segmented into 700-ms stimulus time-locked epochs (-100/+600 ms). The ICA procedure and the toolbox ADJUST were applied to identify, select, and discard the components representative of eye movements and other artifacts.

The segments were then baseline-corrected using the average pre-stimulus activity (-100/0 ms) and the two electrodes used to detect eye movements were removed from following processing. Electrodes which surpassed at least 40% of artifactual activity were replaced using spherical spline interpolation. Then, epochs with activity exceeding 80 μV were rejected and trial-by-trial data were visually inspected for residual artifact. This procedure led to the rejection of 18.54% of data. Epochs were re-referenced offline to the algebraic mean of the left and right mastoids and FCz activity was reconstructed. Epochs were thus averaged separately for each of the six conditions; i.e., Pain Inhibition (PI), Pain Baseline (PB), Pain Facilitation (PF), No-Pain Inhibition (NPI), No-Pain Baseline (NPB), No-Pain Facilitation (NPF). The statistical analysis consisted of a two-by-three factorial design: Stimulus (two levels: pain vs. no-pain) by Instructions (three levels: inhibition vs. baseline vs. facilitation).

Mass univariate t-tests and appropriate multiple comparison corrections permit to identify condition differences across a wide range of electrodes and time windows, avoiding the use of a priori spatial or temporal regions of interest (Crowley et al., 2012; Lage-Castellanos et al., 2010). If analyses are guided only by previous studies, it may be possible that some effects remain undiscovered. The procedure, implemented through the "Mass-Univariate ERP Toolbox" (Groppe, Urbach, & Kutas, 2011), consisted of computing the difference between waveforms associated to two conditions,

performing t-statistics at each time point and electrode and, finally, applying the BH FDR correction method (Benjamini & Hochberg, 1995) to adjust the threshold for rejection or acceptance of the null hypothesis.

3.4.5 Behavioral results

In the pain judgment task, the behavioral performance was measured by mean reaction times (RTs) of corrected answers and mean d-prime (d')[7]. ANOVAs on both measures showed significant Pain by Instruction interactions (RTs: $F_{(2,34)} = 41.67$, partial $\eta^2 = 0.71$, $p < 0.001$; d': $F_{(2,34)} = 41.67$, partial $\eta^2 = 0.60$, $p < 0.001$; Fig. 4.1 and 4.2). Compared to Baseline, PI (i.e., hypoalgesic effect) and NPF (i.e., allodynic effect) induced slower RTs and decreased target detection rates. The opposite pattern was found for PF and NPI, which were associated with faster RTs and increased target detection rates. No differences in mean RTs or d' were found between PI and NPF, as well as between PF and NPI, suggesting homogeneity in inhibitory and facilitatory effects. Faster RTs were associated to PB and PF compared to NPB and NPF conditions, respectively, revealing that pain discrimination is speeded up at baseline and when the pain is cognitively exacerbated. Instead, there was no difference in RTs between PI vs. NPI, suggesting that this pain effect is disrupted when top-down pain inhibition occurs (Fig. 4.1). As revealed by d', pain and no-pain detectability was similar in baseline (PB=NPB), but decreased for PI vs. NPI and NPF vs. PF (Fig. 4.2).

[7] The statistic d-prime or *d'* is an index used in signal detection theory that depends on a combination of response sensitivity (i.e., the individual sensitivity in detecting a signal) and bias (the inclination of the subject to say "yes" or "no"). Thus, d-prime is an estimation of response sensitivity considering the individual bias in decision making. It is calculated as the difference between the z-transforms of the hit rate and the z-transforms of the false alarm rate: $d' = Z(\text{hit rate}) - Z(\text{false alarm rate})$. The function $Z(p)$, $p \in [0,1]$ is the inverse of the cumulative Gaussian distribution. A higher *d'* indicates a higher sensitivity in detecting the signal (Heeger, 1997).

Fig. 4.1

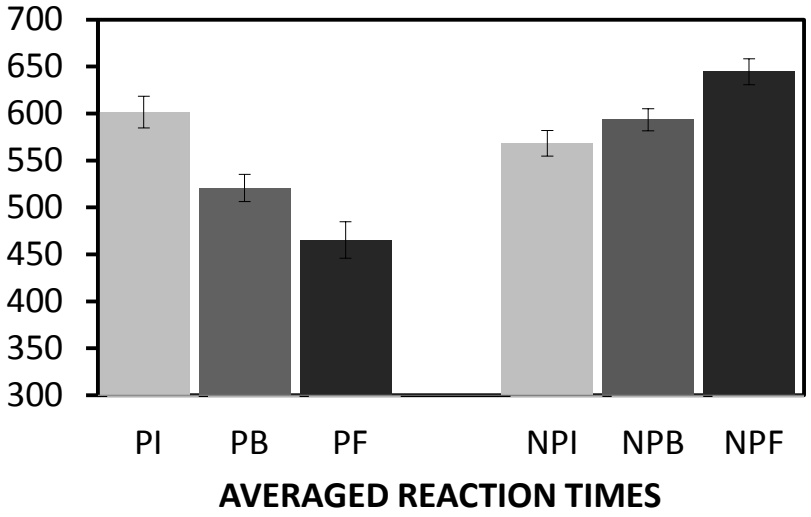

Fig. 4.1 Analysis of mean reaction times associated with the six conditions: PI (pain inhibition), PB (pain baseline), PF (pain facilitation), NPI (no-pain inhibition), NPB (no-pain baseline), NPF (no-pain facilitation).

Fig. 4.2

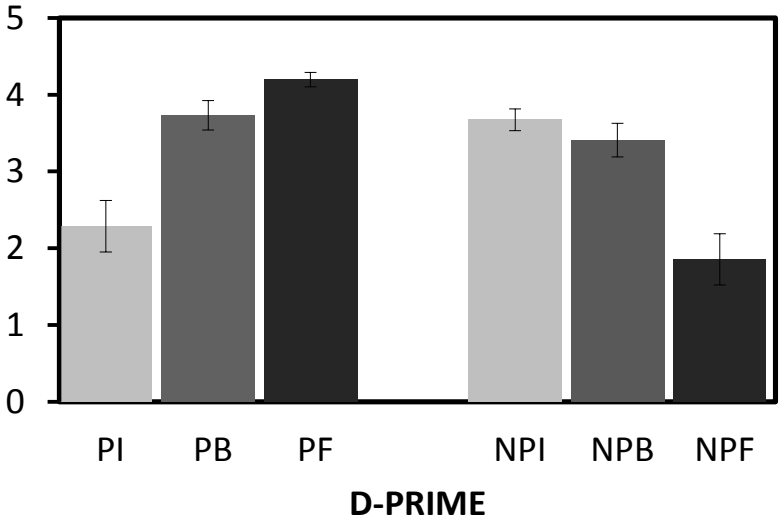

Fig. 4.2 Analysis of d-prime associated with the six conditions: PI (pain inhibition), PB (pain baseline), PF (pain facilitation), NPI (no-pain inhibition), NPB (no-pain baseline), NPF (no-pain facilitation). For a definition of d-prime, see footnote 7, p. 110.

3.4.6 Subjective results

Although the painful and non-painful stimuli had the same intensity throughout the experiment, participants reported significant differences in the experienced pain intensity and unpleasantness according to the given instruction. Compared to Baseline, Inhibition and Facilitation Blocks were associated with significant decreased and increased intensity and unpleasantness ratings, respectively (pain intensity main effect: $F_{(2,34)} = 22.61$, partial $\eta^2 = 0.57$, $p < 0.001$; pain unpleasantness main effect: $F_{(2,34)} = 32.98$, partial $\eta^2 = 0.66$, $p < 0.001$; Fig. 4.3). Reappraisal efficacy ratings showed no difference for the Inhibition and Facilitation Blocks (reappraisal main effect: $t_{(34)} = 0.19$, p = n. s.; Fig. 4.3 and 4.4). Interestingly, reappraisal ratings associated with Inhibition and Facilitation conditions showed a significant correlation, which explains the 87% of the variance ($R^2 = 0.87$, $p < 0.001$). A regression model showed that reappraisal ratings were not predicted by either Intensity or Unpleasantness evaluations ($R^2 < 0.14$, p = n. s.). However, Intensity and Unpleasantness Ratings were highly correlated in all conditions ($R^2 > 0.57$, $p < 0.001$).

Fig. 4.3

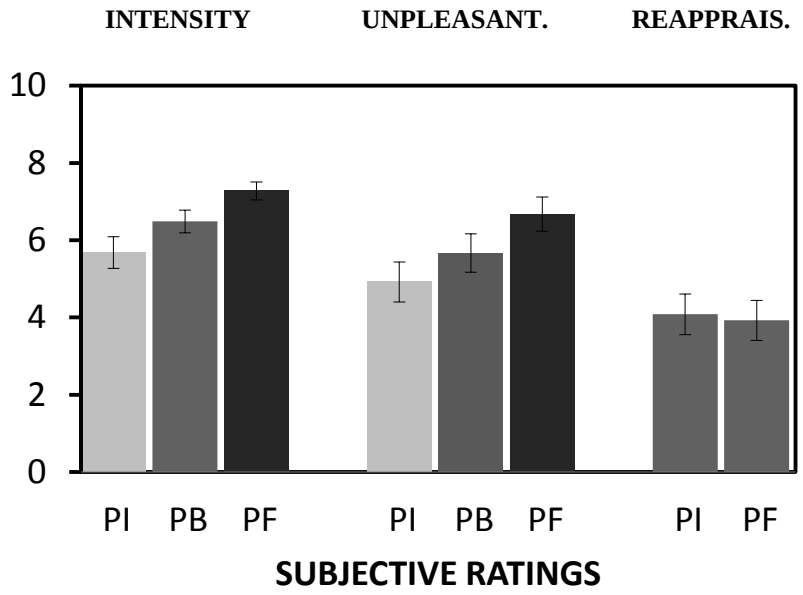

Fig. 4.3 Analysis of subjective ratings with blocks with the same instruction: PI (pain inhibition), PB (pain baseline), PF (pain facilitation). On the left: analysis of the intensity ratings. In the middle: analysis of the unpleasantness ratings. On the right: analysis of the reappraisal ratings.

Fig. 4.4

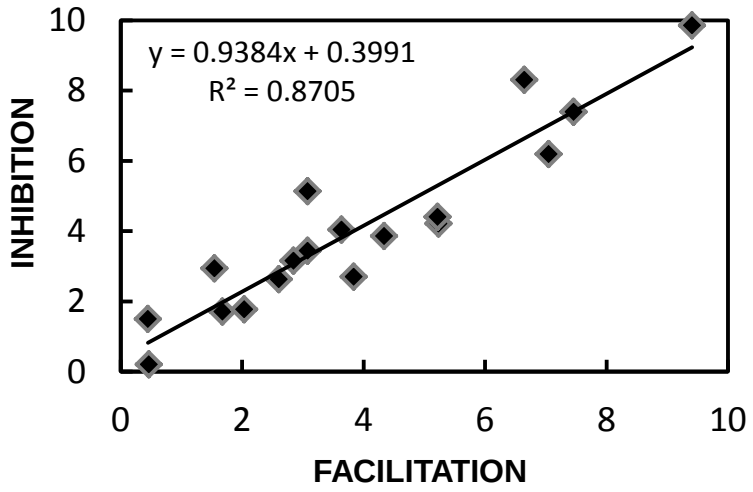

Fig. 4.3 Correlation plot between the mean reappraisal efficacy ratings associated with the inhibition and the facilitation conditions.

3.4.7 Electrophysiological results

Analyses of the electrophysiological data were carried through point-by-point t-tests performed on each electrode in the time interval from 0 to 600 ms. The contrasts were chosen to assess the Stimulus main effect, the Instruction main effect, and the Stimulus by Instruction interaction. To control for the false discovery rate in the multiple comparisons testing, we applied a FDR correction with a value set at 0.05.

The waveforms resulting from the averaging of the epochs time-locked with the pain stimulation are depicted separately for the pain main effect (difference between pain and no-pain conditions, Fig. 4.5) and the instruction main effects (differences between inhibition and baseline in Fig. 4.6, and between inhibition and facilitation in Fig. 4.7). No differences emerged for the Instruction main effect in the contrast between baseline and facilitation, as well as no stimulus-by-instruction interaction emerged.

The significant Stimulus main effect (Fig. 4.5) showed that that painful vs. non-painful stimuli elicited greater N1 amplitudes (around 40-50 ms), P1 (around 70-80 ms), N2 (100-150 ms) and P2 (200-400 ms).

Fig. 4.5

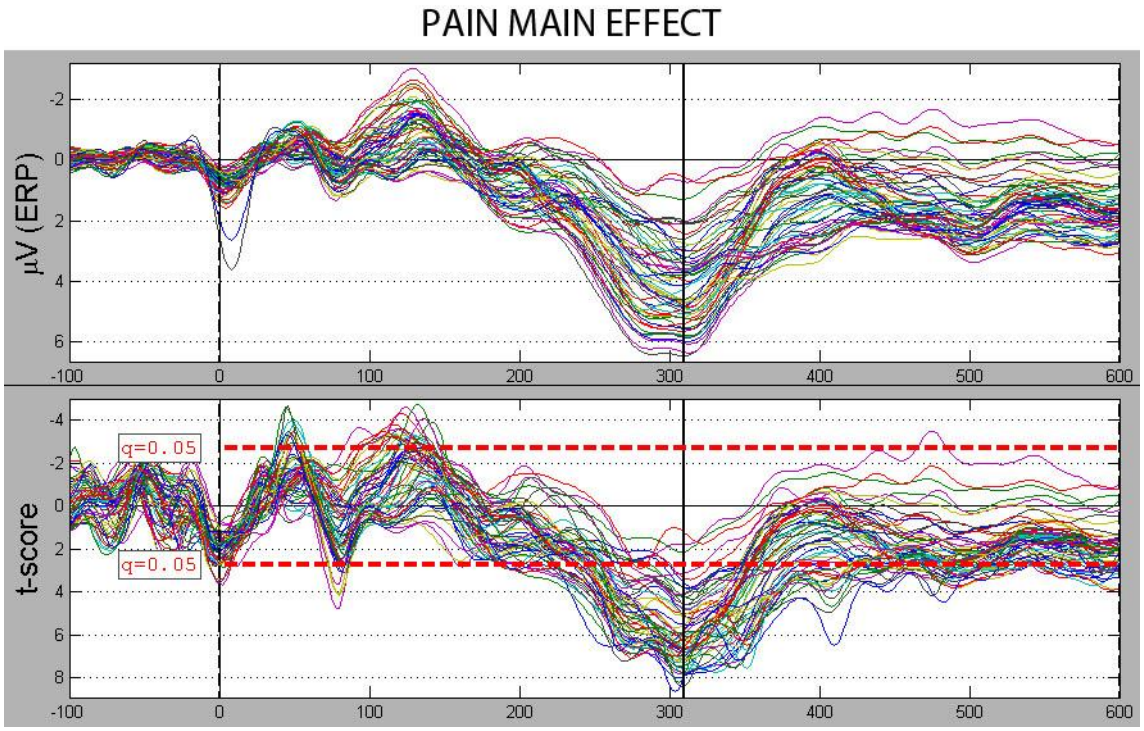

Fig. 4.5 The top of the panel depicts the ERP waveforms of the difference between painful and non-painful conditions. Instead, the bottom of the panel dispays the point-to-point mass univariate t tests, performed on 59-channels and 600 ms-time window. Time-scale is from -100 to 600 ms. Negativity is displayed upward. For each analysis, the FDR procedure was used to control for multiple comparisons using an FDR <.05. Significant differences between the two conditions emerge when the waveforms cross the red dot lines (t-score).

The significant Instruction main effect (Fig. 4.6) indicates that both painful and non-painful stimuli during inhibition vs. baseline elicited greater N1 amplitudes (around 50 ms) in anterior right-lateralized electrodes (F4, F6, FC6), ipsilateral to the stimulation site. However, the greater N1 amplitudes are elicited in anterior central and left-lateralized electrodes (F1, FC3, FC1, Cz). The central-left location for the maximal peak is associated with the stimulation of the right forearm. Indeed, early potentials are usually greater in the contralateral hemisphere with respect to the site of the stimulation. Thus, inhibition and baseline conditions are associated with greater early activity in left-lateralized electrodes, but differed in the early recruitment of ipsilateral (right) cortical activity. Moreover, during inhibition stimuli induced greater negative potentials from around 130-140 ms to 280 ms in central electrodes. This time window included the descending part of the N2 potentials and the ascending part of the P2 potentials. For instance, at the time point 140 ms, the electrodes F2, FC3, FC1, FCz, FC2, C3, C1, Cz, CP1, CPz exhibited greater negative amplitudes, whereas at the time point 240 ms, the

electrodes F1, Fz, F2, FC5, FC3, FC1, FCz, FC2, FC4, C5, C3, C1, Cz, C2, C4, CP5, CP3, CP1, CPz, CP2, CP4, P3, P1, Pz, P2 displayed diminished positive amplitudes (Fig. 4.6). In summary, during inhibition vs. baseline both painful and non-painful stimulation elicited greater N2 potentials, but reduced P2 potentials.

Similarly, the significant Instruction main effect for the contrast inhibition vs. facilitation (Fig. 4.7) indicates that both painful and non-painful stimuli elicited greater N1 amplitudes (around 50 ms) in the anterior right-lateralized electrode FC6. Therefore, also inhibition and facilitation conditions differ in the early recruitment of ipsilateral (right) cortical activity, but in a restricted area with respect to the contrast inhibition vs. baseline. During inhibition vs. facilitation, painful and non-painful stimuli induced greater negative potentials from around 100 ms until 330-340 ms in central electrodes. Thus, the differences between the inhibition and facilitation conditions started earlier and lasted longer compared with the difference between the inhibition and the baseline conditions. This time window included both the ascending and the descending part of the N2 potentials and the ascending part of the P2 potentials. For instance, at the time point 120 ms, the electrodes F3, F1, Fz, FC5, FC3, FC1, FCz, FC2, FC4, C5, C3, C1, Cz, C2, CP3, CP1, CPz exhibited greater negative amplitudes, whereas at the time point 230 ms, the electrodes FP1, AF3, F7, F5, F3, F1, Fz, F2, F4, FC5, FC3, FC1, FCz, FC2, FC4, FC6, C5, C3, C1, Cz, C2, C4, C6, CP5, CP3, CP1, CPz, CP2, CP4, P3, P1, Pz, P2, POz displayed smaller positive amplitudes (Fig. 4.6). Similarly to the contrast inhibition vs. baseline, also the difference inhibition vs. facilitation was associated with greater N2 potentials, but reduced P2 potentials.

Fig. 4.6

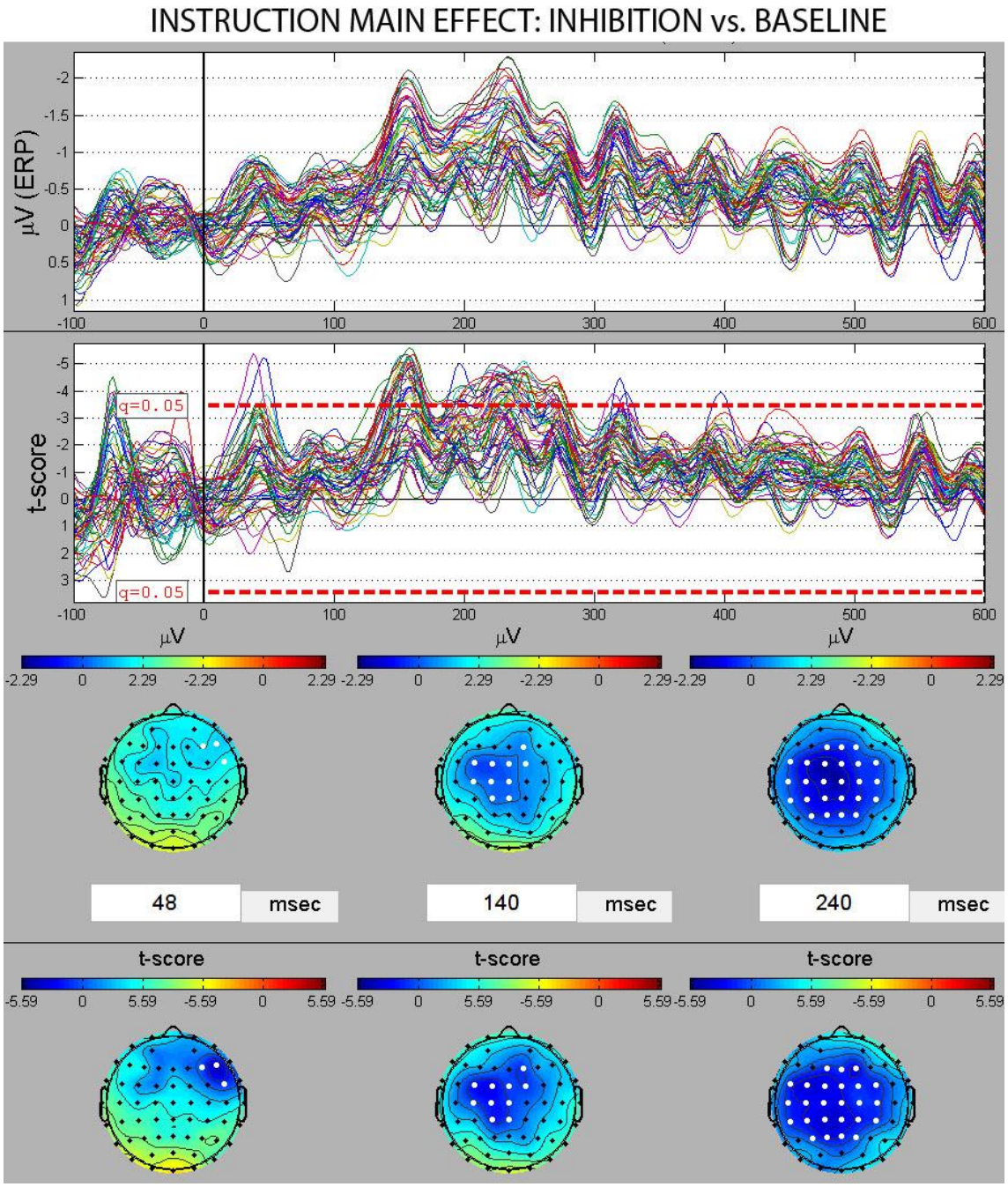

Fig. 4.6 The first row of the panel depicts the ERP waveforms of the difference between the Inhibition and the Baseline conditions in μV. The second row of the panel displays the point-to-point mass univariate t tests, performed on 59-channels in the time window from 0 to 600 ms. Time-scale is from -100 to 600 ms. Negativity is displayed upward. For each analysis, the FDR procedure was used to control for multiple comparisons using an FDR <.05. Significant differences between the two conditions emerge when the waveforms cross the red dot lines (t-test). In the third and fourth row of the panel, the electrodes which showed the significant Instruction main effect are displayed in white. The third raw depicts the spline maps of the neural activity in μV associated with the difference between the Inhibition and the Facilitation conditions at 48, 140 and 240 ms. Instead, the fourth row displays the spline maps of t-scores associated with the difference between the Inhibition and the Facilitation conditions at 48, 140 and 240 ms. The blue color indicates a negative difference. Thus, Inhibition elicited greater negative and less positive potentials than Baseline.

Fig. 4.7

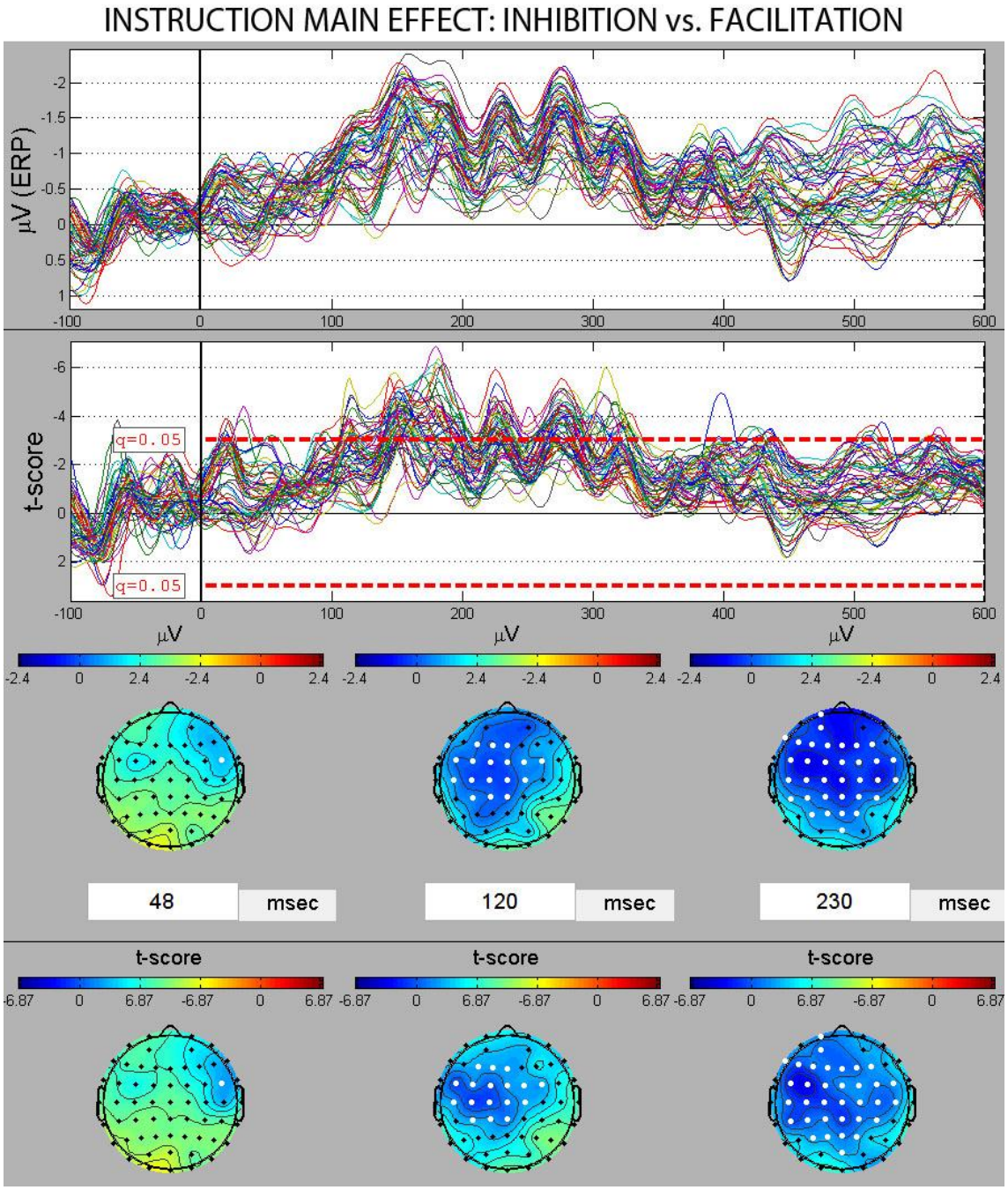

Fig. 4.7 The first row of the panel depicts the ERP waveforms of the difference between the Inhibition and the Facilitation conditions in μV. The second row of the panel displays point-to-point mass univariate t tests, performed on 59-channels in the time window from 0 to 600 ms. Time-scale is from -100 to 600 ms. Negativity is displayed upward. For each analysis, the FDR procedure was used to control for multiple comparisons using an FDR <.05. Significant differences between the two conditions emerge when the waveforms cross the red dot lines (t-score). In the bottom panel, the electrodes which showed the significant Instruction main effect are displayed in white. The third raw depicts the spline maps of the neural activity in μV associated with the difference between the Inhibition and the Facilitation conditions at 48, 120 and 230 ms. Instead, the fourth row displays the spline maps of t-scores associated with the difference between the Inhibition and the Facilitation conditions at 48, 120 and 230 ms. The blue color indicates a negative difference. Thus, Inhibition elicited greater negative and less positive potentials than Facilitation.

3.4.8 Discussion

The present study investigated a mental imagery driven pain modulation in behavioral and ERPs measures elicited by electrical painful and non-painful stimulation of the right forearm. Mental images of an anesthetized gloved or a hurting wounded forearm were suggested to attenuate or to exacerbate pain perception. The two reappraisal conditions (Inhibition and Facilitation) were compared with a neutral bottom-up condition (Baseline), where instructions were to imagine the skin of the forearm and passively experience the stimuli. This procedure permitted to examine the role of top-down and bottom-up processing both in subjective pain modulation and in pain-related ERPs, as well as to elucidate parallelisms between inhibitory and facilitatory pain and somatosensory processes.

Painful and non-painful stimulus detection, indexed by mean reaction times and d-prime, disclosed opposed patterns of responses associated with inhibitory and facilitatory mental images as compared with Baseline. Imaging a glove covering the forearm and attenuating its sensations rendered pain discrimination slower and less accurate, but increased the discrimination of non-painful stimuli. Conversely, imaging a wound hurting the forearm and exacerbating painful sensations increased the discrimination of painful stimuli, but impaired the discrimination of non-painful stimuli. Indeed, painful stimuli in the Inhibition condition and non-painful stimuli in the Facilitation condition needed more time to be accurately detected and were associated with decreased sensitivity as revealed by the d' index (Fig. 4.1 and 4.2). In the baseline condition, painful stimuli were detected faster than non-painful ones (Fig. 4.1). This effect reflects the salience-inherent bottom-up capture of attention by pain, indeed brain activity is naturally tuned to prioritize the processing of the stimuli that pose a threat to the individual's integrity (Legrain, Perchet, et al., 2009). The pain effect was exacerbated during Facilitation and reduced during Inhibition, revealing interplay between top-down reappraisal processes and bottom-up attentional mechanisms. Thus, focused hypoalgesia and allodynia may depend on an active inhibition or facilitation of salience-related mechanisms by cognitive strategies. Stimulus sensitivity, measured by a combination of hit and false alarms responses, namely d-prime or d' (Fig. 4.2), was similar for PB and NPB conditions, revealing an equal ability to detect both kind of stimulation. However, target detection rates were radically dropped in the PI and NPF conditions, compared with NPI and PF, as well as with Baseline (PB and NPB),

revealing hypoalgesic and allodynic responses. The behavioral impairment observed in the two critical conditions (i.e., PI and NPF) may be related to a lack of congruence between the prediction intrinsically encoded in the mental image and the actual stimulus.

Both top-down instructions have been revealed to effectively change, either decreasing or increasing, the pain intensity and unpleasantness experience in comparison to Baseline (Fig. 4.3). As shown in the correlation plot, the reappraisal ability showed large inter-individual differences, covering the entire range of evaluation (Fig. 4.4). Interestingly, the ratings on top-down efficacy in the two reappraisal conditions were highly correlated, showing that individuals reported to be equally able to inhibit or to facilitate their pain experience. Moreover, the consistency between behavioral and subjective measures suggests that the effects on pain ratings are not simply due to response bias. Noteworthy, the symmetrical results between inhibitory and facilitatory mechanisms, observed both in behavioral and subjective measures, excludes the possibility that differences between inhibitory and facilitatory responses in ERPs measures may depend on task difficulty.

Furthermore, we examined ERP effects in the 600-ms post-stimulus time window. This time window includes the typical N1, P1, N2 and P2 potentials, elicited by electrical stimulation (Fig. 4.5). We applied a false discovery approach to explore ERP effects across an array of 59 electrodes. The pain vs. no-pain contrast confirmed the classical pain effects, consisting of increased N1, P1, N2 and P2 potentials (Fig. 4.5). In the Inhibition condition, painless and painful stimuli elicited greater negative potentials since 50 ms. Both contrasts Inhibition vs. Baseline and Inhibition vs. Facilitation point to early differences at the level of a right anterior cluster, ipsilateral to the stimulation site, and later differences associated with N2 and P2 amplitudes in a central array of electrodes. In particular, the Inhibition condition was associated with greater N2 negative potentials and reduced P2 positive potentials. The finding of an increased N2 potentials during Inhibition compared with the other two conditions is counterintuitive, since greater N2 amplitudes are typically associated with increased pain sensations, especially in the context of distraction manipulations (Bromm & Lorenz, 1998; Legrain et al., 2012). However, this pattern of results was previously reported by other studies, which suggested that the increased N2 potentials associated with reduced pain sensations can be interpreted as indicating an increased inhibitory

processing (De Pascalis et al., 1999; De Pascalis, Magurano, Bellusci, & Chen, 2001). It is possible that the N2 potentials reflect different mechanisms involved in reappraisal and distraction, likely related to the involvement of dorsolateral prefrontal regions (McRae et al., 2010). Contrary to the behavioral data, we found no specular patterns of neural responses for Inhibition and Facilitation. However, it is worth noting that the behavioral results concern the inhibition of painful stimuli and the facilitation of painless stimuli. Because ERPs are strongly influenced by the intensity of the stimulation, it is likely that facilitation of painless stimuli elicit subtle differences that do not surpass the significance threshold. Thus, the ERP sensitivity to the physical stimulus intensity may be a confound masking the painless facilitation effect.

In summary, in pain contexts reappraisal is one of the most effective cognitive strategies for pain modulation. It operates by increasing focused attention to the hurting body area and by cognitively changing the stimulus meaning to reshape the pain experience. Here, an imagery-based reappraisal strategy designed to either reduce or amplify pain sensations has been shown to induce consistent analgesic and allodynic effects for painful and non-painful conditions, respectively, as revealed by the behavioral and subjective responses. Pain inhibitory mechanisms were associated with early and late ERP modulations involving N1, N2 and P2 potentials. The behavioral and the subjective findings strongly suggest that inhibitory and facilitatory pain mechanisms reflect closely related neurocognitive processes.

GENERAL DISCUSSION AND CONCLUSIONS

Pain is a complex phenomenon, with severe consequences on both individual well-being and management of public healthcare. Acute pain is an unpleasant experience associated with many forms of injury and inflammation. It plays a crucial role in organism survival, by signaling that an event is threatening the integrity of the body, forcing rest, and driving pursuit of pain relief. Usually, as soon as the source of potential or actual damage is removed and the body has healed, pain sensations disappear. However, pain could cease to function as warning signal when it persists beyond the expected period of healing, leading to chronic pain states.

Individuals often conceive of pain as an objective entity, beyond their own control, and recur to anti-inflammatory/analgesic treatments or other drugs to control painful sensations. In cases of persistent or chronic pain states, this objectification can lead directly to loss of well-being associated with feelings of helplessness. However, pain is not limited to the mere transduction of noxious stimuli in physical symptoms, but constitutes a multifaceted experience including perceptual feelings, affect, motivation, cognition and attention. Importantly, pain modulatory mechanisms can be engaged non-pharmacologically, through emotional, cognitive and attentional manipulations.

The present thesis investigated the modulating effects on pain of the supine body position, emotions and gender differences, placebo in conventional and alternative medicine, and cognitive reappraisal in pain modulations. In each experiment, subjective and pain-related somatosensory potentials (i.e., pain-related ERPs) were collected to evaluate the effects of these variables on pain modulation. Collectively, the findings point to the importance of non-pharmacological interventions for achieving pain modulation, in increasing our knowledge of pain cortical mechanisms, and enhancing awareness of medical caregivers and patients in developing an active attitude towards pain interventions.

In the first study (*Horizontal body position reduces cortical pain-related processing,* Par. 3.1*),* we considered the influence of short-term horizontal body position on cortical pain processing (i.e., sensory-motor modulation of pain). The results demonstrate that, compared to a sitting position, the supine body posture significantly reduced subjective sensitivity to painless stimuli, but at the cortical level inhibited the

late cortical processing elicited by both non-painful and painful stimuli. The late cortical processing in question refers to frontal negative and posterior positive amplitudes, between 300 and 600 ms, whose magnitude were reduced following ninety minutes of bed-rest compared to the sitting position. Moreover, the late negative amplitudes in the anterior clusters were significantly correlated with the subjective pain ratings in the experimental BR group, but not in sitting controls. The correlation revealed higher variability in cortical responses elicited by electrical stimulation in the BR participants, showing no alteration in the perceived pain in the BR participants with negative cortical potentials similar to the control group, but reduced amplitudes for the participants which reported reduced pain perception. Importantly, as revealed by the sLORETA analysis, this late cortical modulation was observed within the right anterior cingulate cortex and superior frontal gyrus that are frontal regions typically involved in cognitive, affective, and motor aspects of pain processing. The results of this study have important implications for the clinical treatment and diagnosis of medical complications arising in bedridden patients, suggesting that prolonged supine position may affect patient's ability to report pain-related symptoms.

In the second study (*Gender differences in pain responses under emotional stimulation*, Par. 3.2), we investigated the effects of emotion and gender on pain perception (i.e., emotional modulation of pain), by highlighting the general roles of emotion, attention, and their interaction on cortical and subjective pain responses. Given the known clinical observation of a link between emotions and pain modulation, we examined the influences of appetitive (erotic, sport/adventure), aversive (fear/threat, mutilation) and neutral backgrounds on pain perception and pain-related ERPs. We revealed the specific role of erotic stimuli in maximizing pain reduction, compared to other pleasant (sport and adventure) or equally arousing pictures (mutilation and injuries). However, no reliable pain facilitation emerged during the vision of aversive contents, suggesting that pain facilitatory effects may be counteracted by increased attention levels during the viewing of aversive arousing pictures. Both in males and females, the N2 modulation (100-150 ms) mirrored the valence of the emotional background and the pattern of subjective pain ratings, with the lowest pain-related cortical processing during the vision of erotic pictures and with the greatest pain-related cortical processing during the vision of mutilation pictures. In addition, the P2 modulation (200-300 ms) mirrored the arousal of the emotional background, showing

reduced pain processing associated with high-arousing stimuli (both erotic and mutilation), and increased processing associated with neutral images. Of particular interest, was the finding related to gender differences in emotional pain modulation. Males showed differentiated cortical responses for erotic pictures and similar patterns for all the other emotional contexts. In contrast, females showed cortical pain responses modulated by all emotional contents both for the N2 and the P2 amplitudes. In particular, women displayed reduced cortical processing during the viewing of fear/threat pictures compared with neutral and mutilation scenes in the electrodes Cz and CPz. These results suggest that qualitative gender differences in emotional processing may lead to differentiated pain outcomes and that affect-related modulation of pain may depend upon different neural pathways in men and women.

In the third study (*Placebo effect in participants with high and low confidence in homeopathy*, Par. 3.3), to test the hypothesis that an unconventional homeopathic treatment may enhance placebo effects, we directly compared pain responses in individuals with either high or low trust in homeopathy in the laboratory. Expectations of effectiveness of a treatment (i.e., beliefs on the efficacy of a homeopathic analgesic treatment on the modulation of pain) were studied in three groups: (1) participants who trusted traditional medicine and expected analgesic effects after the administration of a supposedly active Ibuprofen pill; (2) participants who trusted traditional medicine, but did not expect analgesic effects after the administration of a supposedly active homeopathic substance; (3) participants who trusted homeopathy, and expected effective analgesic effects after the administration of the supposedly active homeopathic analgesic. We found no subjective differences in pain responses among the different groups. However, at the cortical level, compared with the low-trust homeopathy group taking homeopathic substance, the high-trust congruent expectancy-drug groups (taking their trusted remedies) showed reduced pain-related potentials at a late stage around 200-250 ms, that is an inhibited P2 component. The larger P2 amplitudes observed in the low-trust homeopathy group taking the homeopathic treatment, points to the lack of placebo responses in individuals who do not trust the administered treatment. These results indicates the key role of expectations in placebo effects and suggests that personal belief in the effectiveness of a treatment is a critical confound in clinical practice of both traditional and alternative medicine. A further interesting result revealed that the group of participants who trusted homeopathic treatments, compared

with the other two groups who instead trusted the traditional medicine, revealed higher scores on the absorption and unlikely virtues scales of the Multidimensional Personality Questionnaire. The high suggestibility found in the homeopathic group complements other empirical findings showing that the preference of natural treatments is influenced by the fear of side-effects typically associated with traditional treatments.

Finally, in the fourth study (*Reappraisal of pain and Mental Imagery induce hypoalgesic and allodynic effects*, Par. 3.4), we investigated the inhibitory and facilitatory processes elicited by an imagery-based reappraisal strategy designed to either reduce or amplify pain sensations. Participants were instructed to use mental images to either Inhibit (through imagery of a glove) or Facilitate (through imagery of a wound) triggered pain responses, or to experience pain without modulation (Baseline; through imagery of the skin). This procedure resulted in consistent analgesic and allodynic effects for painful and non-painful conditions both at the behavioral and the subjective level. Individuals reported feeling less pain during the Inhibition condition, linked to a reduced ability to correctly detect painful stimuli, which were conversely judged as non-painful. In contrast, the same individuals, reported higher perceived pain during the Facilitation condition, due to a reduced ability to process non-painful stimuli that were detected and judged as such. Painful and non-painful stimulation in the reappraisal and non-reappraisal conditions were associated with ERP modulation of N1 (50-60 ms), P1 (70-80 ms), N2 (100-150 ms), and P2 (200-400 ms) potentials. In particular, the pain main effect was associated with the modulation of each component: painful vs. non-painful stimulation elicited greater N1, P1, N2 and P2 potentials. The instruction main effects revealed similar differences in the contrasts inhibition vs. baseline and inhibition vs. facilitation. During inhibition, both painful and non-painful stimuli elicited greater N1 and N2 amplitudes, but reduced P2 potentials. Moreover, compared with the contrast inhibition vs. baseline, the differences between inhibition and facilitation displayed a limited N1 modulation, but increased N2 and P2 modulations, which were associated with an early onset, a later offset and involved a larger array of electrodes. These findings suggest that inhibitory and facilitatory pain mechanisms may be two sides of a coin, reflecting closely related neurocognitive processes. An important implication of this study is that in individuals suffering from greater pain due to cognitive amplification, resulting for example from pathological attentional bias, may have the potential to counteract and reverse hyperalgesic states.

Learning to cognitively modulate pain may thus represent a cost-effective addition to pharmacological pain intervention.

In conclusion, the studies here presented provide evidence for complex modulation of pain-experience and pain-related somatosensory processing depending on whether pain is determined by primarily bottom-up, such as peripheral sensory-motor modulation, or more top-down processes, such as distraction related to emotional contents, placebo and cognitive modulation associated with attention, expectations and reappraisal. The findings show that pain modulation has clear effects on subjective ratings and on the amplitudes of pain-related responses (i.e., N1, P1, N2, P2 and/or LPP), increasing early effects and the magnitude of the ERP modulation with a greater involvement of top-down control processes. In the study on the horizontal body position, which was associated with the lowest top-down involvement, subjective effects regarded the painless condition, whereas cortical modulation of both painless and painful stimuli involved a late component between 300 and 600 ms. The studies on emotional and placebo pain modulation, associated with middle top-down recruitment, (i.e., the second and third study, on emotional and placebo modulation) revealed a discrete subjective pain effects and a modulation of the late N2 and P2 components, between 100 and 300 ms. Finally, the study on imagery-related pain modulation, which was associated with the largest top-down influence, showed the greatest pain modulation at the subjective level, as well as the earliest and long-lasting cortical pain modulation, by affecting N1, N2, and P2 potentials. Interestingly, distraction-based strategies, involved in the emotional and placebo manipulation, were associated with reduced pain perception, decreased N2 and P2 potentials. Conversely, the reappraisal-based strategy adopted in the fourth study lead to reduced pain perception and P2 amplitudes, but increased N2 potentials. Thus, the N2 modulation may be related to specific differences across strategies that limit the extent to which pain is processed (distraction, expectations of reduced pain) vs. strategies that promote focused attention on pain in order to change the interpretation of its meaning (reappraisal). Indeed, an important difference between distraction and reappraisal is the degree to which the stimulus is attended. Instead, the P2 modulation may refer to pain-related processing that is common in both types of strategies, such as the appraisal processing. Distraction from pain and pain reappraisal, even if in different ways, lead the individual to appraise pain as less intense and less unpleasant.

On the note here, the reappraisal inhibition strategy increased hypoalgesic effects both at the subjective and cortical levels, suggesting that this strategy is one of the most powerful and flexible forms of cognitive pain regulation. In line with the findings of the present thesis, reappraisal of negative affect in the context of emotion regulation has been revealed to exert greater effect than distraction (McRae et al., 2010). Learning how to regulate pain by exploiting cognitive strategies may represent an effective tool for health and well-being. Undeniably, non-pharmacological interventions do not represent the only solution for any acute or chronic pain states, but together with pharmacological treatment they may have remarkable implications on analgesic outcomes. In the best scenario, a combination of multiple disciplines would maximize the probability of a successful treatment, by limiting idiosyncratic reactions, improving subjective well-being, and reducing the cost of clinical trials. A multidisciplinary perspective taking into account the needs of a person, in terms of biological, psychological and social dimensions may have extraordinary consequences on the outcome of a treatment.

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