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PAIN PERCEPTION AND ITS MODULATION

THEORETICAL MODELLING AND EXPERIMENTAL FINDINGS

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This thesis is dedicated to my grandmother Rina.

A piece of my heart stopped beating, the day you left us.

ABSTRACT

Pain is a highly subjective conscious experience and studies in this field are focused on brain regions involved in pain perception and on the developing of models to better understand the influence of cognitive factors on pain experience.

We investigated the domains of this field with three different studies.

Firstly, we studied the modulation of pain perception in a case of chronic pain, specifically a patient affected by painful phantom limb syndrome (right foot). The patient's brain activity during the execution of tasks or at rest was investigated with functional magnetic resonance imaging (fMRI). In particular, the tasks performed by the subject were imagination tasks, where he had to imagine a high level of pain in the phantom limb or something pleasant in order to modulate pain. Moreover, both task-dependent and resting-state activity were recorded in two subsequent days, characterized by different level of persistent pain intensity referred by the patient: a low intensity in Day1 and an higher intensity in Day2.

Significant activations of pain-related brain areas were found with pain imagination task in Day1 but not in Day2. Furthermore, regions of increased activity were found in cortical domains usually related with attention and executive control. These results indicated which brain areas were involved in the effective modulation of patient's chronic pain and which areas of the pain matrix were possibly related with perception in the phantom limb. The analysis of fMRI data acquired during the execution of the distraction task revealed in both days a network of activated areas that resembled the network of brain regions normally active at rest (default-mode network). This data supported the hypothesis that the distraction task was able to interfere with the ongoing mechanisms of persistent pain sensation, shifting the patient's brain state toward a condition similar to that described in healthy subjects in the absence of specific input or output. These findings were further confirmed by the results obtained with the analysis of the data recorded when the patient laid in the MRI scanner, without performing any specific task (resting state). In fact, correlation analysis revealed that neuronal activity of the default-mode network had opposite dynamics compared with the activity within the pain matrix and, moreover, the level of activation of pain-related areas was higher when the patient perceived an high persistent pain level.

The study of phantom limb pain gave a preliminary result about brain activity during chronic pain, investigating neuronal processing at rest, an fMRI approach developed only in recent years. Moreover, analysing data recorded at different pain intensities and using different pain-modulating tasks, this study showed which brain areas could be responsible for effective pain modulation in this pathological condition.

In the context of how several factors can influence pain perception, we developed a theoretical model based on Bayesian decision theory. Bayesian models have been effectively developed to explain processes that occur in the visual system or during learning. We were aimed to develop a model able to explain how the pain perceptive system integrates peripheral noxious information data with previous experience and the subject's expectation, driven by visual cues. To test the correctness of the proposed model, two experiments were performed with healthy subjects. Specifically, in both experiments a classical conditioning session with an analgesic treatment was used to inform the subjects about painful stimuli applied during the experiment. This provided the previous experience for subsequent test sessions. The occurrence of an placebo analgesic effect was tested in a subsequent session, demonstrating the efficacy of this experimental procedure in the modulation of pain perception.

The predictions of the model were compared with data measured in the group of volunteers. Two integrative processes were considered: firstly, the integration of peripheral noxious signals and information derived by previous experience, subsequently the integration of noxious signals, previous experience and the current expectation of incoming pain, guided by visual cues. In both cases, the predictions of the model were in agreement with observed data, corroborating the validity of the proposed model and suggesting that the tasks performed by the sensory-cognitive system may be described by a Bayesian integrative process. Moreover, the experiments demonstrated how pain perception could be influenced by previous experience even in the absence of current suggestion about the incoming stimulus. A further relevant point is that the predictions of the Bayesian model can open new perspectives in the investigation of placebo analgesic effect also in a clinical context.

Finally, we investigated the relationship between pain perception and physical activity. Two groups of healthy volunteers (trained and untrained subjects) were recruited and performed an exhaustive incremental exercise at cycle ergometer. Pain pressure thresholds were recorded before, immediately and 15 minutes after the physical activity. The results demonstrated how fatiguing exercise induced the modulation in pain perception and how this effect depended on the fitness level of the subjects. In particular, in trained subjects the exercise induced an

hypoalgesic effect, that lasted for few minutes after the exercise. Such hypoalgesic effect was not significant in untrained subjects, where, on the contrary, an hyperalgesic effect was observed 15 minutes after the execution of the fatiguing exercise. Other authors have studied the pain-modulation effect of physical exercise, but they have focused their attention on low or medium-high level of physical activity, without investigating the role of the fitness status. The findings we obtained were a first demonstration of the role of training level and fatigue in inducing significant pain modulation, suggesting the hypothesis that, according to the degree of the adaptation of the organism to the stressful activity, different mechanisms could be involved in these effects, or that the pain perceptive system responded differently to pain-modulating substances, released during the physical exercise. The differences found between trained and untrained healthy volunteers also suggested that pain perception could indicate how the organism would respond to an intensive exercise and that information provided by the pain perceptive system could be related to the general state of the organism. Moreover, the results found in this study have important implication in relation to the possibilities offered by physical activity as non-pharmacological treatment of pathological conditions characterized by pain as a symptom. In this context, the hyperalgesic state induced by an excessive exercise has to be taken into account when the modality of the exercise are evaluated.

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1 - PAIN PERCEPTION AND ITS MODULATION

Pain is the sense that warns about a possible or real damage to the tissues. More precisely, the International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" and it underlies that pain "is unquestionably a sensation in a part or parts of the body, but it is also always unpleasant and therefore also an emotional experience" (IASP 2011).

Pain is vital to avoid dangerous situations, protect the human body and allow healing processes to occur. Due to its importance as key mechanism of body defence and protection, pain has evolved as an intricate interplay between sensory and cognitive mechanisms, distinct from the classical senses: it is inherently variable and multifaceted, it is a discriminative sensation, an affective motivation, a potent autonomic drive and a reflexive motor stimulus (Craig 2003b; Perl 2011). Unlike other senses such as vision, hearing, and smell, pain has an urgent and primitive quality, a quality responsible for the affective and emotional aspect of pain perception. Moreover, the intensity with which pain is felt is affected by surrounding conditions, and the same stimulus can produce different responses in different individuals under similar conditions (Tracey and Mantyh 2007; Wiech et al. 2008).

From a clinical point of way, pain is the main reason for visiting the emergency department in more than 50% of cases and is present in 30% of family practice visits (Breivik et al. 2006). Between the two main form of pain (figure 1.1), acute pain is a direct outcome of a noxious event, such as a trauma or a surgery, and its management is reasonably good; on the other hand, chronic pain can be described as a condition in which pain persists long after its usefulness as an alarm signal and the healing of the tissue damage, and constitutes one of the major medical health problem in the developed countries (Tracey and Mantyh 2007).

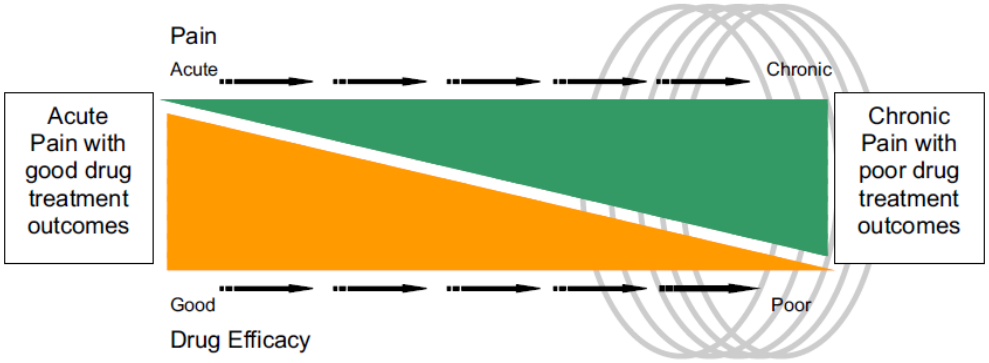

Figure 1.1 – The problem with chronic pain. Therapy for acute pain (e.g., acute inflammation, trauma, post-surgical pain) is overall excellent. However, in chronic pain (e.g., neuropathic pain, fibromyalgia, complex regional pain syndrome), therapy is poor. This group thus falls into a zone (circles) of "therapeutic failure" or "therapeutic impasse" where multiple therapies are tried with overall little success. Functional imaging appears poised to open up new approaches to the understanding of chronic pain conditions. An improved basic understanding of the mechanisms underlying chronic pain is likely to suggest new avenues for the development of novel pharmacotherapies. *From (Borsook and Becerra 2006)*

Only in Europe, for example, epidemiological studies have revealed the 19% of the population suffers from chronic pain, with a greater prevalence in women or in adult aged between 41 and 50 years (figure 1.2A and figure 1.2B). Furthermore, it has been reported a varying prevalence rates for this chronic condition among counties, ranging from 12 to 30% (figure 1.2C), but also within the same country. In Italy, for example, the prevalence is above 32% in the northern part of the country and less than 22% in the southern part (Breivik et al. 2006).

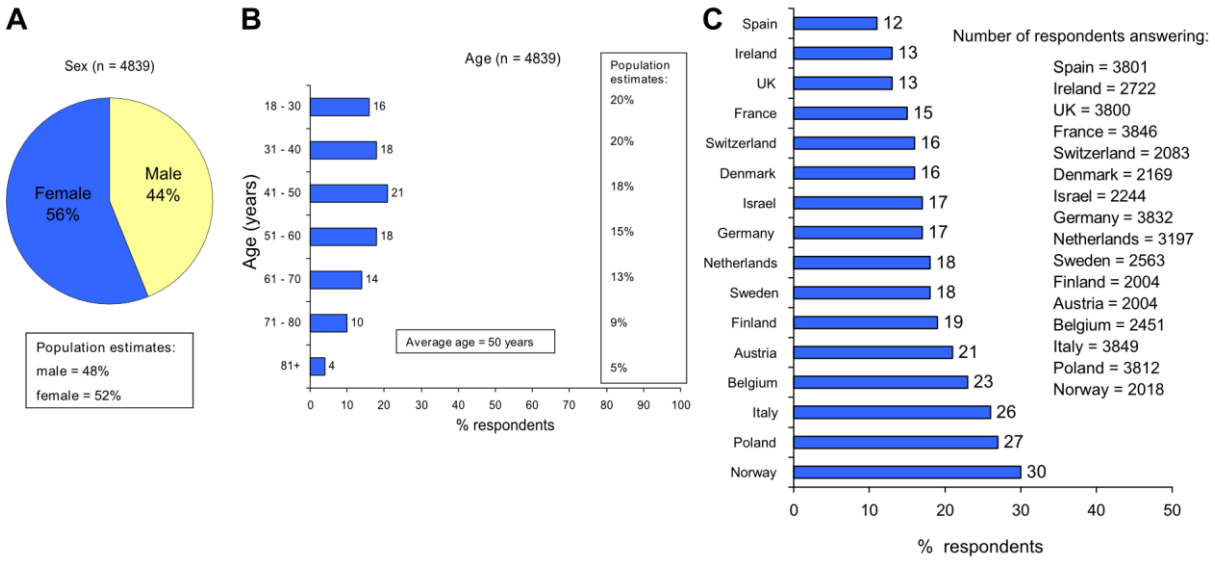

Figure 1.2 – Prevalence of chronic pain among 46,394 adults (>18 years) in 15 European countries and Israel responding to a computer-aided telephone screening interview. Chronic pain was defined as pain lasting more than 6 months, having pain during the last month, several times during the last week, and last experienced pain having an intensity 5 or more on a Numeric Rating Scale: 1 (no pain) to 10 (worst pain imaginable). The prevalence in the two sexes and in seven age categories is reported in *A* and *B*; data are calculated considering a total of 4839 responders that were interviewed in-depth. The prevalence of chronic pain in the 16 countries is presented in *C*. *Modified from (Breivik et al. 2006)*

The needs of chronic pain sufferers are largely unmet, creating an enormous emotional and financial burden to sufferers, carers and society. Improvements in our ability to diagnose chronic pain and develop new treatments are needed, together with robust and less subjective "readouts" of the pain experience. Brain imaging techniques have provided novel insights into functional, anatomical and chemical changes in the human nervous system that allow to define new approaches that may assist current drug development efforts. Though, our knowledge about the mechanisms of pain perception, especially in pathological conditions, is still far from be clear and complete, as well the processes involved in the fine modulation of pain perception to adapt the appropriate behavioural responses to the surrounding environment are not completely understood (Borsook and Becerra 2006; Borsook et al. 2007; Borsook and Becerra 2011).

This first chapter of this thesis is mainly addressed to review the current knowledge about the sensory and cognitive processes involved in pain sensation. In particular, the main neuronal pathways and brain areas will be presented, together with the factors and mechanisms that modulate the perceptive response evoked by a noxious stimulus. This first chapter is therefore thought to introduce the context in which the results presented in this thesis are inserted and to be useful for the following discussion.

1.1 - Pain and the nociceptive pathway

Pain is an highly subjective and personal experience. Classically, pain sensation is classified as a sub-modality of somatic sensory system, together with touch, pressure, position and temperature senses (Basbaum and Jessell 2000; Panerai 2011). However, pain has peculiar aspects that make it a unique sensation. Firstly, pain involves not only a sensory-discriminative component, but also affective and emotional components (Ingvar 1999; Bingel and Tracey 2008) that make pain sensation variable not only between subjects, but also within the same individual. Secondarily, pain has a role both as a sensory/detection system and as a protective system that alerts the body about a real or possible incoming dangerous event. Considering the difference between the first and the second roles, it is possible to understand the difference between the terms 'nociception' and 'pain': 'nociception' refers to the detection of stimuli that are capable of producing tissue injury, whereas 'pain' refers to the unpleasant sensory and emotional experience associated with actual or potential tissue damage, that drives subsequent effective behavioural responses; in other words, pain is the product of the brain's abstraction and elaboration of sensory input (Basbaum and Jessell 2000; Craig 2003c).

To fully understand why pain is a unique sensation, it is important to think about the role of this sensation. Senses like vision and touch are needed to collect information from surrounding environment with the aim of producing a good representation of the world, useful for planning effective movements and behaviours. Similarly, sensory-discriminative component of pain (nociception) contributes to improve the effectiveness of world representation, adding information about harmful stimuli or conditions. Though, the contribution of nociception goes beyond the planning and execution of effective movements, in fact it is the first step that, together with affective and emotional components, makes pain fundamental to protect the organism from dangerous or even deadly situation. Pain alerts the organism about a dangerous situation, warns about a condition that should be avoided and contributes to organize and maintain a record of previous experience in order to prevent possible future harmful situation. In a more general meaning, pain is one of the homeostatic mechanisms that organisms have to maintain the integrity of the body. For this reason, the human feeling of pain is both a distinct sensation and a specific emotion that reflects homeostatic behavioural drive, similar to temperature, itch, hunger and thirst (Craig 2003b).

Considering all these factors, it is possible to understand why pain perception involves a diffuse neuronal network and it is not just the collection and organization of incoming stimuli, but a complex processing that involves the integration with autonomic system and affective

and emotional spheres. Pain processing involves the contribution of different brain areas, within all the central nervous system. In fact, neuroimaging studies have identified a complex assembly of brain areas (figure 1.3) which includes primary (SI) and secondary (SII) somatosensory areas, insula, anterior cingulate and prefrontal cortices, but also subcortical structures such the thalamus, the brainstem, the amygdalae and the cerebellum (Bingel and Tracey 2008).

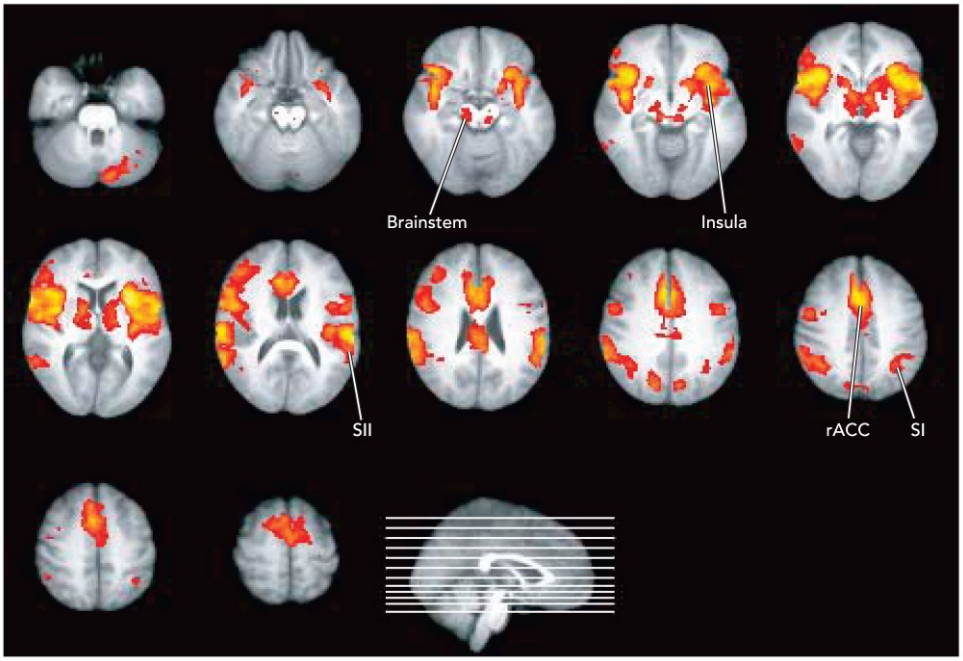

Figure 1.3 – The pain matrix. Increased blood-oxygen-level-dependent activity in response to thermal painful stimuli overlaid on a structural T1-weighted MRI. *From (Bingel and Tracey 2008)*

The areas included in the "pain matrix" are not only involved in the elaboration of pain perception, but also in its modulation according to many factors, like expectation, anxiety and mood. Examples of brain nuclei with a pain-modulatory effect are the midbrain periaqueductal grey (PAG) and the rostral ventrolateral medulla (RVM). Specifically, they constitute the starting point of a descending pain-inhibiting pathway that ends in the spinal cords with positive and/or negative effects on spinal cord excitability (Tracey and Mantyh 2007). Modulatory pathways are fundament if one considers the biological role of pain as homeostatic emotion. In fact, these mechanisms have evolved for a fine tune of the pain sensation in order to produce the appropriate behaviour for the stimulus and the context in which it is presented (Bee and Dickenson 2007).

1.1.1 - Afferent nociceptive pathways

The process that leads to pain perception is typically initiated by the activation of peripheral receptors, which selectively detect intense, potentially tissue-damaging stimuli (Fields 2004). These receptors, called nociceptors, have unencapsulated free nerve endings (figure 1.4) and constitute the starting point of a specific sensory channel that start at the periphery and ascend in a central afferent pathway to the brain (Schaible and Richter 2004).

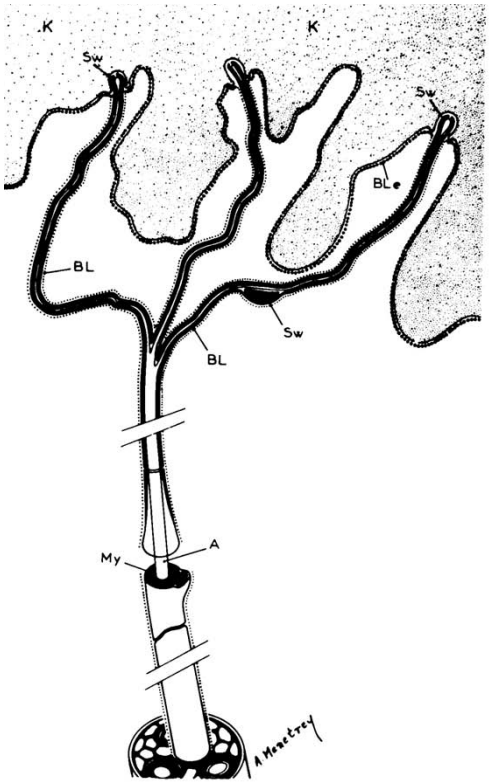

Figure 1.4 – Structure of a nociceptive terminal. Reconstruction of the terminals of an Aδ mechanical nociceptor, based on electron microscopic observations. The afferent fibre has several different unencapsulated endings that penetrate the basal lamina (BLe) and then extend into the epidermis. The myelin sheath (My) ends within the dermis. Most of the terminal membrane of the endings is covered by Schwann cell (Sw) processes. Other abbreviations: A, axon; K, keratinocytes. *From (Willis and Westlund 2004)*

Nociceptors, present in skin, muscles, joints, deep visceral tissues, teeth and dura mater, have been classified according to their stimulus specificity and the conduction velocity of their afferent fibers.

Considering cutaneous sensory neurons, nociceptors that respond preferentially to mechanical or thermal stimuli have been found: the first are highly responsive for intensive pressure applied to the skin, whereas the latter are specific for extreme temperatures (>45°C or < 5°C). However a third type of nociceptors is widely distributed in the skin and includes those neurons that are sensitive to several types of mechanical, thermal and chemical noxious stimuli, such as protons (acid pH) lactic acid, ATP, histamine and bradykinin. These receptors have been thus called polymodal nociceptors (Craig 2003c; Schaible and Richter 2004). Finally, some nociceptors are silent in normal conditions, but respond to subsequent tissue damage or inflammation (Basbaum and Jessell 2000).

Nociceptors have unmyelinated (C-fibers) or thinly myelinated axons (Aδ-fibers), that conduct the electric information, transduced by the sensory terminals, at velocities of 5-30 m/s and 1 m/s, respectively. Because of different conduction velocity, C-fibers and Aδ-fibers carry different information about the status of the various tissue of the body and differentially contribute to the pain perception. In fact, it has been frequently observed that two different sensations of first and second pain can be evoked in a subject after a single painful stimulation of the skin, specifically short-latency pricking pain, followed by long latency pain of a burning and less bearable quality (Craig 2003c). In according with the latency of these different pain components, it has been demonstrated that they are related with nociceptive input carried by Aδ-fibers and C-fibers respectively (Willis and Westlund 2004), supporting the hypothesis that differences in the pain processing are present since the first structures included in the pain perceptive system .

The primary sensory neurons have cell bodies in the dorsal root ganglia (DRG) or the trigeminal ganglia. In particular, axonal projections of those neurons that have the cell bodies in the DRG terminate in the dorsal horn of the spinal cord (figure 1.5), which has an important role in the integration and modulation of pain-related signals and where second-order neurons with different properties have been found (Riedel and Neeck 2001).

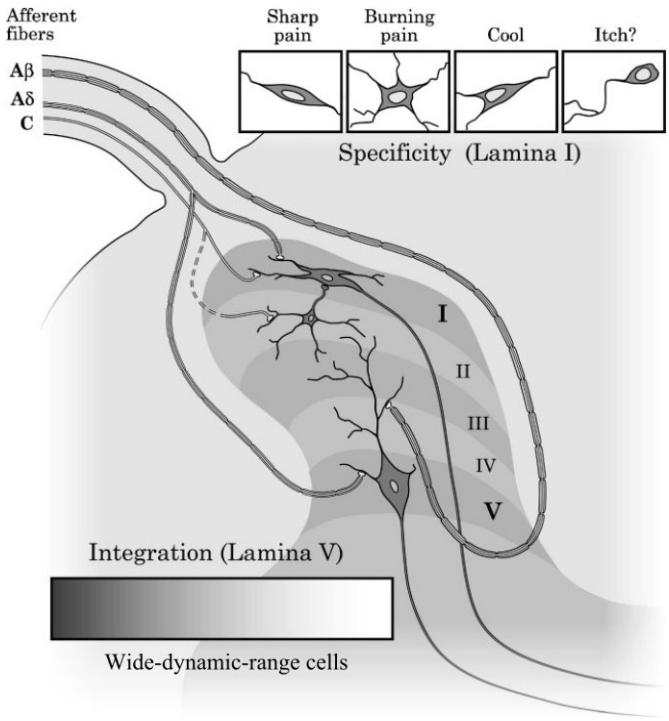

Figure 1.5 – Afferent nociceptive fibers in the spinal cord. Summary diagram representing the anatomical basis for afferent inputs to specific cells in lamina I and integrative cells in lamina V of the spinal cord. *Modified from (Craig 2003c)*

Both Aδ and C fibers enter the spinal cord via the Lissauer's tract, ending in different layers (figure 1.5); the majority of these fibers terminates in the superficial region of the dorsal horn

innervating cell bodies of laminae I and II, while Aδ fibers terminate also in lamina V (Riedel and Neeck 2001). Second-order neurons in these spinal sites can be differentiated mainly in two types, on the basis of their sensitivity for the noxious stimuli and the characteristics of the nociceptive information processed by them (Riedel and Neeck 2001). Lamina I has several distinct, modality-selective neurons that receive input from particular subsets of small-diameter fibers and transmit information about the ongoing physiological status of the body tissues. This group of neurons constitutes virtual labelled lines because their activity corresponds to distinct sensations, albeit after integration in the forebrain. On the other hand, lamina V neurons are large cells with dendrites that receive large-diameter (myelinated) primary afferent input from cutaneous and deep sources, as well as direct Aδ-nociceptor input. They respond to pressure, noxious heat, noxious cold, and noxious deep and visceral stimulation, therefore they are called wide-dynamic-range cells (figure 1.5). Moreover, in contrast to lamina I neurons, they are not somatotopically organized (Craig, 2003 labelled). Finally, the dorsal horn is also the terminal site of descending modulatory pathways (figure 1.6), that originate in brainstem centres, such as PAG and RVM (Tracey and Mantyh 2007).

Both lamina I and lamina V neurons projects mainly to the contralateral thalamus through the spinothalamic tract in the anterolateral system (figure 1.6), although dorsal pathways have been described (Willis and Westlund 2004). Although there is no absolute clear anatomical separation in the spinothalamic tract, it can be subdivided in two systems, according to the pain components in which they are involved. On one hand, the lateral thalamocortical system consists of the relay nuclei in the lateral thalamus, that then project mainly to SI and SII in the postcentral gyrus; on the other hand, the medial thalamocortical system consists of relay nuclei in the central and medial thalamus that have connections with the anterior cingulate cortex, the insula, and the prefrontal cortex (Schaible and Richter 2004; Tracey and Mantyh 2007). Considering the different components that characterize the pain perception, the lateral system is mainly involved in the analysis of the location, duration and intensity of the noxious stimuli, on the contrary, the medial system, integrating spinal cord inputs with brainstem homeostatic activity, is mainly involved in the affective aspects, related to the unpleasant feelings associated with the noxious stimulus and with the aversive reactions that it causes (Tracey and Mantyh 2007). Together these two pathways generate the feeling and the motivation, respectively, that constitute the homeostatic emotion of pain (Craig 2003b).

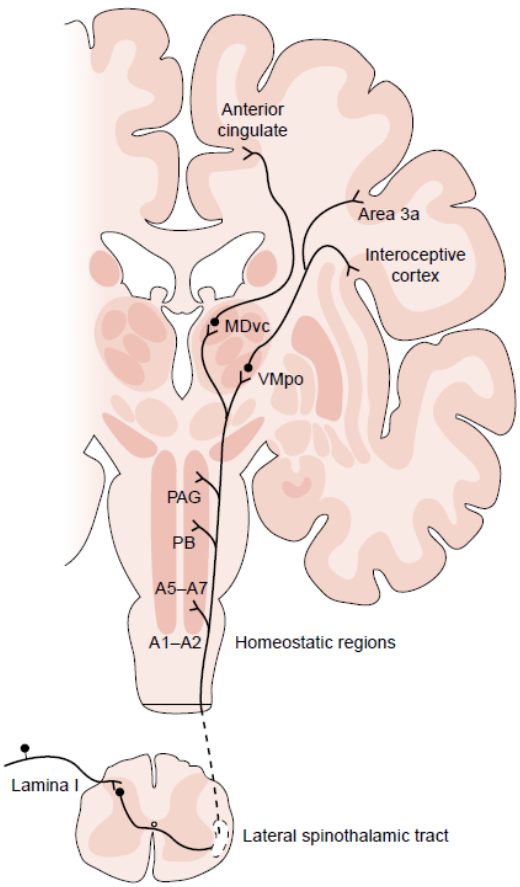

Figure 1.6 – Summary of the projections, ascending from the spinal cord. Brainstem and thalamic sites are abbreviated as follows: A1-A2 and A5-A7, noradrenergic cell groups; PB, parabrachial nucleus; PAG, periaqueductal grey; VMpo, posterior part of the ventral medial nucleus; MDvc, ventral caudal part of the medial dorsal nucleus. *From (Craig 2003b)*

1.1.2 - The pain matrix

The development of neuroimaging techniques, like functional magnetic resonance imaging (fMRI) and positron-emission tomography (PET), have given the possibility to investigate the cerebral representation of pain and delineate the functional anatomy of different aspects of pain perception in the brain. Since the first studies, a diffuse assembly of brain structures that commonly activate in the processing of acute pain was described and thus was named "pain matrix" (Bingel and Tracey 2008). As shown in figure 1.7, the brain areas most commonly observed in the context of pain include SI, SII, insular, anterior cingulate and prefrontal cortices as well as the thalamus (Apkarian et al. 2005; Borsook and Becerra 2006; Bingel and Tracey 2008). However, even not systematically found to be involved in perceptive processing, it should be noticed that subcortical areas like the brainstem, amygdalae and cerebellum also play a role in the multi-factorial experience of pain (Bingel and Tracey 2008).

Finally, the pain network should not be seen as an isolated and static entity, but rather as a substrate that is significantly and actively modulated by several other brain regions depending on the precise interplay of factors that contribute to the individual perception of pain, such as cognition, mood and injury (Tracey and Mantyh 2007; Wiech et al. 2008).

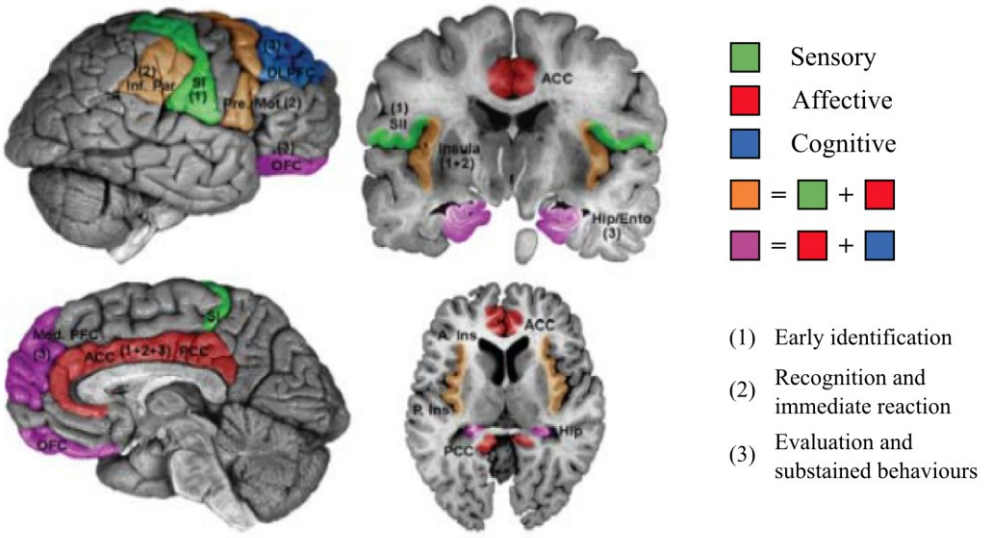

Figure 1.7 – The cerebral signature of pain. The picture summarizes the areas found to be active in fMRI studies: color-coding reflects the hypothesized role of each area in processing the different dimensions of pain, whereas numbers in parenthesis indicate their relative involvement during different temporal stages of the pain experience. The names of the structures are abbreviated as follows: ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; SI, primary somatosensory cortex; SII, secondary somatosensory cortex; Inf. Par, inferior parietal lobe; DLPFC, dorsolateral prefrontal cortex; pre-motor cortex; OFC, orbitofrontal cortex; Med. PFC, medial prefrontal cortex; P. Ins, posterior insula; A. Ins, anterior insula; Hip, hippocampus and Ento, entorhinal cortex. *Modified from (Borsook et al. 2007)*

Primary and secondary somatosensory cortices

The primary and secondary somatosensory cortices (SI and SII) receive projections from the thalamic relay nuclei of the lateral pain system and are the main cortical sites involved in the processing of the discriminative-sensory properties of the experience of pain. Although electrical stimulation of SI did not evoke painful responses in neurosurgical patients nor the ablation of SII causes detectable sensory deficits (Ingvar 1999), several neuroimaging studies have demonstrated that these two cortical structures have the primarily function to discriminate the location and intensity of a painful stimulus (Porro et al. 1998; Porro et al. 2004). For example, Bornhövd and colleagues (2002) studied the relation between BOLD signal and the intensity of a laser stimulus. In particular, they chose five intensities: from a not noticed stimulus (P0) to the highest painful stimulus (P5), comprising a stimulus intensity that the subjects perceived as warm, but not painful (P1). With this experimental protocol, they demonstrated that SI was involved in the coding of the stimulus intensity, independently from its harmful quality, whereas SII and the posterior portion of the insula were involved in the coding of the pain intensity (figure 1.8).

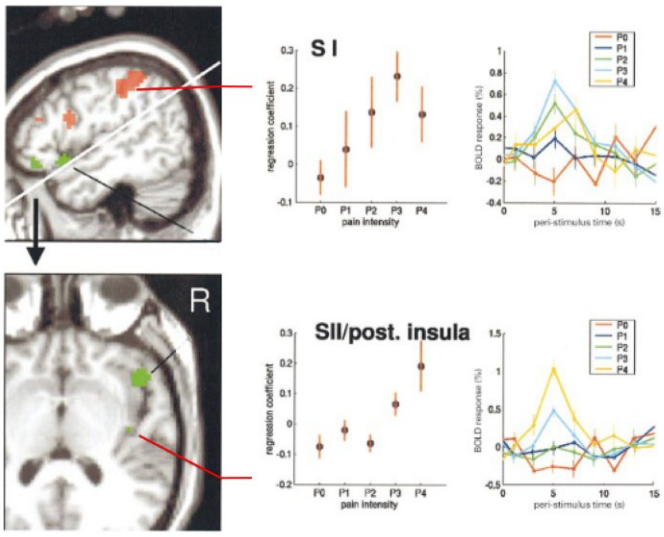

Figure 1.8 – Relationship between stimulus intensity and BOLD signal in SI and SII. Although, both somatosensory cortices are involved in the coding of stimulus intensity, only the activation of the secondary somatosensory cortex is related to the pain-related spatially discriminative aspects of noxious stimulus. *Modified from (Bornhovd et al. 2002)*

More recently, Nir and coworkers (2008) published a study in which they recorded electrical scalp signal with electroencephalography (EEG) and reconstructed the cortical activity evoked by five different intensities of thermal stimuli, randomly applied on the back of healthy volunteers. As shown in figure 1.9, the activation magnitude of the contralateral SI significantly correlated with the subjective perception of noxious stimuli, proving that the contralateral primary somatosensory cortex has a key role in pain intensity coding.

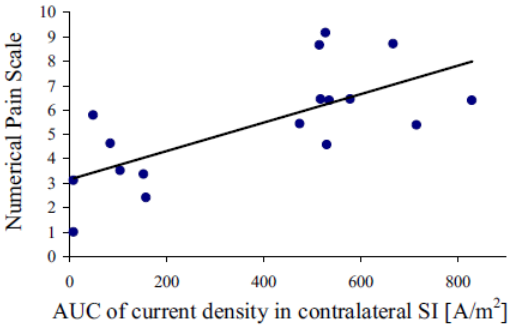

Figure 1.9 – Correlation between the mean activation in SI and the mean pain rating on a numerical pain scale. Mean activation was calculated as the area under the curve (AUC) of the SI waveform reconstructed with EEG source imaging. *From (Nir et al. 2008)*

Insula

Insular cortex is located bilaterally within the brain, tucked away under the posterior part of the frontal lobe and the anterior part of the temporal lobe. The most relevant anatomic landmark is the central insular sulcus, that separates the insular cortex in two main divisions, anterior and posterior insula, with functional distinct features (Singer et al. 2009). In fact, it has been shown that anterior insula (AI) is involved in emotional and affective dimensions of pain experience as well as in the representation and integration of autonomic and visceral

signals and in interoception (Singer et al. 2009; Craig 2011), whereas somatosensory discrimination is performed by posterior insula (see figure 1.9). These functional subdivision of the insula is reflected in the dense anatomical connectivity that anterior and posterior insula have with many subcortical and cortical areas: AI is interconnected with subcortical structures, such as the nuclei of the brainstem, limbic structures and the basal ganglia, while the posterior insula is connected with the thalamus and basal ganglia (Lamm and Singer 2010).

Neuroimaging studies have demonstrated a bilateral activation of anterior insular cortex during the experience of pain, that has a crucial role for integrating cognitive, emotional and vegetative activities and the affective dimension of pain (Wiech et al. 2010). For example, the investigation of empathy's substrates in the central nervous system revealed the involvement of anterior insula in the processing of emotional state felt when a loved person experienced pain (figure 1.10). On the contrary, activity in the posterior insula/SII, the sensorimotor cortex(SI/MI) and the caudal anterior cingulate cortex was specific to receiving pain (Singer et al. 2004).

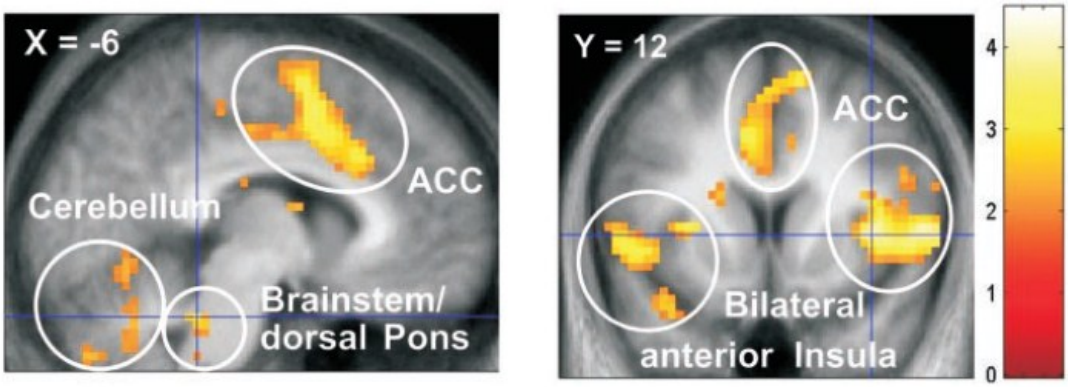

Figure 1.10 – Shared networks observed when pain was applied to self or to the partner. Only part of the network mediating pain experience is shared when empathizing with pain in others, suggesting a segregation of sensory-discriminative and emotional-affective attributes of the pain, in which the anterior insula and anterior cingulate cortex (ACC) are mainly involved in the latter. *From (Singer et al. 2004)*

Furthermore, it has been suggested that AI has also a role in the anticipation of pain, in particular, in the integration of the perceived threat value of the stimulation into the decision about the salience of the impending stimulus (Wiech et al. 2010).

These experiments, together with other functional imaging studies, have accumulated evidence that the anterior insula of humans is associated with subjective emotional feelings, not only in the experience of pain, but also in all task-related and attention-related aspects of behavior, including subjective time estimation, music appreciation, mental effort, and behavioral salience (Lamm and Singer 2010; Craig 2011).

Anterior cingulate cortex

The anterior cingulate cortex (ACC) is the anterior part of the cingulate gyrus, located in the medial wall of the brain hemisphere, immediately above the corpus callosum (figure 1.11). This cortex domain can be separated in specialized parts on the basis of cytoarchitecture, connectivity and functions. ACC integrates input from various sources, including motivation, evaluation of error and modulation of executive and attention functions, and acts by influencing activity in other brain regions, modulating cognitive, motor, endocrine and visceral responses (Bush et al. 2000). In particular, it has been shown that cognitive and emotional information are processed separately in the two main subdivisions of ACC: a dorsal cognitive division and a rostral-ventral affective division (respectively in red and blue in figure 1.11)

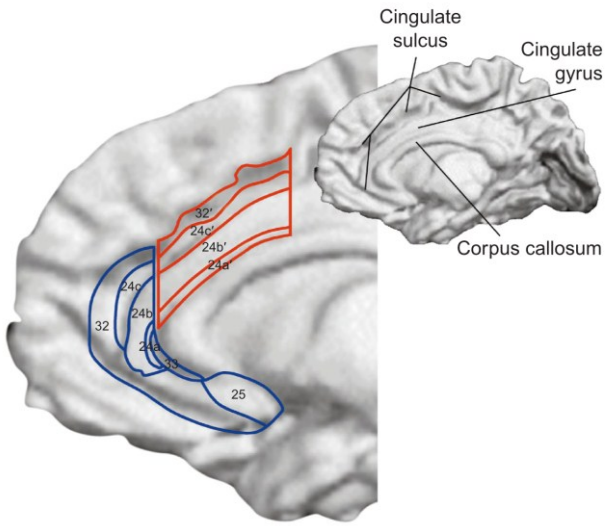

Figure 1.11 – Anterior cingulate cortex anatomy. The right part of the figure contains a reconstructed MRI of the medial surface of the right hemisphere of a single human brain (anterior towards the left, posterior towards the right). The cortical surface has been 'partially inflated' to allow simultaneous viewing of gyri and sulci. The cingulate gyrus lies between the cingulate sulcus and the corpus callosum. A schematic representation of cytoarchitectural areas (numbered) of ACC is shown on the left. Cognitive division areas are outlined in red and affective divisionareas are outlined in blue. *Modified from (Bush et al. 2000)*

ACC is activated during the majority of PET or fMRI studies of pain and it has been implicated in the affective processing of pain (Apkarian et al. 2005; Chen 2008). This role has been confirmed by lesion studies (Talbot et al. 1995; Greenspan et al. 2008) and its connections with other brain structures. Specifically, ACC receives nociceptive afferent inputs from the thalamic nuclei of the medial pain system and projects to other regions with executive and cognitive functions such as the prefrontal cortex, the amygdala or brainstem structures (Craig 2003a). Together with the insular cortex, ACC is believed to act as a neural "alarm system" or conflict monitor, detecting when an automatic response is inappropriate or in conflict with current goals. Moreover, these two regions can be seen as the sensory and motor components of an interoceptive system for detecting potentially or current dangerous stimuli and organizing coherent and effective behavioral responses. In this context, it has been shown that rostral-ventral division ACC is primarily involved in assessing the salience of

emotional and motivational information and the regulation of emotional responses (Panerai 2011). Finally, ACC is also involved in the cognitive modulation of pain. For example, it has been demonstrated that expectation-induced analgesia is mediated by an enhanced coupling of ACC, in particular its rostral part (rACC), with subcortical brain structures such as the periaqueductal grey. On the basis of these findings, it has been proposed that rACC directly act on the descending modulatory system through the activation of midbrain structures, inducing an opioid-dependent modulatory effect on pain (Petrovic et al. 2002).

Prefrontal cortex

The prefrontal cortex (PFC) is an heterogeneous brain area, where different subdivisions are thought to play specific roles in various cognitive, emotional and memory functions (Apkarian et al. 2005). Considering its role in relation to the experience of pain, PFC has a substantial role in the cognitive regulation of pain, as demonstrated, for example, by the activations of prefrontal cortex regions during the performance of tasks that involve attention toward or away from a concurrent noxious stimulus, resulting in an enhanced or decreased evoked perception (Apkarian et al. 2005; Brooks and Tracey 2005; Wiech et al. 2008). Moreover, its anatomical position supports this role as an essential source of modulation: it receives sensory information from all modalities and is associated with limbic structures that are crucial for affect and emotion. Furthermore, it has connections with motor system structures, resulting in a direct translation of PFC outcome into behaviour (Wiech et al. 2008). The dorsolateral prefrontal cortex (DLPFC), in particular, is involved in maintaining and appropriately updating internal representations of expectations, which modulate processing in other brain areas. In this sense, many authors have described the pivotal role of DLPFC in anticipation and expectation mechanisms that mediate the placebo effect. Using fMRI, Wager and colleagues (2004) were the first in describing a significant increase in DLPFC activity in the period immediately before the delivery of a noxious stimulus, when a visual cue anticipated the subjects about the intensity of the stimulation. On the contrary, the authors found significant reduction in neural activity in pain-responsive structures, such as the ACC, insula and thalamus, only during the shock period.

DLPFC is not the only subdivision of the prefrontal cortex that participates in initializing pain modulation. In fact, evidence suggest that the ventrolateral prefrontal cortex (VLPFC) is also involved in cognitive modulations of pain, reflecting reappraisal of the emotional significance of a stimulus resulting in a decrease in subjective pain intensity (Wiech et al. 2008).

Finally, orbitofrontal cortex (OFC) contains neurons responsive to negative emotional stimuli, furthermore, the electrical stimulation of the orbitofrontal and the medial prefrontal cortices results in analgesia. These findings are supported by fMRI studies that showed a negative correlation between experimental pain intensity ratings and activation of these areas, so that lower activations in these cortical demines occurred with higher pain ratings (Bantick et al. 2002; Brooks and Tracey 2005).

All together, the functionally different subdivisions of PFC underlie a complex interplay between cognitive and executive areas that initializes and regulate the pain processing within the other areas of the pain matrix (figure 1.12).

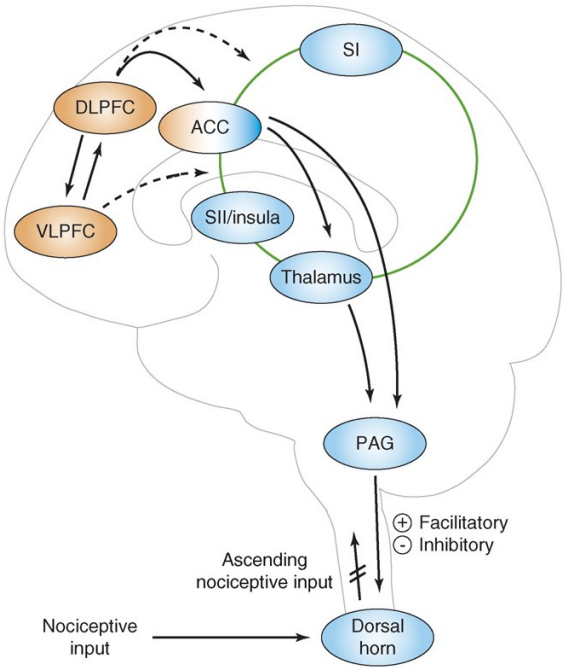

Figure 1.12 – Possible neural pathways of cognitive pain modulation. Cognitive modulations of pain are related to activation of prefrontal brain areas (DLPFC, VLPFC and ACC; shown in orange), which modulate activation in pain-associated regions in the cortex (ACC, SI, SII and insula), brainstem and dorsal horn (the PAG and dorsal horn; shown in blue). Abbreviations are as follows: ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; PAG, periaqueductal grey; SI, primary somatosensory cortex; SII, secondary somatosensory cortex; VLPFC, ventrolateral prefrontal cortex. *From (Wiech et al. 2008)*

1.2 - Modulation of pain perception

Because the biologically relevant output of the nervous system is behaviour, circuits are meaningfully defined only in relation to a specific behaviour. In the case of pain perception, signals related to noxious or potentially noxious input could receive enhanced attention or be subordinated to other bodily needs of higher priority. In other words, nociceptive inputs can be regulated in order to produce the most effective behaviour as response to stimuli that harm or threaten to harm the body (Brooks and Tracey 2005).

1.2.1 - Pathways for pain modulation

Several cortical structures have been found in studies aimed to identify the neuronal substrates underlying the cognitive modulation elicited by the factors such as attention or expectation. As mention in paragraph 1.1.2, the prefrontal cortex has an essential role in controlling the activity of pain-processing areas such as the insula or the primary somatosensory cortex; furthermore, the anterior cingulate cortex has been described as source of inputs that modulate the activity in other areas of the pain perceptive system.

Since the studies performed by Sherrington, showing that nociceptive reflexes were enhanced after transection of the spinal cord (Sherrington 1906), the existence of pain-inhibitory pathway has been postulated. This pathway originates in the brain and descends in the spinal cord, terminating in the dorsal horn and modulating the transmission of nociceptive information between first- and second-order nociceptors. Most of the knowledge about the descending control of nociception derives from studies in animals, like mice and rats; however, in recent years the development of non-invasive methods for investigating in humans the functionality of structures such as the spinal cord have opened new perspectives for understanding the complex mechanisms that regulate the transmission and the processing of nociceptive information from the spinal cord to higher-order brain structure, in normal and pathological condition (Tracey et al. 2002; Tracey and Iannetti 2006).

Many of the brain regions that modulate the perception of pain act through a common pathway that originate in the periaqueductal grey matter (PAG), a midbrain region located around the cerebral aqueduct. The first direct demonstration that this area has an active role in changing the experience of pain came from the work Mayer and Price (1976) that demonstrated that stimulation in the PAG produced analgesia without any concurrent effects on alertness or motor performance. In the context of the PAG activation, opioids such as morphine and heroin are one of the best known and most studied group of substances able to induce analgesic effect through the binding with receptors on PAG neurons.

Investigations about the effects of these substances on pain have focused largely on the μ-opioid receptor (MOR), because its activation is necessary for the action of these potent analgesics (Fields 2004). MOR agonists produce analgesia through both pre- and postsynaptic mechanisms at multiple CNS sites. MOR agonists can directly inhibit pain transmission at spinal levels, furthermore the MOR is present in all of the known supraspinal components of the pain-modulation circuit including the insular cortex, amygdala, hypothalamus and brain stem structures (Fields 2004).

In the context of physiological activation of opioid receptors, one of the best example is the placebo effect that has been shown to be induced by the release of endogenous opioids and the activation of the descending modulatory system (Zubieta et al. 2005).

PAG does not control nociceptive transmission directly, but through connections with the rostral ventromedial medulla (RVM), that is defined functionally, as the midline pontomedullary area in which electrical stimulation or opioid microinjection produces behavioral antinociception (Heinricher et al. 2009). It contains serotoninergic neurons that, together with noradrenergic neurons in the dorsolateral pontine tegmentum, project through the spinal cord dorsolateral funiculus and selectively target the nociceptive neurons in the dorsal horn. Thus, it can be considered the final output of the top-down pain-modulation system (Bee and Dickenson 2007). In particular, the RVM shapes spinal processing acting through a direct action on presynaptic terminals of the primary afferent fibers, on postsynaptic receptors on second order projection neurons and also on intrinsic interneurons within the spinal cord (Bee and Dickenson 2007).

RVM neurons can be classified on the basis of their effect on nociception; in particular, stimulation or inactivation of these neurons can suppress or enhance nociception, depending on the functional context. By means of whole-animal electrophysiological experiments, three classes of neurons have been identified (figure 1.13): ON-cells, that facilitate nociception, OFF-cells, that suppress nociception and NEUTRAL-cells that seems to have no effect on nociception (Fields 2004). For example, it has been demonstrated the stressful factors can induce both analgesia and hyperalgesia, according to the type of stressor involved, and that OFF-cells are implicated in stress-induced analgesia related to conditioned fear, while ON-cells are activated by what is referred to by various authors as “psychological”, “mild”, or “emotional” stress (Martenson et al. 2009; Cornelio et al. 2011).

As in the case of the PAG, also RVM neurons have receptors for opioid ligands, that mediate the analgesic effect induced by these substances. In this case, this effect is due to several types

of synaptic actions by MOR ligands, such as the postsynaptical inhibition of ON-cells or disinhibition of OFF-cells through presynaptic inhibition of GABAergic neurons (Fields 2004).

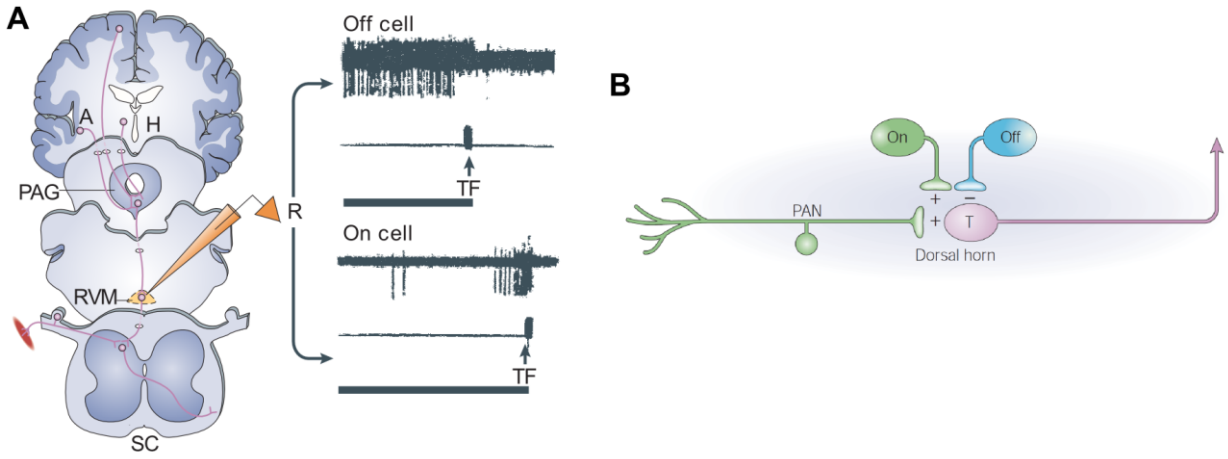

Figure 1.13 – Two populations of neurons exert opposing modulatory actions have been identified in the midbrain. In *A*, recordings from neurons in the rostral ventromedial medulla (RVM) of rats during application of noxious heat to the tail reveals one class that pauses (off cells) and one that bursts (on cells) just prior to the withdrawal tail flick (TF), demonstrating both facilitatory and inhibitory control of nociceptive transmission (*B*). Abbreviations are as follows: A, amygdala; H, hypothalamus; PAG, periaqueductal grey; PAN, primary afferent nociceptor; R, recording electrode; SC, spinal cord; T, pain transmission neuron. *From (Fields 2004)*

Although the involvement of PAG and RVM in pain modulation are well-known, other structures have been identified as origin of descending pain modulatory pathways (figure 1.14 Heinricher, 2009). Moreover, cortico-cortical interactions among regions involved in sensory and emotional information of pain should be considered to fully understand the neuronal mechanisms responsible for perception of pain and its modulation (Bingel and Tracey 2008).

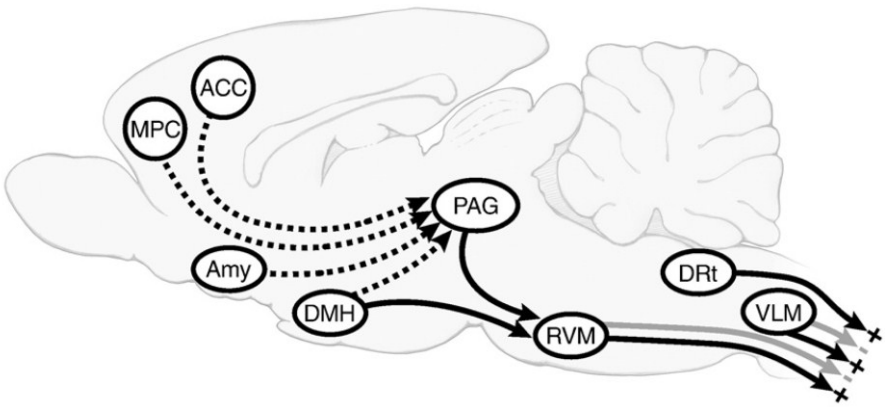

Figure 1.14 – Schematic representation of the main pain modulatory descending pathways. The periaqueductal gray matter (PAG) especially, but also the rostral ventromedial medulla (RVM), the lateral and caudal dorsal reticular nucleus (DRt) and the ventrolateral medulla (VLM) receive important direct and indirect inputs from limbic forebrain areas including anterior cingulate cortex (ACC), amygdala (Amy), dorsomedial nucleus of the hypothalamus (DMH) and medial prefrontal cortex (MPC). *From (Heinricher et al. 2009)*

All the brain areas and pathways described in the above paragraphs acts, in normal condition, to ensure a fine equilibrium between the opposing descending controls to support spinal gain that is large enough to detect noxious stimuli, yet not large enough to permit excessive sensory transmission. On one hand, this can be advantageous to finely tuning the processing of nociceptive information, in order to generate an appropriate behavioral response. However, it may alternatively become maladaptive and permit long-lasting pain, possibly in the absence of any identifiable cause, so that sensitization of the nervous system at different levels can contribute to the development and/or maintenance of some chronic pain states (Zambreanu et al. 2005; Heinricher et al. 2009).

1.2.2 - Factors that shape the perception of pain

As mentioned in the previous paragraph, the descending pain-modulating pathways are the final brain output that regulate the spinal transmission of nociceptive information to supra-spinal structures. On the brainstem pathways, many high-order brain areas exert their action. These brain areas are involved in the modulation of the relationship between reported pain intensity and the peripheral stimulus induced by many factors, such as the level of arousal, anxiety, depression, attention and expectation or anticipation, that establish the significance of the stimulus and help determine an appropriate response to it (Brooks and Tracey 2005).

Attention modulates perception and cognition by allocating processing resources to relevant external and internal events. It therefore amplifies behavioural and physiological responses to relevant events and attenuates responses to irrelevant events. The highly subjective and

behaviourally relevant experience of pain is particularly susceptible to these attentional modulations and ever simple manipulations with attention can alter the experience of pain. (Tracey and Mantyh 2007; Wiech et al. 2008). The neuronal substrates of these modulation have been extensively studied and several brain structures have been identified. In particular, for its clinical importance, several authors have investigated the neuronal circuits involved in the pain-inhibiting effects of distraction. The main effect of distraction appears to be the increased activity in regions of the medial pain system, such as the orbitofrontal and the dorsolateral domains of the prefrontal cortex and the rostral part of the anterior cingulate cortex, and a corresponding reduction in activation in the lateral pain system, like the thalamus and the insular cortex (Bantick et al. 2002; Porro et al. 2004; Brooks and Tracey 2005). Furthermore, it has been suggested that these areas decrease pain perception via the descending pain modulatory system (Tracey et al. 2002; Valet et al. 2004).

These findings demonstrated the effects of attention on pain-related brain areas; however, a direct evidence of the involvement of higher-order frontal and parietal areas, which mediate top-down attentional changes in other sensory and cognitive processes are inconclusive, probably because of the inherently difficulty in grading attention in experiments on pain. In fact, pain attracts attention *per se* and can rarely be ignored, making difficult to isolate the attention-related brain activity neuroimaging experiments. For example, in experimental paradigms in which subjects are actively engaged in distracting tasks and these distraction conditions are compared to conditions in which subjects attended to pain, the level of attention might be similar for attention and distraction conditions, so that the comparison of these conditions does not enable identification of brain areas that specifically influence pain processing via attentional mechanisms. Moreover, even distraction paradigms cannot ensure that attention is effectively diverted from pain (Wiech et al. 2008).

Attention is not the only factor that can have an effect on pain perception. Expectation of high pain intensity and anticipation of a painful experience can influence the pain-related activities in several brain areas. The effects of anticipation of a painful stimulus on brain activity were observed in the rostral anterior insula and the prefrontal cortex, in particular its medial subdivision, but also in pain discriminative areas such as the primary somatosensory cortex and the thalamus, where significant increases in neuronal activity were found during the anticipation of pain (Ploghaus et al. 1999; Porro et al. 2002). Expectations enable an organism to be prepared for the incoming noxious stimulus, therefore it is essential to detect discrepancies between expected and perceived features (figure 1.15), so that expectation can be updated if necessary (Wiech et al. 2008). The mechanisms of expectation and comparison

between sensory information and internal representation of the incoming input lead to one of the best example of the cognitive modulation of pain: the placebo analgesic effect (Enck et al. 2008; Price et al. 2008).

A placebo can be an inert treatment that is given as it has an effective action on the organism. The placebo effect, or response, is the outcome after the sham treatment. Because the placebo has an effect on the perception of pain, this can derive exclusively from the action of the contextual non-noxious stimuli, associated with the administration of the sham treatment. Therefore, the study of the placebo effect is the study of the psychosocial context around the patient (Benedetti et al. 2005). In this sense, expectations can induce an internal representation of the incoming noxious stimulus that is different from the actual stimulation, resulting in a biased perception of pain evoked by the stimulus, as in figure 1.15B and figure 1.15C.

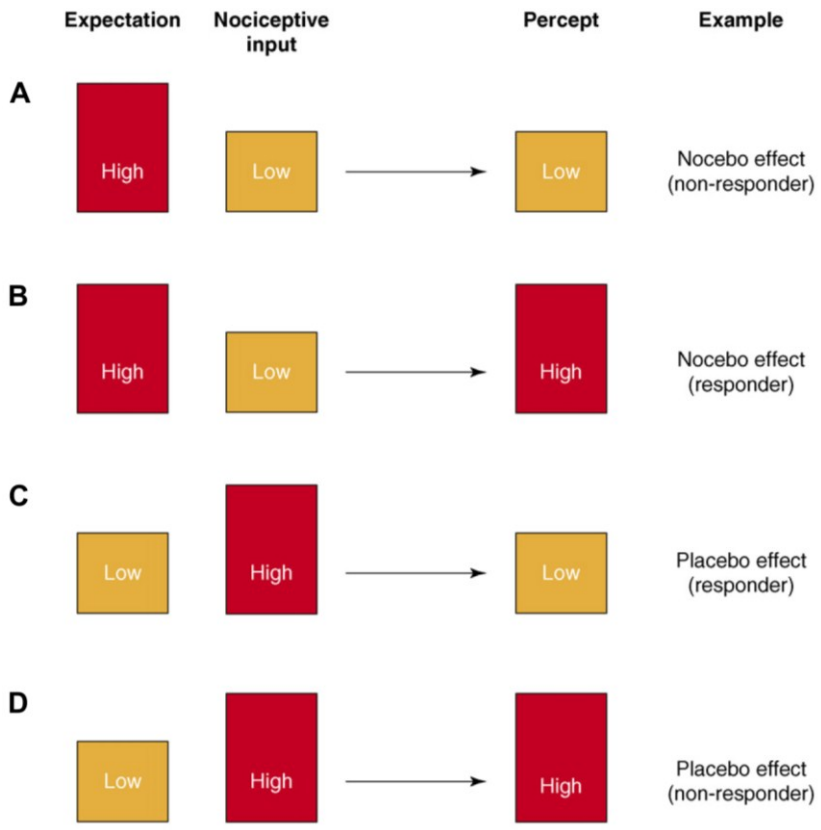

Figure 1.15 –Violation of expectations and possible consequences. In an experimental setting, pain-intensity-related expectations can be induced by presenting the subject with cues that signal the intensity level of the next stimulus ('expectation'). The subsequently applied stimulation ('nociceptive input') can violate the induced expectation in both directions: it can either be less intense (*A* and *B*) or more intense (*C* and *D*. In both cases, the expectation can bias the perception so that subjectively rated stimulation intensity ('percept') follows the cued intensity level and is either rated as higher (*B*) or lower (*C*) than the actual nociceptive input. Alternatively, the nociceptive input can over-rule the induced expectation (*A* and *D*). An example of this pain-modulating effect of expectation is the placebo effect, that is induced by an inert substance together with the suggestion that the pain might be relieved. From (Wiech et al. 2008)

Furthermore, the role of expectation in inducing the placebo effect is further supported by the results that demonstrated a correlation between reported placebo effects and prefrontal activation involved in generating and maintaining expectations like the dorsolateral prefrontal cortex (Wager et al. 2004).

Expectations can be induced by verbal suggestion, but also by previous knowledge about the effect of a real analgesic treatment (Benedetti et al. 2003; Colloca and Benedetti 2006). The previous experiences of pain and learning are also implicated in the unconscious mechanism of Pavlovian conditioning that has been demonstrated to have an essential role in inducing an effective placebo analgesic effect. Specifically, the learning of relations between events allows a better representation and therefore prediction of environmental stimuli (Colloca et al. 2008).

First insights of the neurobiological basis of the placebo effect came from pharmacological studies that showed that placebo analgesia could by blocked by naloxone, an antagonist of opioid receptor, suggesting the involvement of endogenous opioid system in the placebo response (Benedetti et al. 2005). This hypothesis was further confirmed by functional neuroimaging experiments that have demonstrated a shared neuronal network between placebo and opioid analgesia. In particular, both opioid and placebo analgesia are associated with increased activity in the rostral anterior cingulate cortex and with an increased correlation in the activities of this cortical area and brainstem nuclei, such as the periaqueductal grey matter (Petrovic et al. 2002). Finally, using PET and a μ-opioid receptor-selective radiotracer, Zubieta and colleagues (2005) demonstrated a reduced receptor availability in several brain structures following administration of a placebo, providing a direct evidence of the involvement of endogenous opioid system in the cognitive modulation of pain perception induced by a placebo (figure 1.16).

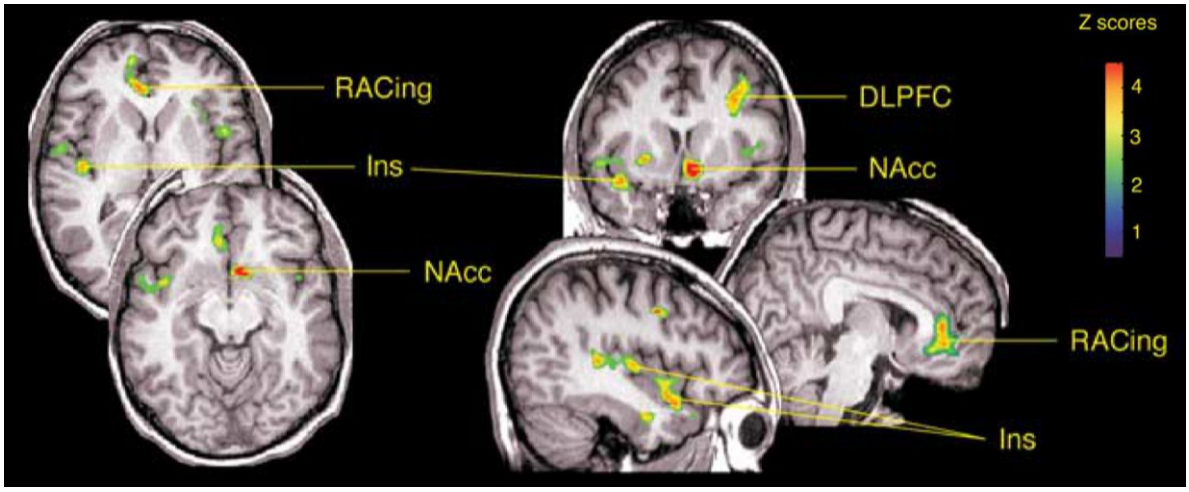

Figure 1.16 – Effects of placebo on the activation of μ-opioid receptor-mediated neurotransmission. Significant effects of placebo on the activation of the μ-opioid system were detected in the left dorsolateral prefrontal cortex (DLPFC), rostral anterior cingulate cortex (RACing), left nucleus accumbens (NAcc) and the insula. Z-scores of statistical significance are represented by the pseudocolor scale; the left side of the axial and coronal images corresponds to the right side of the body (radiological convention). *From (Zubieta et al. 2005)*

Although it has been demonstrated that psychosocial factors acts on the pain-perceptive processing through the endogenous opioid system, more recent studies have underlined the role of non-opioid mechanisms, as for example, the subject was previously exposed to non-opioid substances (Benedetti et al. 2005; Price et al. 2008). Moreover, placebo analgesia is not the only effect observed after the administration of a placebo. For example, placebo motor responses have been observed in Parkinsonian patients, after administration of a sham antiparkinsonian medicine; furthermore, cardiac and respiratory placebo responses or immunomodulatory effects have also been reported (Benedetti et al. 2005; Pacheco-Lopez et al. 2006; Price et al. 2008).

Finally, the study of the placebo effect acquire greater relevance for its clinical and ethical implications, because the use of inactive (placebo) conditions in clinical trials when effective treatments are available has created an ethical controversy (Price et al. 2008). However, it has been well documented that placebo effects can obscure those of active conditions, even for treatments that were eventually demonstrated to be effective. Thus, placebo effects may represent points of either strength or vulnerability for the expression and maintenance of various pathological states and their inherent therapeutic interventions (Benedetti et al. 2005).

2 - AIMS OF THE THESIS

This thesis is mainly aimed to present and discuss the results of experiments conducted during the PhD course. The main context that underlies the studies here presented is the investigation of cognitive processes involved in pain sensation and its modulation, from different point of view. For this reason, the work is subdivided in three parts, each concerning the investigation of a particular aspect of pain modulation:

- I part: investigation of brain activity in a pathological case of pain sensation without incoming noxious stimulation, using functional magnetic resonance imaging. In physiological conditions, pain sensation is triggered by a noxious stimulus. However, pain sensation can be produced even in absence of a real stimulus, determining a pathological conditions often observed in chronic pain. In this sense, brain activity in a patient with phantom limb pain was investigated, both when the persistent pain was modulated by attention and during a resting state period.
- II part: evaluation of a theoretical model of pain modulation, based on Bayesian decision theory. The proposed model tries to explain how cognitive processes integrate several factors, such as the incoming noxious stimulus, contextual information and the previous experience, in order to produce the perception of pain. The hypothesis was that this integration is based on a statistical inferential process and that the nociceptive system could work as a Bayesian operator.
- III part: modulation of pain perception by external intervention: relationship between pain sensation and physical activity. Several authors have demonstrated that a stressful event, such as physical exercise, can activate pain-inhibiting descending pathways, leading to an hypoalgesia state. However recent findings in animals have also reported that high-intense exercise can produce an opposite effect of hyperalgesia. Therefore, the third study was aimed to investigate the influence of exercise on pain perception and to test whether an exhaustive exercise would be able to induce hypoalgesia, hyperalgesia or both, subsequently in time.

3 - BRAIN ACTIVITY IN PHANTOM LIMB PAIN

3.1 - Introduction

Pain has always fascinated neuroscientists for its peculiar characteristics that make it different from the other sensory modalities. One of these is its intrinsic connection with diseases and pathological conditions. In fact, pain has the fundamental function to alert the body when an event is damaging or is potentially able to hurt the organism. However, in many cases, the experience of pain persists long after its usefulness as an alarm signal and often, after the tissue damage has healed. Chronic pain in these patients is probably not directly related to their initial injury or disease condition, but rather to secondary changes including ones that occur in the pain perceptive system itself (Borsook et al. 2007). Moreover, the chronic pain is frequently associated with comorbid psychosocial disorder, such as depression and anxiety, sleep disturbances, and decision-making abnormalities which significantly diminish their quality of life (Borsook and Becerra 2011).

Given that the brain is a highly interconnected dynamical network, and that chronic pain conditions are states that are maintained over years and even a whole lifetime, it is not surprising that many studies have identified injury-induced reorganization at any level of the central nervous system (figure 3.1) and that these alterations are not only functional but may be due also to a modification of the structural organization of the nervous system (Borsook et al. 2007; Vranken 2009). Moreover, the wide variety of peripheral and central nervous system lesions corresponds to highly heterogeneous clinical conditions, that could be classified in two main categories, according to the causes of the disease: nociceptive/inflammatory pain, in which pain is associated with somatic lesions, and neuropathic pain, which is the consequence of a peripheral or central nervous system lesion. The latter represent the more challenging form, especially because of the aetiology of the dysfunction and the difficulty in the treatment. In fact, neuropathic pain still represents a clinical challenge due to the fact that it poorly responds to conventional analgesics (Dworkin et al. 2003; Vranken 2009).

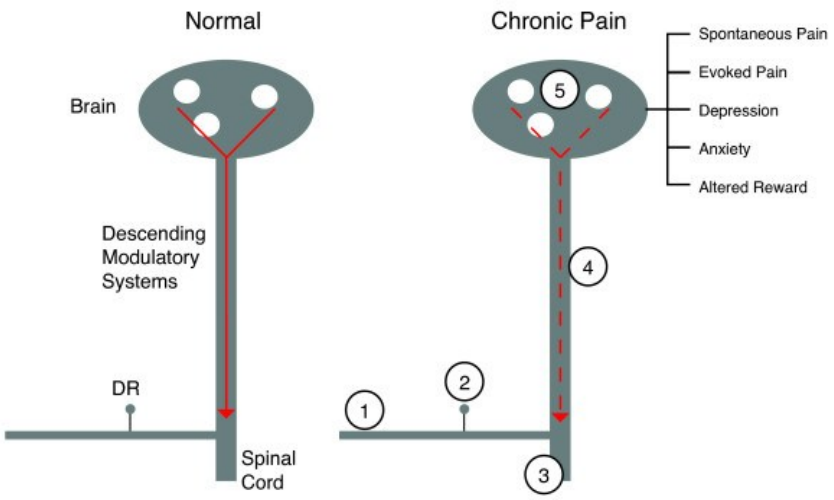

Figure 3.1 – Summary of changes in neural function that could be responsible for chronic pain. The several forms of chronic pain can be divided in two main categories: those caused by chronic activation of nociceptors (1) and those caused by damage to or malfunction of the nervous system. In these case, changes can occur along the neural axis. In the peripheral nerve there may be loss of fibers or (1), changes in function of neurons in the dorsal root ganglia (2) and alterations in the dorsal horn (3). In the central nervous system, there are changes in dorsal horn (non-afferent inputs) as well as changes in sensory processing (e.g., hyperalgesia (3 and 5)) and emotional processing (5). These changes also result in an alteration of descending modulatory influences (4). The constellation of changes in peripheral and central nervous systems result in changes to the pain intensity during spontaneous and evoked pain, and emotional processing (e.g., altered reward or hedonic state) that may lead to changes in comorbid disease in chronic pain (e.g., depression or anxiety). *From (Borsook et al. 2007)*

Neuropathic pain is a multidimensional clinical entity probably mediated by a number of different pathophysiological mechanisms. The heterogeneity of neuropathic pain syndromes is apparent from the clinical examination of patients who may present with various painful symptoms (figure 3.2), including spontaneous pain and evoked pain that, in some case, can be even more distressing than spontaneous pain (Moisset and Bouhassira 2007).

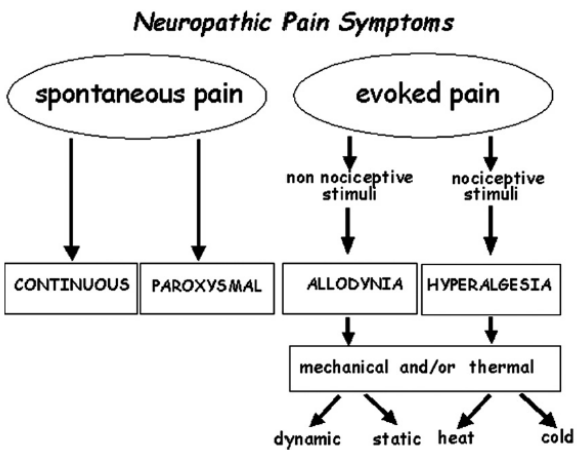

Figure 3.2 – Multiple symptoms in patients with neuropathic pain. The patients may present with various combinations of spontaneous and evoked pains which may depend on and different mechanisms. Evoked pain includes both allodynia and hyperalgesia induced by various types of mechanical and thermal stimuli. *From (Moisset and Bouhassira 2007)*

Despite this heterogeneity, the brain represents the final common pathway in behavioural responses to pain, both conscious and unconscious, therefore understanding and disentangling

the cerebral mechanisms of chronic pain could be of great help in characterizing and treating the different types of chronic pain. Non-invasive brain-imaging techniques, such as functional magnetic resonance (fMRI) and positron emission tomography (PET) can help to understand the processing of pain in normal and pathological conditions, hopefully improving the success of clinical interventions (Borsook et al. 2007; Borsook and Becerra 2011).

Given the success in identifying a unique, fairly reproducible, brain activity pattern for painful stimuli in normal subjects, an approach early used in the attempt to characterize clinical pain states is the comparison of brain activity between chronic and physiological pain, with the aim of understanding whether these conditions show the same or different patterns of brain activity (Apkarian et al. 2005; Borsook and Becerra 2006; Friebel et al. 2011). For example, Moisset and collaborators (2007) recently reviewed neuroimaging studies that specifically investigated, both with fMRI and PET, the changes in brain activity associated with brush-evoked allodynia, a condition frequently associated with spontaneous pain in patients, but still poorly understood. The authors underlined that allodynia was predominantly associated with changes in the lateral system, relating to sensory discriminative aspects of pain, rather than the medial system, which relates to the affective-emotional dimension of pain (medial thalamus, ACC and insula). In fact, many of the studies taken into account that investigated the regional cerebral differences between brushing the painful area and rest, in patients with unilateral chronic pain, showed an increased brain response in structures of the lateral pain system, such as the lateral thalamus, primary and secondary somatosensory cortices (SI and SII, respectively) or the posterior insula. Activation of medial thalamus, anterior cingulate cortex (ACC) and insula were observed in few of them. Moreover, together with other authors (Borsook and Becerra 2006; Wiech et al. 2008) the review of Moisset and co-workers highlighted the role of the prefrontal cortex (Brodmann areas 44, 45 and 46) in brush-evoked allodynia. This result is similar to that showed by Apkarian and colleagues (Apkarian et al. 2005) in their meta-analysis, demonstrating that the prefrontal cortex is the cortical domain most frequently involved in chronic pain condition (detected 81% of the times in clinical conditions, 55% in normal subjects), therefore suggesting the participation of the cognitive/evaluative dimension of pain. These studies indicate that acute physiological pain and neuropathic pain may have distinct although overlapping brain activation patterns, but that there is not a unique “pain matrix” or “allodynia network” (Moisset and Bouhassira 2007).

Our knowledge about the mechanisms involved in spontaneous pain is still far from being clear, and methodological limitations make it difficult to study such processes *in vivo* in humans, even with common neuroimaging techniques. For this reason, only in recent years

results have been published on the brain processing in spontaneous chronic pain. A first attempt to shed light on brain activity in spontaneous pain come from the study of Baliki and colleagues (Baliki et al. 2006). They applied the general linear model analysis, commonly used for analysing fMRI data acquired in block-design experiments, to investigate the brain activity during two conditions of the spontaneous pain in the chronic back pain patients: spontaneous pain perceived at a sustained high level and during the increasing in the intensity of the spontaneous pain increased (figure 3.3).

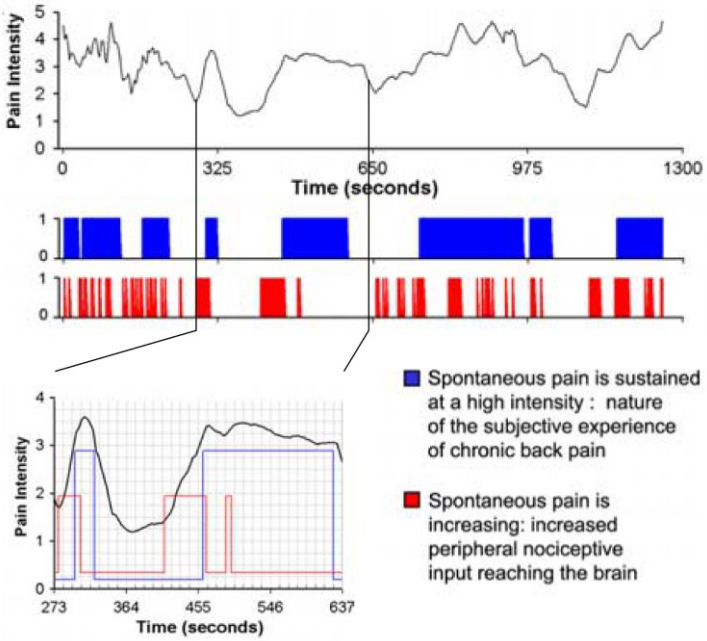

Figure 3.3 – Two distinct phases of spontaneous pain can be separated on the basis of the pain ratings of chronic back pain patients. Example rating of spontaneous fluctuations of pain in one CBP patient. The panel shows two ideal signals derived from such ratings to search for brain activity BOLD signal. The first signal (blue) corresponds to time periods when the subjective rating of spontaneous pain intensity is high, whereas the second one (red) corresponds to time periods where ratings are rapidly increasing, which may correspond to increased nociceptive input to the brain. The bottom panel shows relationships between the three time-curves at a higher time resolution. *Modified from (Baliki et al. 2006)*

With such approach they found that, during high sustained pain, brain activity increased in regions known to be involved in negative emotions and detection of unfavourable outcomes, such as the medial prefrontal cortex and the rostral anterior cingulate. On the other hand, during the increasing phase of chronic back pain, they found a significant and transient increase in cortical domains commonly observed to be activated in acute pain (figure 3.4).

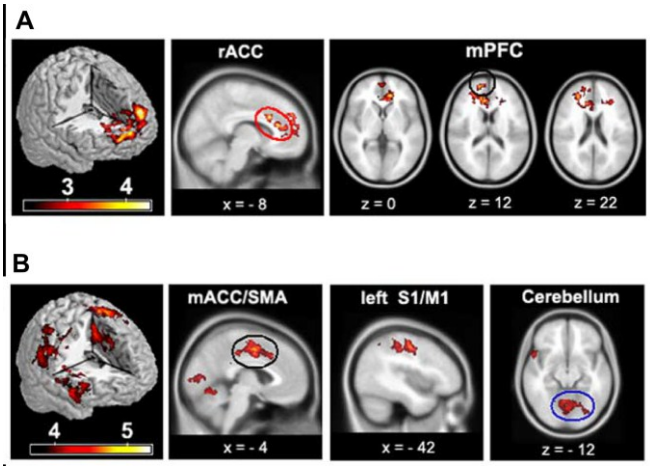

Figure 3.4 – Brain activity for spontaneous chronic back pain in two distinct phases. The regions in which the activity significantly increased during high intensity sustained pain are shown in *A*, whereas those significantly active compare to rest during the increasing phase are shown in *B*. *Modified form (Baliki et al. 2006)*

These findings therefore suggests that the emotion/affective dimension of pain was mainly involved during sustained spontaneous perception, whereas the increasing phase was principally related to the activity in the sensory/discriminative pain areas. Moreover, the authors also suggested that there was a shift in perceptual characteristics in the experience of pain and that perception became dominated by cognitive evaluations of the condition, with decreased emphasis on its sensory properties, when the pain was perceived at a sustained high level (Apkarian 2004).

Changes in the functional relationship among cortical structures have been hypothesised to be responsible for spontaneous chronic pain. Following this idea, two independent studies have been published in 2010 regarding the functional organization during the resting state in the brain of chronic pain patient. These studies demonstrated that the modular organization of the brain is disrupted in chronic pain patients. Specifically, the dynamics of the default mode network (Fox and Raichle 2007) were changed by long enduring pain, and pain-related areas, such as the insular cortex, were found to be significantly involved in cerebral activity during the resting condition (Tagliazucchi et al. 2010) demonstrating the impact of long-lasting chronic condition on the brain function.

Finally, together with the functional impairment within pain-processing areas, some authors have also highlighted that the mechanisms beneath the chronic perception of pain could involve anatomical changes, such as neurodegeneration and atrophy in the neuronal substrate of the pain processing system, and that the duration of the progression of the neuroanatomical modification could be related to the duration of the chronic condition (Apkarian 2004; Kuchinad et al. 2007; May 2011). One of the first examples on this issue comes from a study by Apkarian and colleagues (2004). They have investigated the neurodegenerative changes in the brain of patients affected by chronic back pain, using voxel-based morphometry to measure the loss of cortical grey matter. In their study, the authors observed regional specific morphometric abnormalities, highlighted by the significant grey matter density reduction in the dorsolateral prefrontal cortex and the thalamus, two key regions in pain perception processing and in its control by executive higher-order functions (figure 3.5A). Using the same technique, another study investigated the degree of grey matter atrophy in patients affected by fibromyalgia and observed a significant reduction in cingulate, insular and medial frontal cortices, and in parahippocampal gyri (figure 3.5B). Moreover, the loss in grey matter correlated with the number of years since the diagnosis, further supporting the hypothesis that the pathological disorder had a relation with the neuronal dysfunction (Kuchinad et al. 2007).

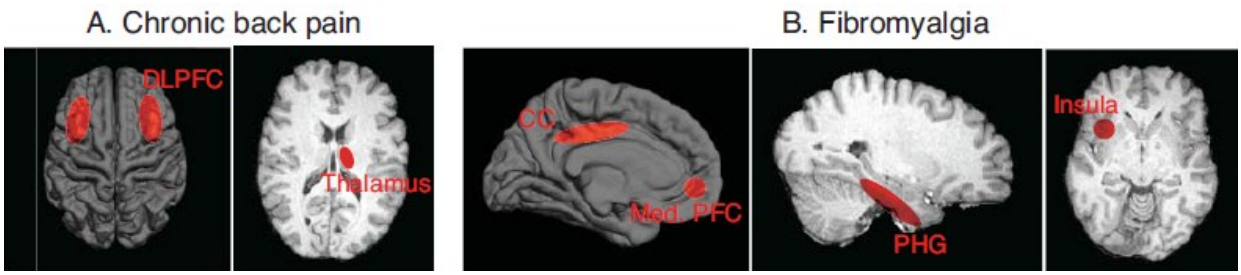

Figure 3.5 – Structural changes in the brain in patients affected by two different chronic pain pathologies. Red circles highlight significant decreases in grey density relative to healthy subjects. The brain structures are abbreviated as follows: CC, cingulate cortex; DLPFC, dorsolateral prefrontal cortex; Med. PFC, medial prefrontal cortex; PHG, parahippocampal gyrus. *From (Borsook et al. 2007)*

However, it is still debated which is the direction of the dependency between chronic pain and the neuronal degeneration, and it is not unlikely that these changes are the consequence and not the cause of the painful syndromes. In fact, they may reverse once the pain is adequately treated. Moreover, structural changes of the brain may not be specific to a particular pain syndrome and only mirror the magnitude or duration of the pain suffered (Baliki et al. 2011; May 2011).

In conclusion, chronic pain is a complex pathological state that has correlates at the cortical level, and is associated with cortical reorganization and perhaps even neurodegeneration. Furthermore, neuroimaging data relating to neuropathic pain tend to confirm that acute physiological pain and clinical pain are associated with different, but overlapping patterns of brain activation.

3.2 - Material and methods

The first study described in this thesis was performed with a patient affected by phantom limb syndrome and a painful sensation in the missing limb. The patient was a 41-year-old Italian male whose right leg was amputated above the knee 4 years before the experiment. The study was aimed to identify brain areas involved in modulation of the persistent pain and in the ongoing processes that characterized this chronic condition.

3.2.1 - Description of the patient

The clinical history of the patient started in 1989 when he referred for the first time sciatic pain in the right inferior limb. In 1990, a magnetic resonance imaging (MRI) scanning allowed to diagnose a right sacro-ischiatic neurogenic neoplasia (schwannoma) that was subsequently removed with the damaging of sciatic nerve. After the surgery, the patient reported a reduction in the strength and the sensation with a right sciatic distribution, but no pain. However, after about one year, the sciatic pain in the right leg restarted due to the reactivation of the neoplasia and therefore the patient underwent a second surgery to remove the renewed tumoral tissue. Immediately after the surgery, a new type of pain was perceived in the right foot, that the patient described as “needles scratching the external part of the right foot”. Together with this ongoing painful scratching sensation, the patient referred periods lasting for 10-30 min in which the pain increased in intensity and distribution, extending from the tip of the foot to the posterior part of the thigh. At the beginning, the scratching sensation was periodical and perceived each second day.

Since the first attempt to remove the tumour, the patient had undergone several surgeries (about 10) to progressively reduce the neoplasia, because it continuously regrow in what remained of the sciatic nerve in the thigh and the calf. At the same time, the patient had also been treated with interleaved cycles of radiotherapy and chemotherapy (Adriamycin and Holoxan).

In June 2002, a transfemoral amputation was performed to the right leg. After the amputation the patient still referred to perceived the missing foot (phantom limb) and the painful scratching sensation in it (phantom limb pain). In October 2002 a temporary prosthesis was applied to the stump, while a permanent one was implanted in April 2005. The patient regularly used the prosthetic leg 4-5 hours in the morning and then he removed it due to fatigue and movement difficulties (lack of muscular strength). The prosthesis did not ameliorate the phantom limb, though it reversed the abnormal positions that the phantom limb takes.

In March 2006, the patient voluntary underwent the experiment described in this thesis, in order to possibly improve the knowledge about the brain processes involved in phantom limb pain. Until that moment, no changes in the phantom sensation had been occurred and the pain was still described as "needles that scratched the superior-lateral border of the right foot, producing a nuisance sensation" quantified as 2 in a 0-to-10 verbal scale. The intensity of this sensation followed daily fluctuations: usually quantified as 2 in the morning, it increased to 6-8 in the afternoon and the evening. The phantom pain was not stable in subsequent days, but it increased for 1-2 days and then decreased in the following 2-3 days, reaching a minimum pain scoring that normally was not below 1/10 (figure 3.6). Moreover, this "scratching perception" was exacerbated by peaks of pain at high intensities (8-9/10) lasting for 10-30 minutes. Two pieces of the patient's descriptions of the phantom limb pain are reported in box 3.1 and box 3.2.

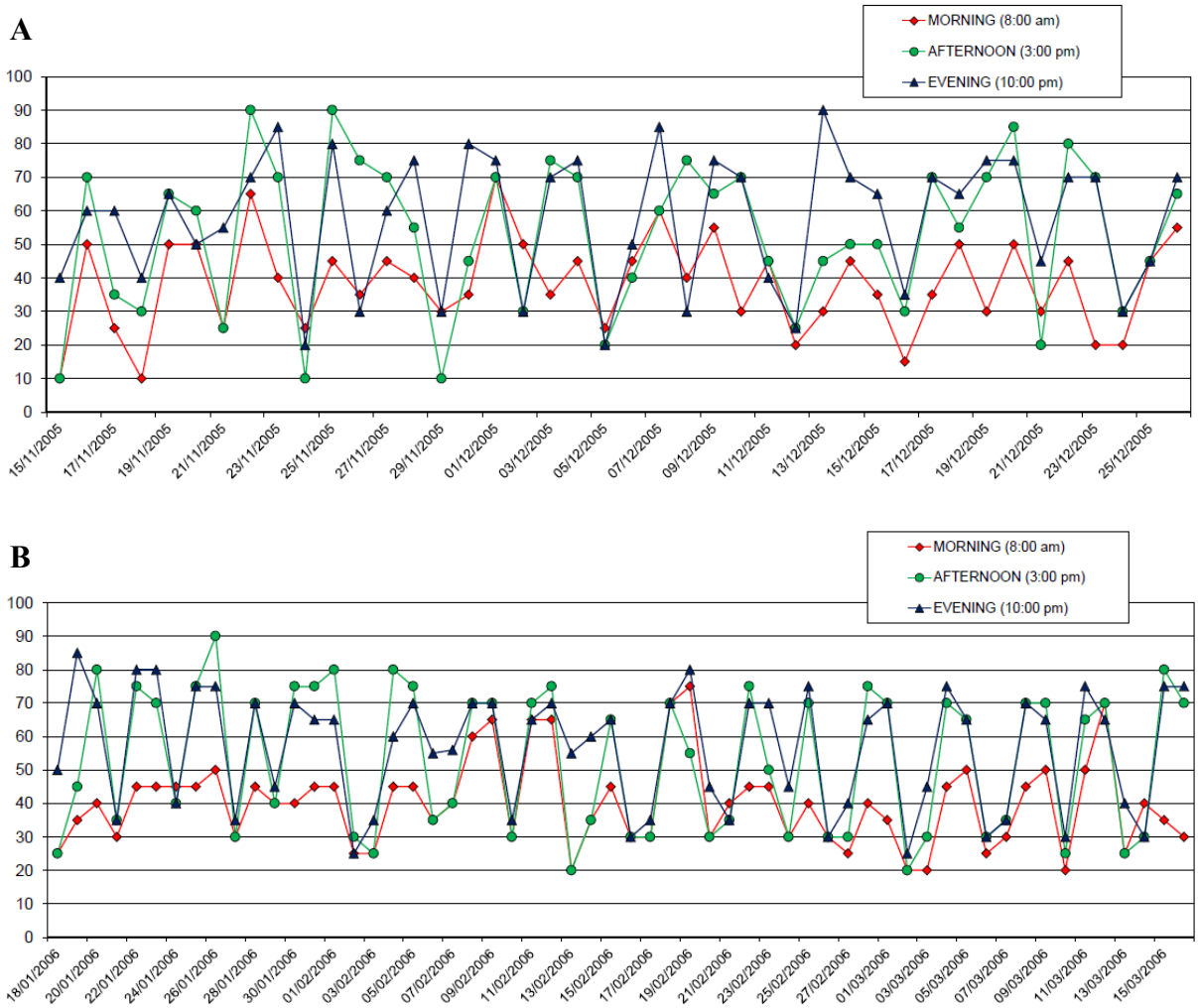

Figure 3.6 - Pain perception reported by the patient in subsequent days. In two separated periods (A and B) the patient scored the ongoing "scratching pain" in the right phantom foot, using a 0-to-100 scale. The rating was performed each day at 8:00 am (red, ◊) , 3:00 pm (green, ○) and 10:00 pm (blue, Δ).

Exogenous factors could modify the pain intensity in the phantom foot, although the patient referred he was not able to identify factors or situations that systematically increased or reduced the phantom perception. Low temperatures could reduce the "scratching perception" in the stump, but not the "bursting pain". In addition, fright or an unexpected event, such as being afraid of falling, could worsen the pain for few seconds. At rest or in absence of distracting stimuli, the pain could sometimes increase, while the "bursting pain" could became worst, indicating a fever state. Movements, such as massages of the stump or the involuntary rhythmic movement of the left leg, were able to reduce the phantom perception. Finally, the light touch of the stump with clothes or sheets could not evoke pain.

At the moment of the experiment, the patient was pharmacologically treated with Laroxyl 7gtts at the evening and Contramal 20-25 gtts when needed (sometimes substituted by Co-efferalgan). When the "bursting pain" became more frequent and lasted for a longer period, Toradol 1fl could ameliorate the intolerable perception.

Box 3.1 – First description of the pathological pain condition, reported by the patient when he introduced himself the first time.

(...)
Le espongo brevemente il mio caso: ho subito una amputazione transfemorale dell'arto destro a causa di un neurofibrosarcoma del nervo sciatico. In precedenza ho subito diversi interventi di sezionamento di tale nervo che mi hanno lasciato uno "strano" dolore localizzato sul piede dell'arto interessato, dolore che non è scomparso con la definitiva amputazione dell'arto malato. La particolarità di questo dolore è che non è continuo ma presenta una certa cadenza periodica comparendo più o meno acuto per alcuni giorni e scomparendo per altri.
Le cure a base di antidolorifici o antiepilettici associati a Garbapentin non migliorano sensibilmente il quadro doloroso. I terapisti del dolore allargano le braccia di fronte al mio caso in quanto dolori di questo tipo, assimilabili al tipico dolore da arto fantasma, sono estremamente difficili da combattere.
(...)
Dimenticavo...
Ho 41 anni, il dolore si è presentato per la prima volta nel 1991 e l'amputazione è avvenuta nel 2002.
(...)

Box 3.2 – Patient's description of the missing inferior limb and the phantom pain.

(...) la sensazione dolorosa è indipendente dall'uso della protesi, il fenomeno si è presentato a seguito di un intervento nella regione glutea, nel corso del quale è stato interrotto il percorso del nervo sciatico senza compromettere ulteriormente l'arto inferiore. Successivamente, dopo innumerevoli interventi, chemio e perfino una perfusione antiblastica si è deciso di sacrificare la gamba destra. Ma il dolore non ha subito modifiche a seguito dell'amputazione. La protesi ha modificato, in positivo, la sensazione spaziale dell'arto, in altre parole normalizza la posizione percepita della gamba che in assenza di protesi a volta sembra sfidare la leggi dell'anatomia assumendo posizioni improbabili. Per quanto concerne la periodicità del dolore le posso dire che non vi è una regola; il dolore compare di solito la mattina, tende ad aumentare durante la giornata raggiungendo i picchi nel primo pomeriggio e la sera. La cosa si ripete per uno, due giorni, poi il dolore scompare per altri due, tre giorni. Ho cercato in tutti i modi un nesso a tale fenomeno ma le assicuro che non ho trovato dei particolari comportamenti che inducono la comparsa, e di conseguenza la scomparsa, del dolore. Attività particolari, alimentazione, stati di ansietà o stress, niente di niente...
Il mio cruccio è capire non tanto il motivo per cui compare il dolore, ma perché esso scompare...

Il dolore si localizza sul lato esterno del piede immaginario destro come se tanti lunghi spilli penetrassero nella pelle. A volte il dolore è così forte che mi impedisce addirittura di pensare.
(...)

3.2.2 - Description of the experimental sessions

In order to investigate the brain activity in the patient affected by phantom limb pain, a functional magnetic resonance imaging (fMRI) protocol comprising three scanning sessions was chosen. The patient underwent it in three subsequent days characterized by different intensities of persistent pain. In Day1 (3:00 pm) he referred a low level of pain sensation (8 on a 0-to-100 verbal scale), whereas in Day2 (7:00 pm) the patient reported a much higher pain level (80 on the verbal scale). The third day, the intensity of pain changed during the execution of the experimental protocol. In fact, in Day1 and Day2, the same pain scoring was referred by the patient before and after the MRI acquisition, on the other hand, in Day3 he reported different pain ratings, meaning that the ongoing "scratching" pain in the missing limb did not remain stable during all the scanning session. For this reason, the data acquired during the third day were excluded by the subsequent analyses.

Each scanning session included three fMRI scans (figure 3.7), that were performed during three conditions: two imagination tasks, each organized in a block design, and a resting-state protocol. In the first task, the patient had to imagine a high pain in his phantom foot (pain imagination task), in particular he reported that performing this task made his pain increase to a higher level, similar to that reported in Day2. In the subsequent task he performed a visual imagination task (distraction task): he thought about something pleasant, in order to move away his attention from pain; he reported that performing this task reduced the pain sensation. Finally, in the last part of the experiment, fMRI images were acquired during a typical brain resting state protocol (Fox and Raichle 2007), in which the subject was lying in the scanner

and asked to not concentrate on specific and recurrent thoughts, but keep his mind as blank as possible, eyes closed and avoid falling asleep. This protocol was chosen in order to investigate the neuronal processes at rest, therefore when the brain is active in the absence of explicit input or output.

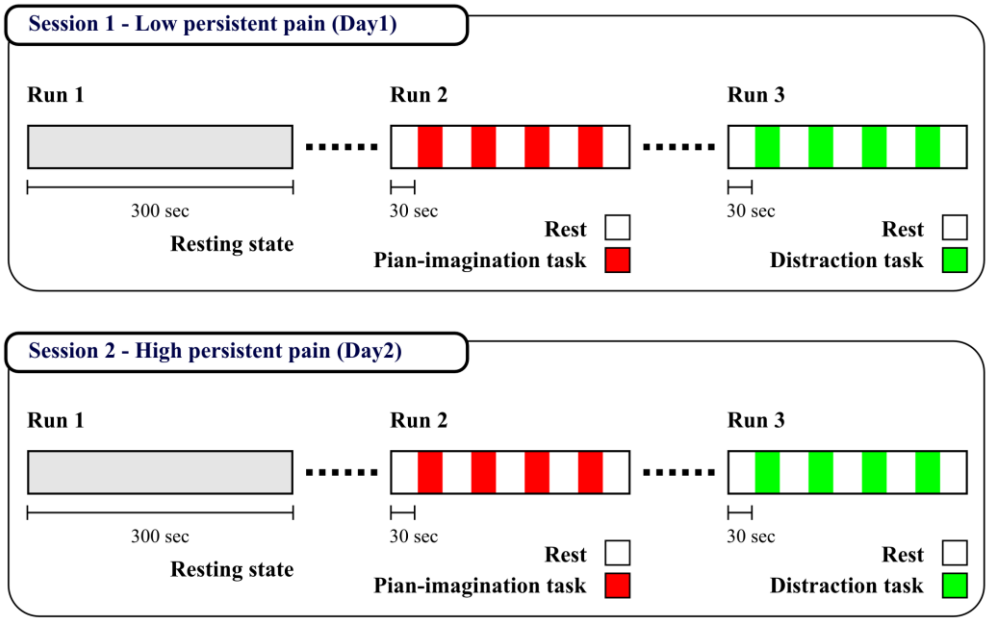

Figure 3.7 – Descriptive representation of the experiment performed by the patient with phantom limb pain. The experiment was composed by two scanning session, performed in two subsequent days. In particular, the patient referred a low persistent pain in Day1, whereas a much higher pain intensity was referred in the following day (Day2). The two sessions comprised the same acquisition protocol: during the first run, the BOLD fMRI signal was acquired when the patient laid at rest, the second run was carried on when the patient performed a pain-imagination task, in which he had to imagine the highest pain level in the phantom foot, finally the patient performed a distraction task in the third run, imagining a pleasant situation. Both tasks where organized in a block design in which task intervals were alternated with periods of rest.

3.2.3 - Acquisition of magnetic resonance data

All imaging was performed on a 3-T magnetic resonance scanner Philips Intera. Functional data were collected using an echo-planar sequence sensitive to BOLD contrast [echo time (TE) = 35 ms, flip angle = 90°, volume repetition time (TR) = 3000 ms]. Whole brain coverage was obtained with 30 axial slices (thickness = 3.75 mm, gap = 0.5 mm), with a 64 × 64 matrix and a field of view (FOV) of 230 mm x 230 mm x 127 mm, resulting in an in-plane resolution of 3.6 × 3.6 mm. Structural data included a high-resolution (1 × 1 × 1-mm voxels) axial T1-weighted (TR = 9.9 ms, TE = 4.6 ms, flip angle = 8.0°) acquisition at the end of the functional sessions of each days, while a T2-weighted scan was performed in Day1 (0.4 × 0.4 × 3.75-mm voxels, TR = 9.9 ms, TE = 80.0 ms, flip angle = 90.0°).

3.2.4 - General linear model analysis

Blood oxygen level-dependent (BOLD) fMRI time series obtained during pain-imagination and distraction tasks were prepared and fully analysed using free available the FSL package (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Before applying general linear model analysis, fMRI signals were pre-processed in order to remove artefacts and confounding signals. Pre-processing were carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98 and included the following steps:

- rigid body correction for interframe head motion within runs using MCFLIRT (Motion Correction using FMRIB's Linear Image Registration Tool). This step provided a record of the six head movement parameters (translation and rotation both on x, y and z axes) within all fMRI runs, subsequently added in general linear model analysis as confounding variables;
- spatial smoothing using a Gaussian kernel of FWHM 5mm;
- grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor, to yield a whole brain mode value of 1.000;
- high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=50.0s).

fMRI signals were linearly modelled on a voxel-by-voxel basis using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al. 2001). Each of the four condition considered in the study (Day1_dol, Day2_dol, Day1_piac and Day2_piac) were analysed separately, obtaining four statistical parametric maps in which positive and negative statistically significant modulation in BOLD activity were displayed.

Figure 3.8 shows a representative design matrix applied to identify significant clusters. Due to the fact that a block design was used, the ideal signal was obtained by the convolution of a Gamma variate (a normalization of the probability density function of the Gamma function), as haemodynamic response function (HRF), and a box-car signal, where rest and task were respectively represented as 0 and 1. A cluster-based correction of the Z (Gaussianised T) statistic images was performed and thresholded at z scores > 3.1. For each resulting cluster of spatially connected voxels surviving the z threshold, a cluster probability threshold of $p = 0.01$ was applied to the computed significance of that cluster, which corrects for multiple comparisons (Friston et al. 1994; Worsley 2001).

Two contrasts were used to identify significant activations and deactivations in task execution compared to rest period, respectively C1 (Imag) and C2 (-Imag) in figure 3.8.

Registration to high resolution structural and standard space images was carried out using FLIRT (FMRIB's Linear Image Registration Tool) (Jenkinson and Smith 2001; Jenkinson et al. 2002).

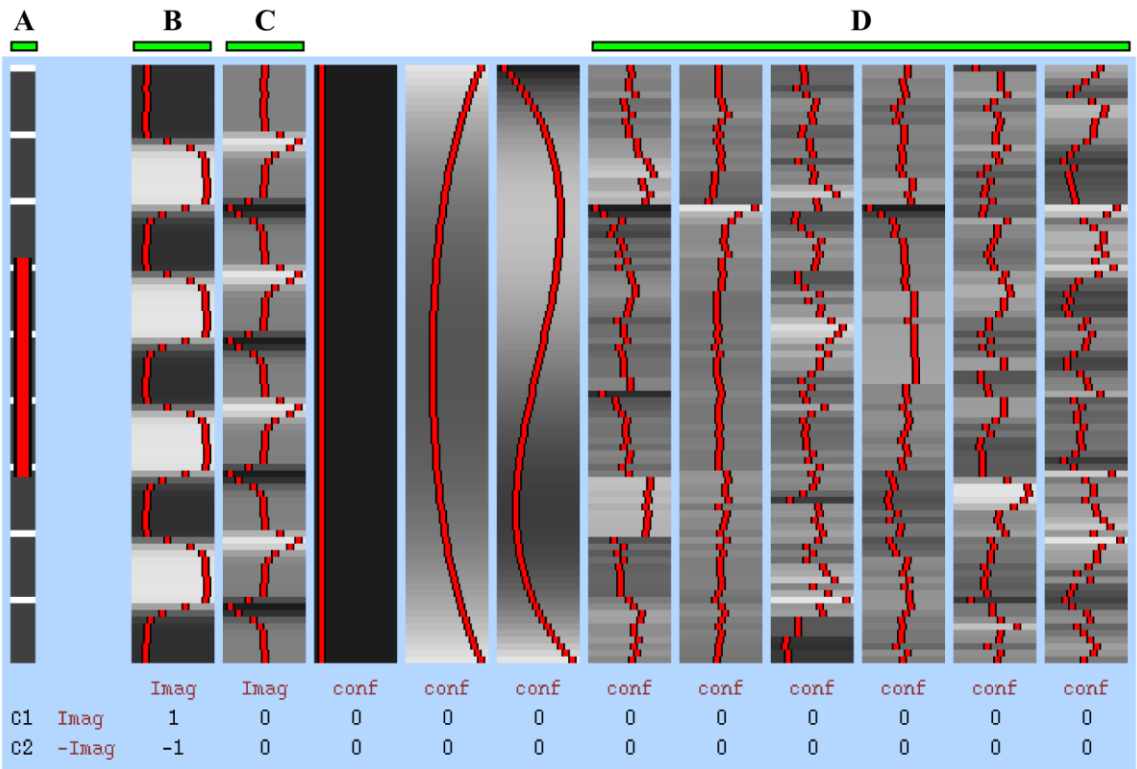

Figure 3.8 – Example of design matrix used in the first-level analysis. The first column (*A*) shows the properties of the high-pass temporal filter, the second one (*B*) is the ideal signal explaining the time course of the BOLD fMRI signal during the task. All the other columns show confounding factors added to improve the fitting of the general linear model. In particular, slice time correction is shown in the third column (*C*), whereas the last six columns (*D*) shows the six parameters of motion artefacts obtained by rigid body correction of head motion.

Conjunction analysis (Nichols et al. 2005) was performed to investigate brain areas that were jointly modulated above the significant threshold. The analysis was carried out with FSL and the script 'easythresh_conj' developed by Dr Thomas Nichols (http://www2.warwick.ac.uk/fac/sci/statistics/staff/academic-research/nichols/scripts/fsl/).

Z-statistic images were thresholded at Z-scores > 3.1 and a cluster probability threshold of p = 0.01 was applied for multiple spatial comparisons according to Gaussian random field theory (Friston et al. 1994)

3.2.5 - Correlation analysis

The fMRI data acquired during the resting-state runs in Day1 and Day2 (see figure 3.7) were analysed computing the Pearson's r correlation coefficient between the time courses of three seeds regions and all other brain voxels' time series, following a procedure modified from Fox et al., (2005)

At first, motion artefacts were removed from BOLD signal through linear regression with the six parameters obtained by body correction of head motion using the FSL MCFLIRT package. Subsequently, after this pre-processing step, data were analysed according to the following procedure:

- band-pass temporal filtering (0.01-0.08 Hz);
- extraction mean signal from a volume of interest (see seeds description reported below);
- computation of Pearson's r correlation coefficient between seed's time course and all other brain voxels' time series.

These three steps were performed using the 'InstaCorr' module (Cox, R. W. 2011) available in the imaging software AFNI (Cox, R. 1996; Cox, R. W. and Hyde 1997), freely available on http://afni.nimh.nih.gov/afni/.

Seed regions were 10-mm-diametr spheres centred in three previously published foci (Fox et al. 2005; Baliki et al. 2008), specifically in the medial prefrontal cortex (mPFC; standard coordinates -1, 47, -4), posterior cingulate cortex/precuneus (PPC; standard coordinates -5, -49, 40) and lateral parietal cortex (LP; standard coordinates: -45, -67, 36). Therefore three correlation maps were obtained from resting-state data recorded in Day1 and Day2. Figure 3.9 shows the position of the selected seeds on the high-resolution T1-weighted structural image.

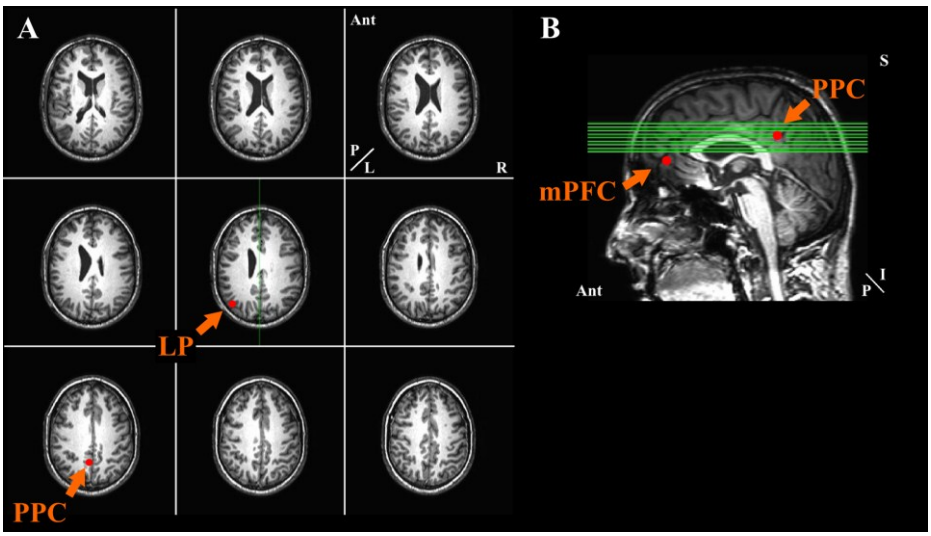

Figure 3.9 - The three seeds chosen to investigate functional connectivity during the resting-state. The time series of three seeds were taken for the voxel-wise correlation analysis applied to BOLD fMRI signals recorded during the resting-state protocol in both days. Specifically, the seeds were spheres with a diameter of 10 mm, centred in medial prefrontal cortex (mPFC), lateral parietal cortex (LP) and in posterior cingulate cortex (PPC). The seeds are shown in the same axial and sagittal slices previously shown in paragraph 3.2.3 ('Functional connectivity during resting state'): nine sequential 3mm-spaced axial slices in *A* and a single sagittal slice in *B*. Anatomical coordinate terms are indicated as follow: Ant, anterior; I, inferior; L, left; P, posterior, R, right; S, superior.

n order to obtain a unique map for each day, in which voxels that significantly correlated or anticorrelated with the three seed regions would be included, the three single correlation maps were averaged and this average was masked by using the following analysis.

- In each single map, correlation coefficients were converted to normally distributed z-scores, firstly using the Fisher's transformation $z = 0.5 + \ln(\frac{1+r}{1-r})$ (where r is the correlation coefficient and z indicates the normally distributed correlation coefficient) and then transforming z in z-score dividing by the square root of the variance $z-score = \frac{z}{\frac{1}{\sqrt{df-3}}}$ (where df corresponds to the degree of freedoms in the measurement). Because BOLD's consecutive samples are not statistically independent, the degrees of freedom were corrected by a factor of 2.86, in accordance with Bartlett theory (Jenkins and Watts 1968), resulting in 100/2.86 = 34.97. degrees of freedom.
- Anticorrelation z-score maps were computed, multiplying z-score maps by -1. This step was needed by the fact that a single-tail statistical test would be applied subsequently, therefore only positive z-scores would be considered.

- Voxels that were significantly correlated or anticorrelated with all the seed regions were identified by mean of a conjunction analysis (Nichols et al. 2005) carried out with the script 'easythresh_conj' developed by Dr Thomas Nichols. Z-statistic images were thresholded at Z-scores > 3.1 and a cluster probability threshold of p = 0.01 was applied for multiple spatial comparisons.
- Finally, the thresholded z-maps of Day1 and Day2 were than used to mask the respective average correlation maps.

3.2.6 - Modelling of brain's cortical surfaces

3D models of the patient's brain surfaces were constructed, in order to facilitate the visualization of the functional regions of the highly-folded cerebral cortex. Cortical reconstruction and volumetric segmentation were performed with the Freesurfer imaging software package (Dale et al. 1999; Fischl et al. 1999; Fischl 2012), freely available for download online (http://surfer.nmr.mgh.harvard.edu/). The process was carried on the high resolution T1-weighted image acquired in Day1 and it included the removal of non-brain tissue using a hybrid watershed/surface deformation procedure (Segonne et al. 2004), automated Talairach transformation, segmentation of the subcortical white matter and deep grey matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) (Fischl et al. 2002; Fischl et al. 2004a) intensity normalization (Sled et al. 1998), tessellation of the grey matter white matter boundary, automated topology correction (Fischl et al. 2001; Segonne et al. 2007), and surface deformation following intensity gradients to optimally place the grey/white and grey/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class (Dale and Sereno 1993; Dale et al. 1999; Fischl and Dale 2000). Once the cortical models were complete, two further procedures were performed: surface inflation (Fischl et al. 1999) and parcellation of the cerebral cortex into units based on giral and sulcal structure (Fischl et al. 2004b; Desikan et al. 2006).

The results of the analyses of functional data, both task-related and resting-state, were visualized with AFNI and SUMA (Saad and Reynolds 2011). In particular, the latter is a freely available software (http://afni.nimh.nih.gov/afni/suma/) distributed with AFNI and allows viewing 3D cortical surface models and mapping volumetric data onto them.

The results of this procedure is shown in figure 3.10. Pial and white matter surfaces are highlighted respectively in green and red on axial and sagittal structural image both in figure

3.10A and in figure 3.10B, whereas the reconstructed 3D pial surface model is shown in figure 3.10C.

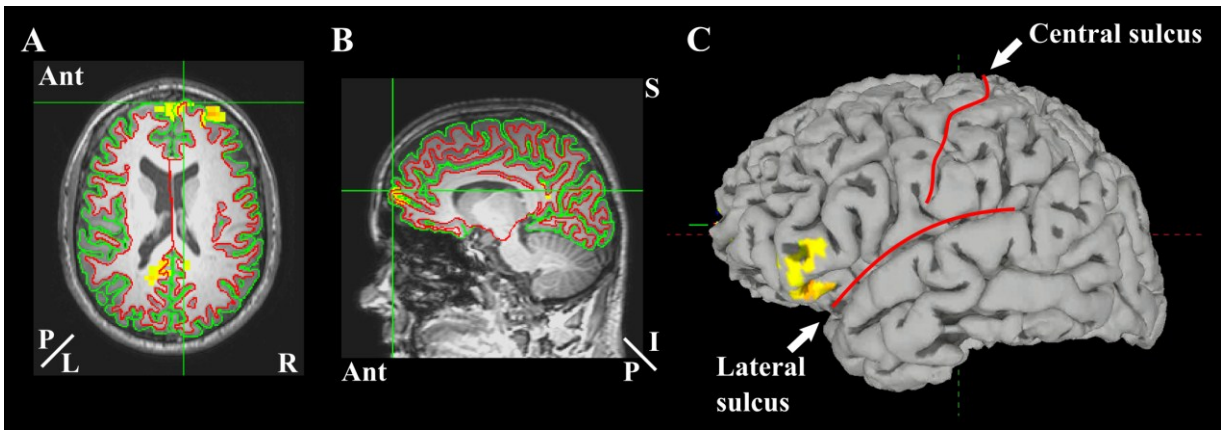

Figure 3.10 – 3D model of patient's brain surface. Freesurfer was used to create a 3D brain model from the high-resolution T1-wheighted structural images of the patient with phantom limb pain. Pial and white matter surfaces are highlighted respectively in green and red in *A* and *B*. C shows the lateral surface of the left hemisphere. Anatomical coordinate terms are indicated as follow: Ant, anterior; I, inferior; L, left; P, posterior, R, right; S, superior.

3.3 - Results

The results presented in this part of the thesis concern a study aimed to investigate the cerebral activity in a specific case of spontaneous neuropathic pain. A patient affected by phantom limb syndrome, with chronic pain in the missing limb, underwent an experiment in which his brain activity during both the execution of pain modulatory tasks and in a resting condition was investigated by means of fMRI.

3.3.1 - Regression analysis of task-related BOLD signal changes

A general linear model was used to identify the brain regions in which the cerebral BOLD signal changed during the execution of the two tasks in Day1 and Day2. This analysis was carried out in order to identify cortical areas that were positively or negatively modulated by distraction and by the imagination of a higher level of pain in the phantom foot; moreover these effects were considered in the light of the different persistent pain intensities referred during the two scanning days. A total of eight statistical parametric maps were obtained, showing regions of increased as well as decreased BOLD signals in each of the two tasks performed in the two subsequent days.

Tables 3.1, 3.2, 3.3 and 3.4 report the brain regions in which the neuronal activity were positively or negatively modulated by the tasks. For each anatomical region, the coordinates in the standard space, the number of voxels in the cluster as well as the z-score and the corresponding corrected p-value are reported. Considering the number and the extent of significant clusters, a first finding was that the two tasks had different effects in the two days, when the patient referred different intensities of persistent pain. This means that the imagination tasks were capable of different modulating effects, depending on the ongoing pain perception and the underlying brain mechanisms. In particular, when the pain perception in the phantom limb was lower and the patient focused his attention on pain, the regression analysis detected a greater extent of activations compared with those found when the pain perception was already higher (see tables 3.1 and 3.2). On the contrary, the pain-inhibiting task had a greater modulating effect in the second day than in the first day.

Table 3.1 – Significant changes in BOLD fMRI signal during pain-imagination task in Day 1

Activations							
X	**Y**	**Z**	**Anatomical localization**	**Brodmann area**	**Cluster size**	**p-value**	**Maximum z-value**
28.4	61.6	2.0	Superior frontal gyrus (R)	10	164	2.36E-26	6.55
-52.4	34.8	-14.8	Inferior frontal gyrus (L)	47	69	2.47E-14	9.44
48.5	-65.3	34.6	Parietal Lobe - Angular gyrus (R)	39	61	4.04E-13	5.81
-46.3	3.8	-41.9	Middle temporal gyrus (L)	38	35	9.88E-09	6.97
-40.6	-9.0	3.8	Insula (L)	13	29	1.19E-07	5.31
-19.5	-11.9	62.9	Superior frontal gyrus (L)	6	21	6.62E-06	4.55
-55.1	-26.9	44.3	Postcentral gyrus (L)	2	20	1.10E-05	4.52
-7.2	-45.7	70.1	Postcentral gyrus (L)	5	18	3.16E-05	4.50
-31.6	-41.1	-3.9	Parahippocampal gyrus (L)	19	17	5.43E-05	4.69
26.2	32.7	43.2	Middle frontal gyrus (R)	8	16	9.43E-05	3.87
-24.7	48.8	-1.3	Superior frontal gyrus (L)	10	15	1.66E-04	4.77
56.0	-10.8	34.4	Precentral gyrus (R)	4	15	1.66E-04	4.72
-43.4	-32.8	55.7	Postcentral gyrus (L)	40	15	1.66E-04	4.63
7.4	35.9	12.8	Anterior cingulate cortex (R)	32	15	1.66E-04	4.06
-59.0	6.5	33.0	Precentral gyrus (L)	6	14	2.95E-04	4.90
31.4	11.3	28.6	Middle frontal gyrus (R)	9	12	9.73E-04	4.43
-9.8	-71.8	51.0	Precuneus (L)	7	11	1.81E-03	4.24
6.8	-41.7	41.7	Cingulate gyrus (R)	31	10	3.44E-03	4.80
-19.4	-53.6	70.7	Postcentral gyrus (L)	7	9	6.66E-03	3.94
-20.4	-30.6	13.9	Thalamus (L)		11	1.81E-03	4.79
27.1	-54.2	-21.3	Cerebellum - anterior lobe, culmen (R)		370	0.00E+00	6.22
-46.4	-71.3	-23.0	Cerebellum - posterior lobe, tuber (L)		47	7.39E-11	5.24
-26.3	-77.7	-15.7	Cerebellum - posterior lobe, declive (L)		22	3.99E-06	5.48
Deactivations							
X	**Y**	**Z**	**Anatomical localization**	**Brodmann area**	**Cluster size**	**p-value**	**Maximum z-value**
49.7	48.8	-10.6	Middle frontal gyrus (R)	47	64	1.40E-13	7.44
47.9	1.9	-40.0	Middle temporal gyrus (R)	38	25	8.94E-07	6.55
22.8	-44.4	48.0	Paracentral Lobule (R)	5	21	6.62E-06	4.29
-47.6	-27.0	-12.4	Inferior temporal gyrus (L)	20	16	9.43E-05	6.17
35.7	12.9	-42.9	Superior temporal gyrus (R)	38	13	5.31E-04	7.79
-34.0	-14.8	-37.6	Uncus (L)	20	37	4.22E-09	7.95
35.4	-9.2	-34.0	Uncus (R)	20	15	1.66E-04	5.13
-36.6	-5.1	3.3	Claustrum (L)		10	3.44E-03	5.89

Table 3.2 – Significant changes in BOLD fMRI signal during pain-imagination task in Day 2

Activations							
X	**Y**	**Z**	**Anatomical localization**	**Brodmann area**	**Cluster size**	**p-value**	**Maximum z-value**
19.9	59.7	-15.8	Superior frontal gyrus (R)	11	26	2.98E-06	4.14
61.6	5.0	3.8	Superior temporal gyrus (R)	22	11	4.26E-03	3.80
-46.2	40.8	8.1	Inferior frontal gyrus (L)	46	11	4.26E-03	4.80
35.6	30.0	27.4	Middle frontal gyrus (R)	9	11	4.26E-03	4.18
35.4	-53.9	-31.1	Cerebellar tonsil (R)		29	8.34E-07	4.39
11.8	-90.1	-36.0	Cerebellum, pyramis (R)		12	2.44E-03	4.12
Deactivations							
X	**Y**	**Z**	**Anatomical localization**	**Brodmann area**	**Cluster size**	**p-value**	**Maximum z-value**
-45.8	-14.4	35.5	Precentral gyrus (L)	4	26	2.98E-06	4.46

Table 3.3 – Significant changes in BOLD fMRI signal during distracting imagination in Day 1

Activations							
X	**Y**	**Z**	**Anatomical localization**	**Brodmann area**	**Cluster size**	**p-value**	**Maximum z-value**
6.0	64.7	21.8	Superior frontal gyrus (R)	10	722	0.00E+00	6.76
0.4	-60.6	16.2	Posterior cingulate cortex (L)	23	1044	0.00E+00	6.59
36.3	-61.4	33.1	Angular gyrus (R)	39	155	8.05E-26	6.56
49.9	25.4	-15.5	Inferior frontal gyrus (R)	47	144	1.56E-24	6.23
-31.8	2.4	54.7	Middle frontal gyrus (L)	6	70	9.29E-15	6.30
-30.7	15.0	33.4	Middle frontal gyrus (L)	8	67	2.65E-14	6.81
-43.7	-47.2	63.2	Postcentral gyrus (L)	5	38	1.83E-09	4.93
-27.2	-20.9	54.6	Precentral gyrus (L)	4	16	7.58E-05	5.06
-7.8	17.4	-8.4	Anterior cingulate cortex (L)	25	12	8.17E-04	4.06
-56.6	-18.2	-5.0	Middle temporal gyrus (L)	21	10	2.96E-03	4.42
-64.3	-0.8	-18.3	Middle temporal gyrus (L)	21	10	2.96E-03	5.57
34.7	-35.4	51.9	Postcentral gyrus (R)	40	10	2.96E-03	4.72
0.5	-18.1	70.2	Medial frontal gyrus (L)	6	10	2.96E-03	3.79
-30.7	-13.1	-28.0	Uncus (L)	20	374	0.00E+00	6.71
12.1	15.5	2.8	Caudate (R)		25	6.56E-07	5.54
33.1	-83.9	-43.9	Cerebellum, inferior semi-lunar lobule (R)		378	0.00E+00	6.44
-25.5	-86.2	-23.6	Cerebellum, uvula (L)		28	1.79E-07	4.66
-13.1	-34.1	-42.0	Cerebellar tonsil (L)		16	7.58E-05	4.62
Deactivations							
X	**Y**	**Z**	**Anatomical localization**	**Brodmann area**	**Cluster size**	**p-value**	**Maximum z-value**
60.8	-15.8	21.4	Postcentral gyrus (R)	3	9	5.80E-03	4.42
53.1	38.9	-3.5	Inferior frontal gyrus (R)	47	9	5.80E-03	4.43

Table 3.4 – Significant changes in BOLD fMRI signal during distracting imagination in Day 2

Activations							
X	**Y**	**Z**	**Anatomical localization**	**Brodmann area**	**Cluster size**	**p-value**	**Maximum z-value**
9.3	71.3	-12.5	Superior frontal gyrus (R)	10	554	0.00E+00	6.65
-43.4	-69.6	20.2	Middle temporal gyrus (L)	39	1312	0.00E+00	7.42
-48.7	19.3	21.1	Inferior frontal gyrus (L)	9	232	1.26E-30	7.78
20.1	34.6	40.8	Middle frontal gyrus (R)	8	121	1.71E-19	6.01
-33.1	-0.7	48.4	Middle frontal gyrus (L)	6	72	1.68E-13	6.91
-0.6	15.9	52.1	Superior frontal gyrus (L)	6	46	9.30E-10	5.78
-40.3	-29.5	33.4	Postcentral gyrus (L)	2	42	3.99E-09	5.45
39.9	-22.7	-30.4	Inferior temporal gyrus (R)	20	24	5.66E-06	5.39
-32.5	61.2	10.9	Middle frontal gyrus (L)	10	22	1.39E-05	5.47
-59.2	-8.2	-18.0	Inferior temporal gyrus (L)	21	20	3.54E-05	5.63
-29.0	48.0	31.9	Superior frontal gyrus (L)	9	17	1.52E-04	4.64
67.9	-50.0	-1.4	Middle temporal gyrus (R)	21	16	2.52E-04	4.57
-21.4	34.9	53.2	Superior frontal gyrus (L)	8	10	6.77E-03	4.56
13.2	1.0	17.0	Caudate (R)		35	5.96E-08	4.91
-19.4	3.4	10.5	Putamen, lentiform nucleus (L)		23	8.82E-06	4.68
11.9	-88.8	-26.6	Cerebellum, uvula (R)		348	1.94E-40	5.93
-16.6	-60.0	-41.6	Cerebellum, inferior semi-lunar lobule (L)		49	3.21E-10	5.90
Deactivations							
X	**Y**	**Z**	**Anatomical localization**	**Brodmann area**	**Cluster size**	**p-value**	**Maximum z-value**
-7.6	-10.1	27.2	Cingulate gyrus (L)	23	56	2.90E-11	4.93
-60.4	-53.4	31.3	Supramarginal gyrus (L)	40	56	2.90E-11	5.52
62.4	-53.9	36.8	Supramarginal gyrus (R)	40	33	1.19E-07	5.04
-44.1	-4.4	15.3	Insula (L)	13	32	1.79E-07	4.93
48.1	-4.0	-37.5	Inferior temporal gyrus (R)	20	24	5.66E-06	6.39
-15.3	-63.6	34.8	Precuneus (L)	7	24	5.66E-06	4.89
29.2	65.7	3.5	Superior frontal gyrus (R)	10	23	8.82E-06	4.51
-3.3	-47.6	37.7	Precuneus (L)	31	23	8.82E-06	5.41
58.6	-2.6	10.1	Precentral gyrus (R)	6	21	2.21E-05	5.53
26.3	-83.0	25.3	Cuneus (R)	19	18	9.28E-05	4.90
-42.3	13.2	-39.4	Superior temporal gyrus (L)	38	13	1.23E-03	5.22
-65.3	-25.4	41.1	Postcentral gyrus (L)	2	12	2.14E-03	4.08
-12.7	59.9	30.9	Superior frontal gyrus (L)	9	11	3.77E-03	4.45
-11.4	45.1	0.0	Anterior cingulate cortex (L)	32	10	6.77E-03	4.98
17.0	13.0	20.2	Caudate (R)		17	1.52E-04	4.55

Brain activity during pain imagination

The cortical areas positively modulated by the pain imagination task in Day1 are shown in figure 3.11. Specifically, this task significantly activated brain areas normally described within the pain matrix (Borsook et al. 2007), such as the contralateral primary somatosensory (SI) and insular cortices, and the inferior parietal lobule, near the parietal operculum. Furthermore, attention-related cortical activations were observed bilaterally in the lateral prefrontal cortex and in the right parietal cortex. In particular, the specific role of parietal cortex in pain processing seems to be directing attention towards the painful stimulus, and this is comparable

with the shifts of visual-spatial attention to unexpected visual targets, while prefrontal cortex seems to be involved in working memory processing such as keeping information about painful stimuli on-line for further processing (Bornhovd et al. 2002).

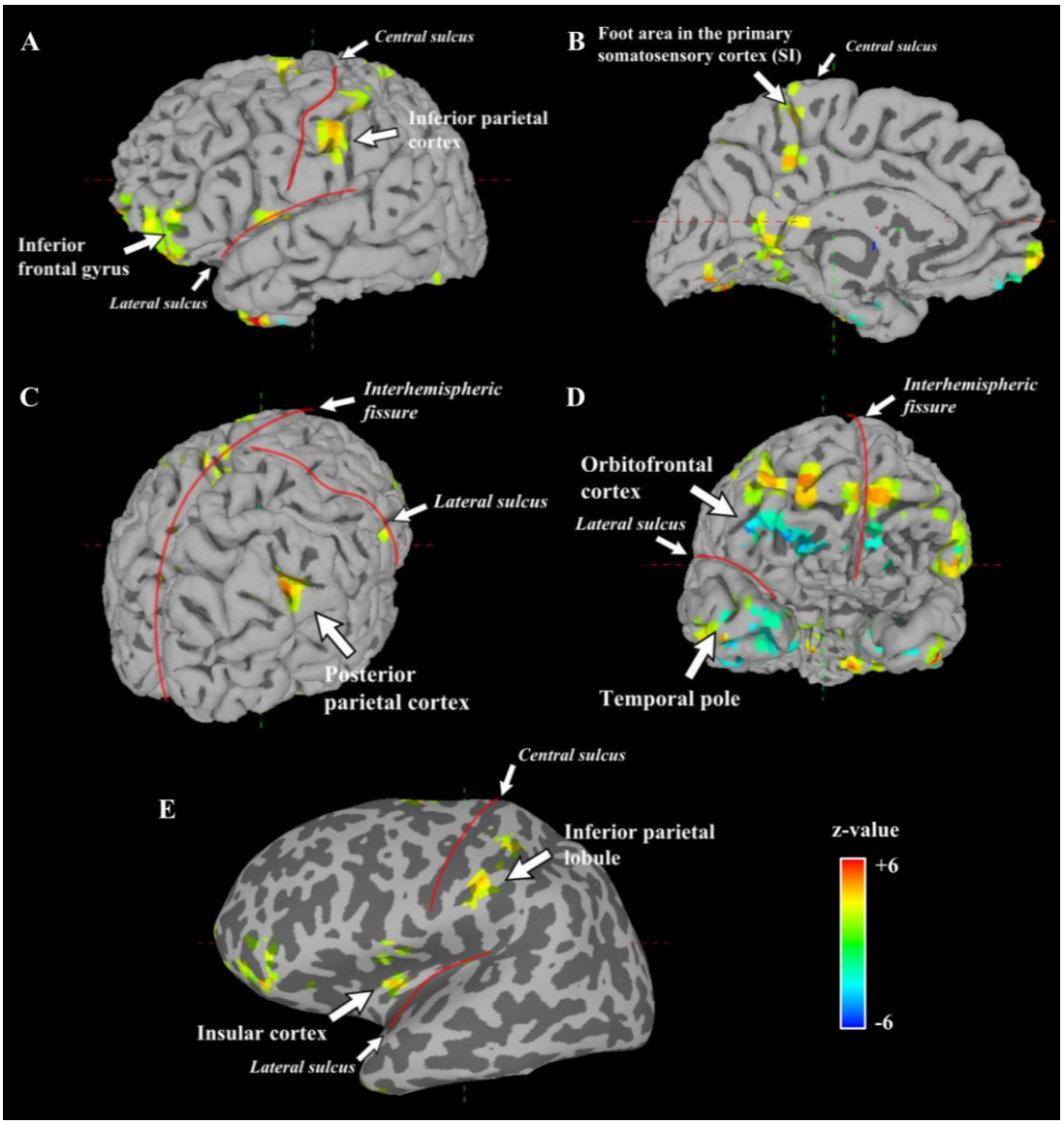

Figure 3.11 – Brain regions activated recalling high pain perception in the phantom limb. When performed with low pain at rest (Day1), the task activated cortical areas of the pain matrix as well as areas previously described as involved in attention and executive processes. *A* and *B* show respectively the lateral and medial surfaces of the left hemisphere, contralateral to the phantom foot. C and D show the activation domains respectively in right parietal and prefrontal cortices. In *E* the left hemisphere is inflated to show activations in the insular cortex; light and dark greys represent respectively circumvolutions and sulci.

Deactivations were observed mainly in the inferior part of the frontal cortex, precisely in the orbitofrontal cortex. The activity in this area was previously described as negatively correlated with the rating of experimental pain in several studies, suggesting that it is involved in pain

modulation induced by attentive tasks or in copying strategies. In particular, it has been suggested that regions like orbitofrontal cortex are involved in the activation of descending pain-inhibiting mechanisms (Petrovic et al. 2000; Bantick et al. 2002).

As mentioned before, the same task did not induce significant changes in neuronal activity in this patient in Day2, when a high pain scoring was reported at rest (see table 3.2 and figure 3.12). This finding can be explain considering the pain level and the task that the patient had to performe. The task required to focus attention on phantom pain and imagine as it was perceived at high intensity. However, the patient reported to perceive in Day2 a high level of pain already at rest, therefore the imagination of a high pain failed to significantly modulate the ongoing pain. This hypothesis is further supported be the fact that the patient referred that he was not able to consistently increase pain perception through imagination in the context of Day2.

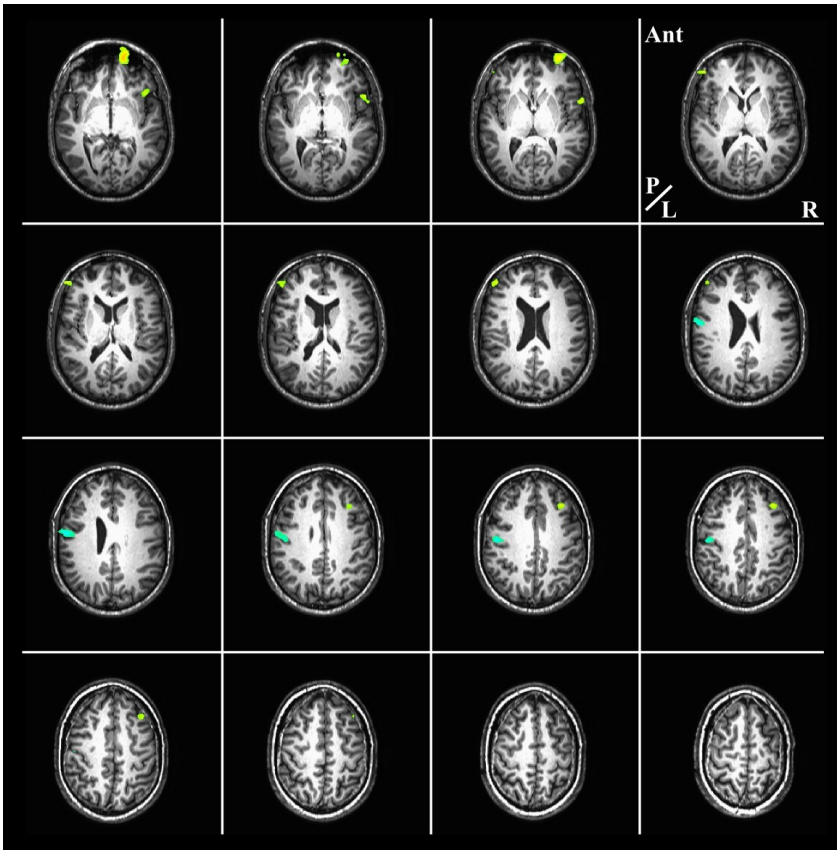

Figure 3.12 – Concentration on pain when the patient referred a high intensity of persistent pain (Day2). On the contrary to what observed in Day1, the pain-imagination task failed to modulated the ongoing brain activity when the pain intensity perceived in the phantom limb was already high. Sixteen subsequent axial slices are reported, each slice is 3-mm spaced from the subsequent and taken in an inferior-superior direction. Anatomical coordinate terms are indicated as follow: Ant, anterior; L, left; P, posterior, R, right.

Brain activity during distracting imagination

Considering the distraction task, the results obtained were similar in the two days (see tables 3.3 and 3.4 and figures 3.13 and 3.15). Regression analysis revealed activations bilaterally, in the posterior and the prefrontal cortices. In particular, when the patient moved his attention away from the persistent pain, fMRI signals increased in lateral parietal cortex, in posterior cingulate cortex and precuneus and in medial prefrontal cortex.

Task-induced deactivations were more consistent in Day2 than in Day1. These results were specular to those observed with imagination of pain, in fact in both cases the task had a greater effect when the pain intensity was such that the induced modulation could effectively change the perceived pain: the pain-increasing task was more effective when pain intensity at rest was low, whereas the pain-inhibiting task had a greater effect when a higher pain scoring was reported at rest.

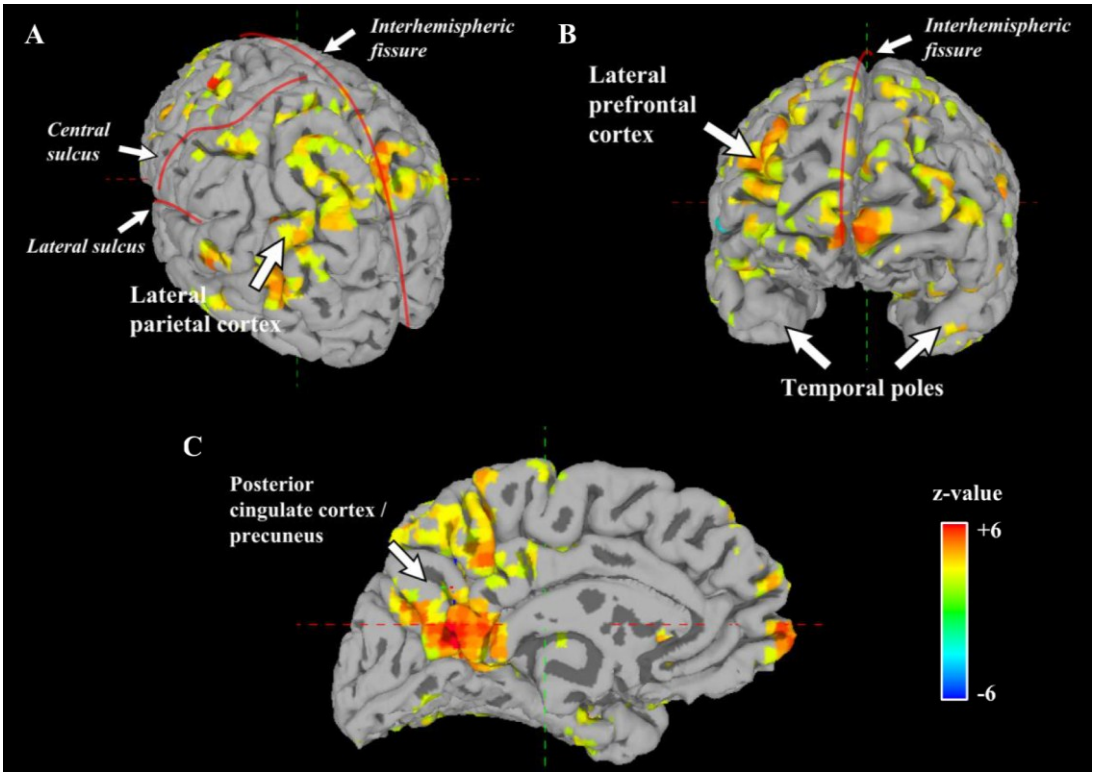

Figure 3.13 – Cortex domains activated by the distracting imagination task in Day1. The main activations were observed in posterior cingulate cortex and precuneus, posterior parietal cortex and lateral prefrontal cortex. Most of this activations are related with the processes involved in imagination and in the shifting of attention from the low-intensity pain toward the distracting thoughts.

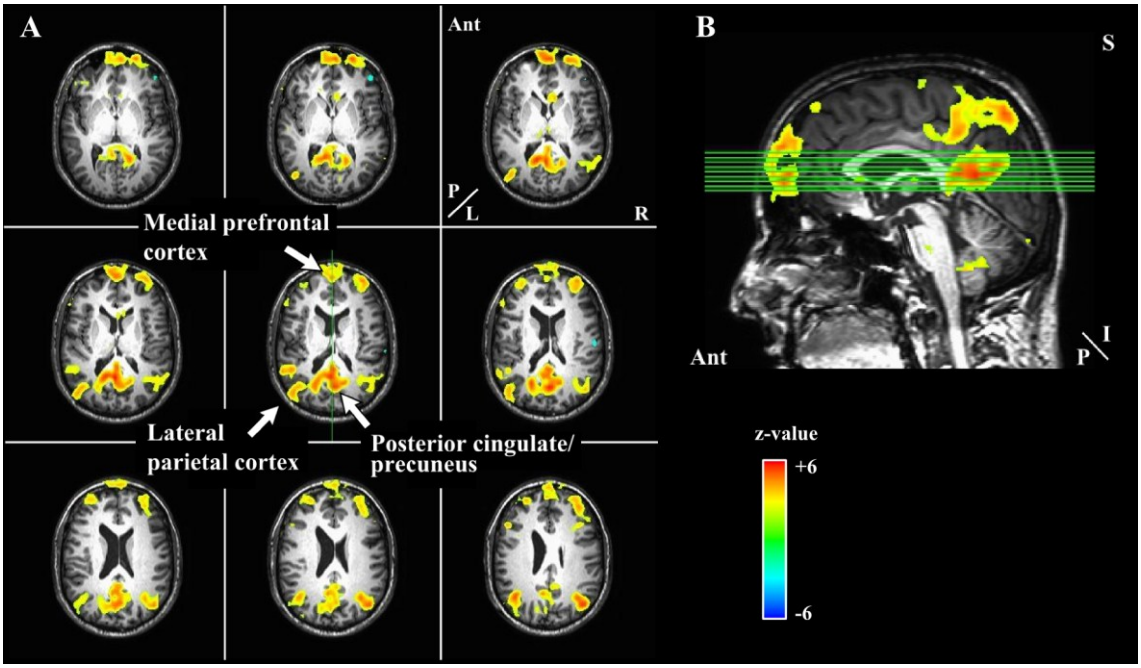

Figure 3.14 – Distracting imagination task in Day1. The significant activations shown in figure 3.5 are overlapped on axial (*A*) and sagittal (*B*) slices. Specifically, nine 3-mm spaced axial slices were taken in an inferior-posterior direction and are indicated with green lines in *B*; in the same way, the sagittal slice is indicated in *A*. Anatomical coordinate terms are indicated as follow: Ant, anterior; I, inferior; L, left; P, posterior, R, right; S, superior.

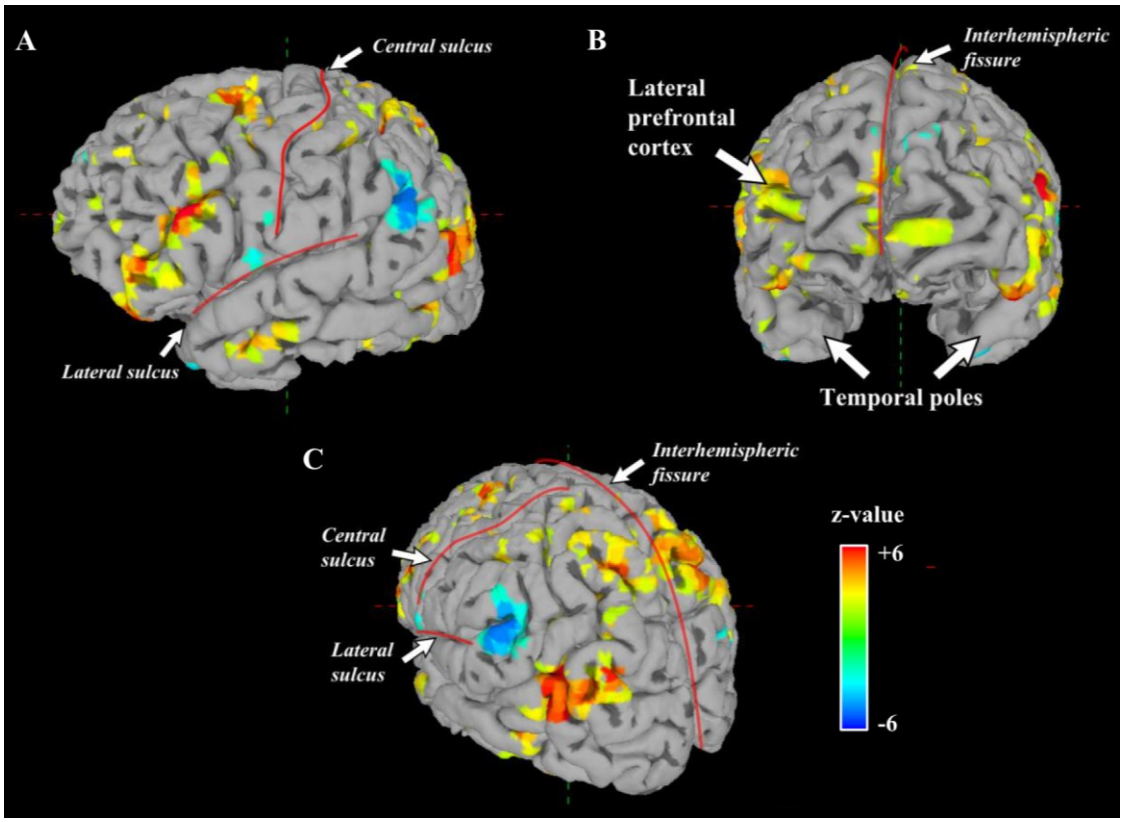

Figure 3.15 – Distracting imagination task in Day2. The task significantly modulate most of the cortical areas found in Day1 (see figure 3.3) in posterior parietal and lateral prefrontal cortices. However, significant negative modulation were observed in inferior parietal lobule, differently to what found when the persistent pain level was low.

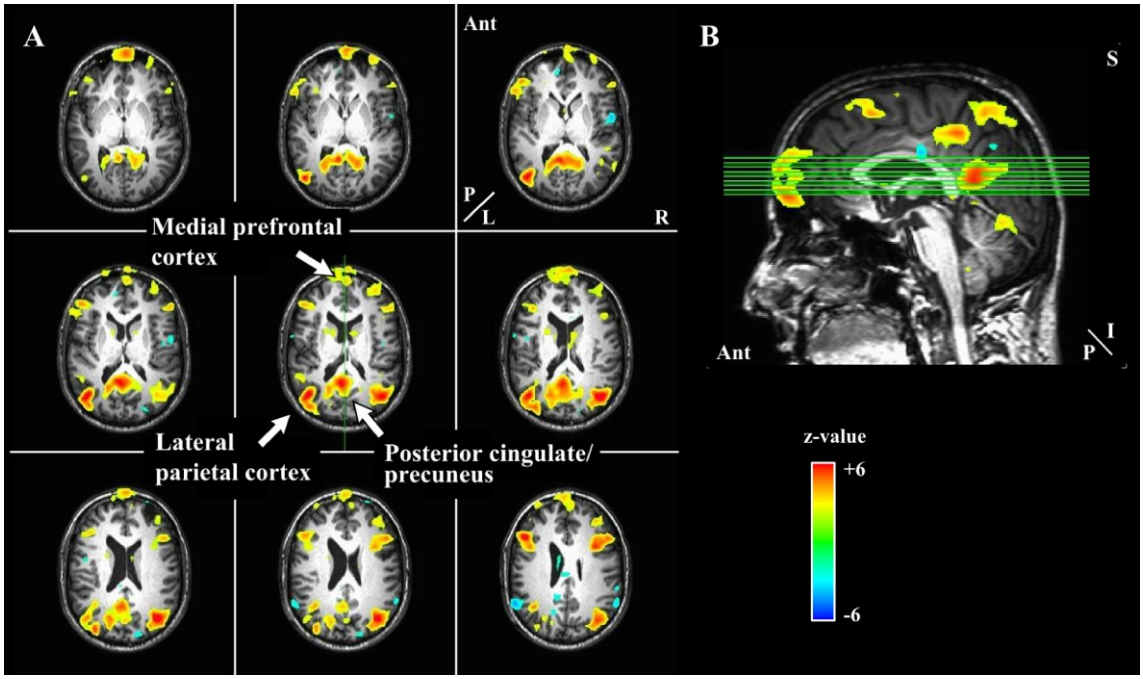

Figure 3.16 – Distracting imagination task in Day2. Clusters of significantly increased and decreased neuronal activity are shown on axial and sagittal slices. Green lines in A and B represent respectively the sagittal slice shown in B and axial slices shown in A. Anatomical coordinate terms are indicated as follow: Ant, anterior; I, inferior; L, left; P, posterior, R, right; S, superior.

3.3.2 - Conjunction analysis

Distraction from pain led to similar activation patterns in both days, and a conjunction analysis was performed to identify regions of similar activations. It is worthy of note that the patient did not imagine the same pleasant situation in the two days, therefore conjunction analysis revealed brain areas jointly activated, but not specific for the task. In other words, this analysis highlighted neuronal regions commonly involved in distraction and reduction of pain.

The activations that were found to be significant in both days are shown in figures 3.17 and 3.18. Significant voxels were mainly observed bilaterally in the lateral parietal cortex and in the medial part of the two hemispheres, within the posterior cingulate cortex and the precuneus. In prticular, the cortical pattern observed in these conditions resambled network observed by other authors when subjects did not perform any specific task an is therefore called default-mode network (Fox and Raichle 2007; Harrison et al. 2008).

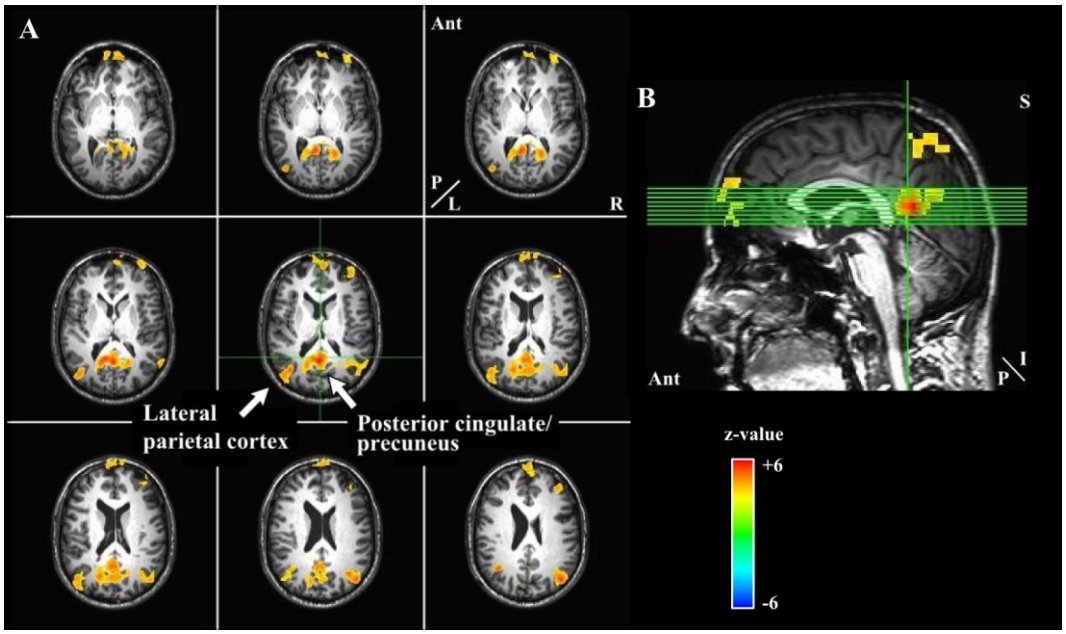

Figure 3.17 – Regions that were active both in Day1 and Day2, during the distraction task. Conjunction analysis revealed voxels that were significantly active in both days within the lateral parietal cortex and in a region that includes posterior cingulate cortex and the precuneus. The statistical map is overlapped on high-resolution T1-weighted and representative 3-mm spaced axial (*A*) and sagittal (*B*) slices are shown. Anatomical coordinate terms are indicated as follow: Ant, anterior; I, inferior; L, left; P, posterior, R, right; S, superior.

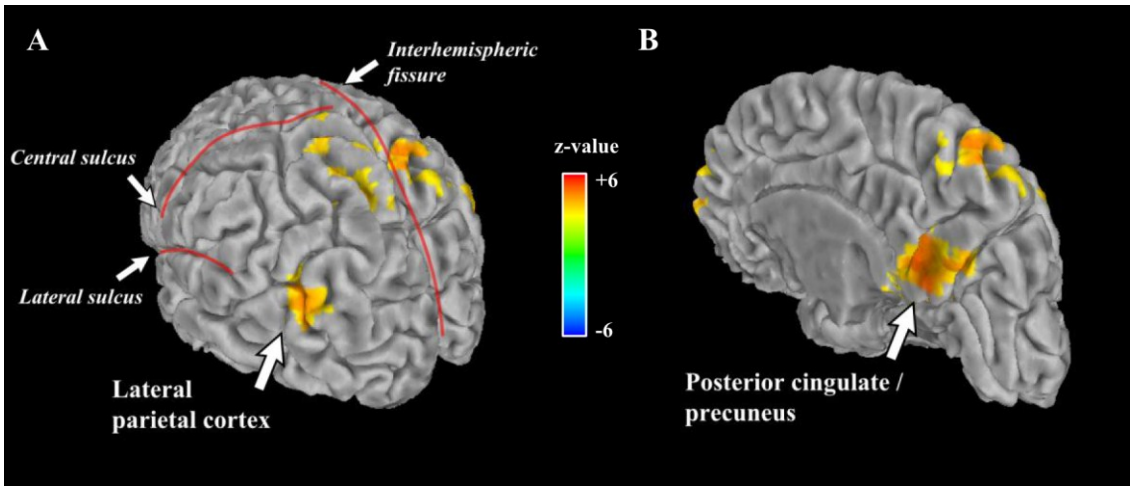

Figure 3.18 – Results of the conjunction analysis presented on 3D model of cortical surface. The 3D reconstruction of pial surface allows to better visualized the common cortical areas that were active in both days, when the patient performed a cognitive distraction task. *A*) lateral surface of the left hemisphere; *B*) medial surface of the right hemisphere.

Because both tasks performed in Day1 and in Day2 involved attentional processes that patient used to shift his attention either toward the ongoing pain, trying to imagine a high pain intensity, or toward pleasant thoughts, imaging to perform activities he enjoyed , a second conjunction analysis was performed in order to identify brain areas active in the three conditions that showed significant modulation in brain activity, specifically the pain-imaginative task in Day1 and pain-distraction task in both days. The result of this analysis indicate the involvement of right inferior parietal lobule in the execution of the two imaginative tasks, as shown in figure 3.19.

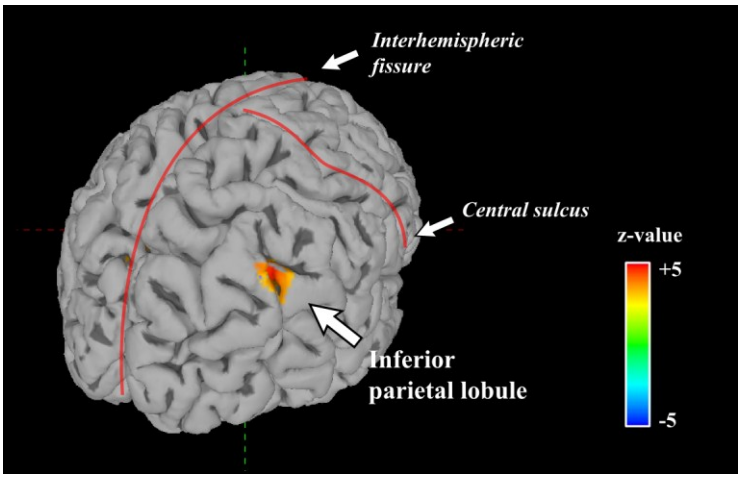

Figure 3.19 – The right inferior parietal lobule was involved in the both imaginative tasks. The result of the conjunction analysis performed on the brain activations that were observed during pain-imaginative task in Day1 and pain-distraction task in both days revealed that the right parietal cortex was commonly active in these three condition, suggesting its role in the attentional mechanisms these tasks share.

3.3.3 - Functional connectivity during resting state

The default mode network was originally identified in positron emission tomography studies (Mazoyer et al. 2001) and refers specifically to a set of cortical regions that show common activity decreases or deactivations when subjects perform cognitively demanding tasks. However, recent studies with fMRI have both confirmed and extended original findings, showing that the default mode network can be identified as a pattern of resting-state functional connectivity in the absence of specific tasks (for a review, see Fox and Raichle, 2007). In the light of the extensive literature on the resting state of the brain and considering the chronic condition of the patient, who reported a pain sensation without specific nociceptive stimulation, fMRI data were acquired at rest in both days. Temporal correlation between the time course of three regions of interest, typically activated during the resting state, and those of all the other brain voxels was calculated, in order to identify spatial patterns of coherent BOLD activity and investigate whether functional connectivity could be altered or depend on the intensity of the persistent pain in the phantom limb (for detailed decription of the procedure, see paragraph 3.2.5 in 'Materials and methods').

Resting-state analysis in Day1

Voxels showing significant correlation coefficients were observed bilaterally in each of the three brain regions involved in the typical cortical network active at rest. Figure 3.20 shows in axial and sagittal T1-weighted slices the functional connectivity of the areas within the default-mode network in Day 1, during which the patient reported a low intensity of persistent pain at rest. Specifically, significant positive correlations were observed in the medial prefrontal cortex, and in the parietal cortex, precisely in the inferior parietal lobule, near the occipital lobe, and in a region comprising the posterior cingulate cortex and the precuneus. In figure 3.21, these three areas are presented on the 3D reconstruction of the pial surface of patient's brain, in order to better visualize the anatomical relationships among the areas of the default mode network. No statistically significant anticorrelation domains were observed in Day1.

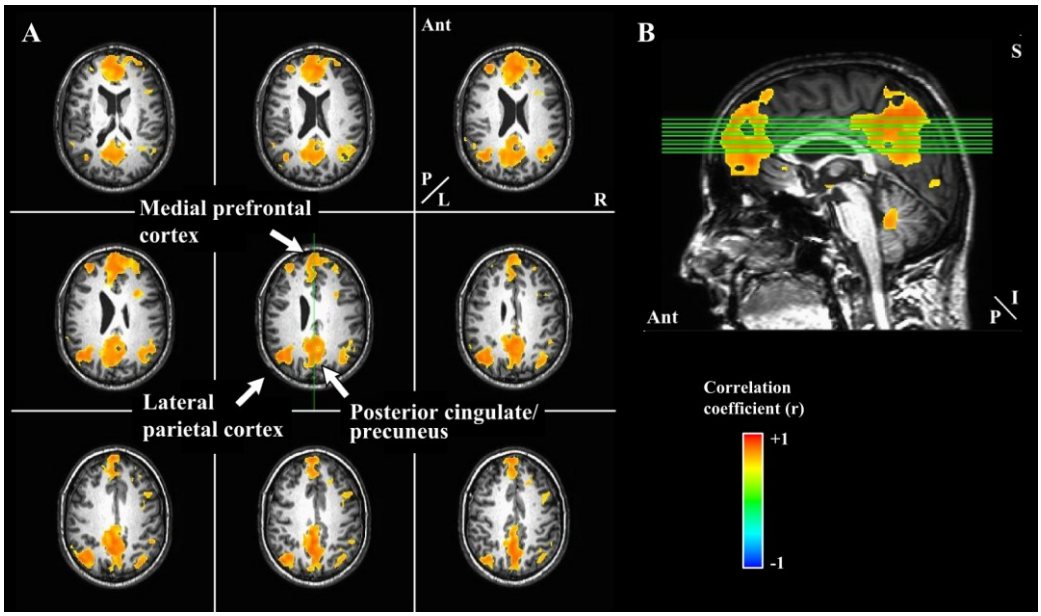

Figure 3.20 – Regions showing significant correlation between BOLD fMRI signals with seeds in the default-mode network (in Day1). Correlation analysis allowed to identified a network of areas whose fMRI signals correlated during the resting state. Significant positive correlation coefficients were observed in regions previously described as belonging to the default mode network. The correlation map is overlapped on high-resolution T1-weighted images, and representative axial and sagittal slices are shown respectively in *A* and *B*. Anatomical coordinate terms are indicated as follow: Ant, anterior; I, inferior; L, left; P, posterior, R, right; S, superior.

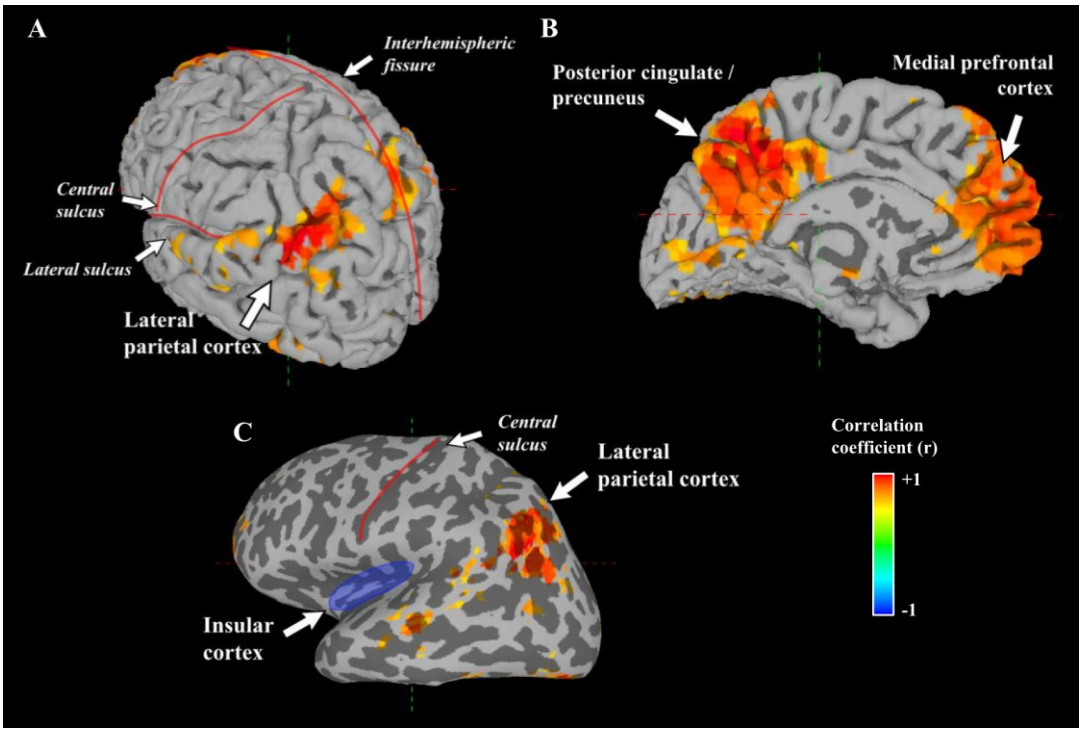

Figure 3.21 – Results of the correlation analysis performed on data acquired at rest in Day1. Significant correlation coefficients were computed considering three seed regions, one in the medial frontal cortex and two in the parietal lobe. Correlation maps is presented on the reconstruction of patient's cortical surface; *A* is a lateral-posterior view the brain, whereas the medial part of the left hemisphere is shown in *B*. In *C* the left hemisphere is inflated to show in the insular cortex; light and dark greys represent respectively circumvolutions and sulci.

Resting-state analysis in Day2

Different correlation patterns were obtained in Day2. In fact, when the phantom limb pain at rest was higher, extensive areas with statistically significant negative correlation coefficients were observed (figures 3.22 and 3.23) together with the positive correlation pattern detected also in Day1. Interestingly, the cortical domains exhibiting negative correlation were observed mainly in the insular cortices, the inferior parietal lobule and the foot area of the primary somatosensory cortex. These areas were consistent with the typical activation resulting from noxious stimulation, suggesting that the painful sensation could be related with the persistent activation of brain areas within the pain matrix.

The results obtained with the correlation analysis showed similar patterns when compared with those obtained with the regression analysis. In fact, cortical domains showing correlated and anticorrelated activities resembled regions in the frontal and parietal lobes whose activity was significantly modulated by attention and distraction. In particular, the correlation analysis demonstrated an activation/deactivation dichotomy between the default-mode network and the pain network, mainly when the intensity of the persistent pain was high, probably because only in this case the ongoing pain processing required a higher amount of cognitive effort and deeply interfered with the neuronal mechanisms within the default-mode network. In addition, this finding is similar to what observed in the regression analysis, where the default-mode network was found to be significantly more active when the patient moved his attention away from pain, whereas the pain network was more active when the patient paid attention on the persistent pain in the phantom foot. Taken together, these results suggest that cortical domains within the default-mode network exhibited opposite temporal patterns of neuronal activity in respect to the areas usually described in the pain network.

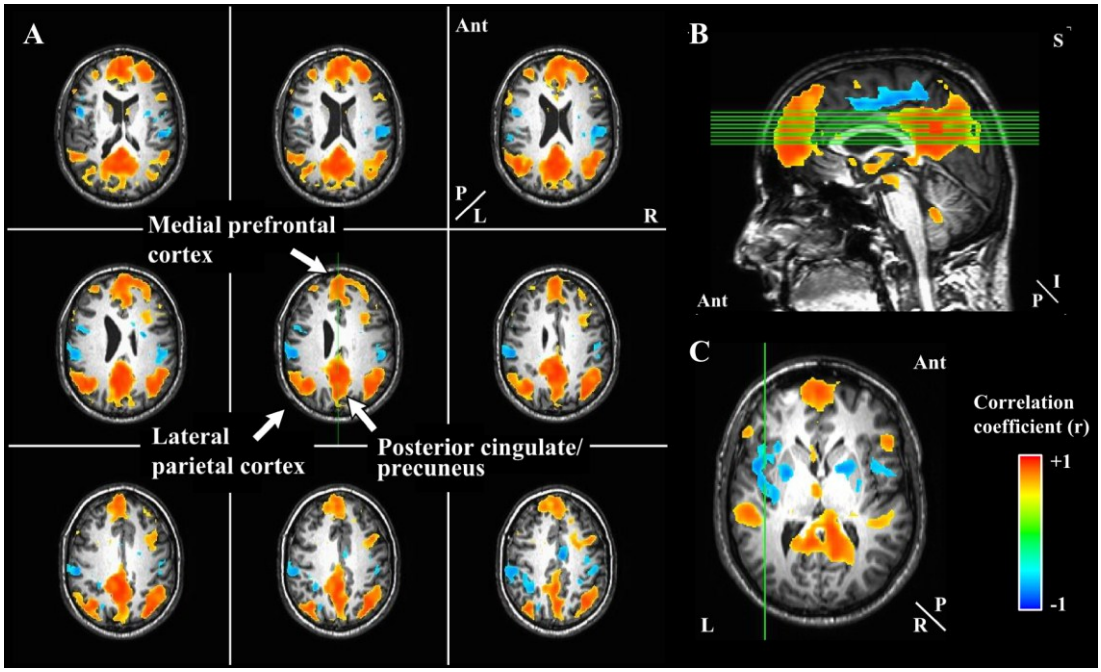

Figure 3.22 – Regions showing significant correlation between BOLD fMRI signals with seeds in the default-mode network (in Day2). In this case, correlation analysis revealed brain regions, whose fMRI signal negatively correlated with the time series acquired from the three seed regions. In particular, positive coefficients were observed in the medial frontal cortex, the posterior cingulate cortex and the lateral parietal cortex (*A* and *C*), whereas anticorrelation pattern included inferior parietal cortex, post-central gyrus and insular cortex (*B*). The correlation map is overlapped on high-resolution T1-weighted and representative axial (*A* and *B*) and sagittal (*C*) slices are shown respectively in A and B. Anatomical coordinate terms are indicated as follow: Ant, anterior; I, inferior; L, left; P, posterior, R, right; S, superior.

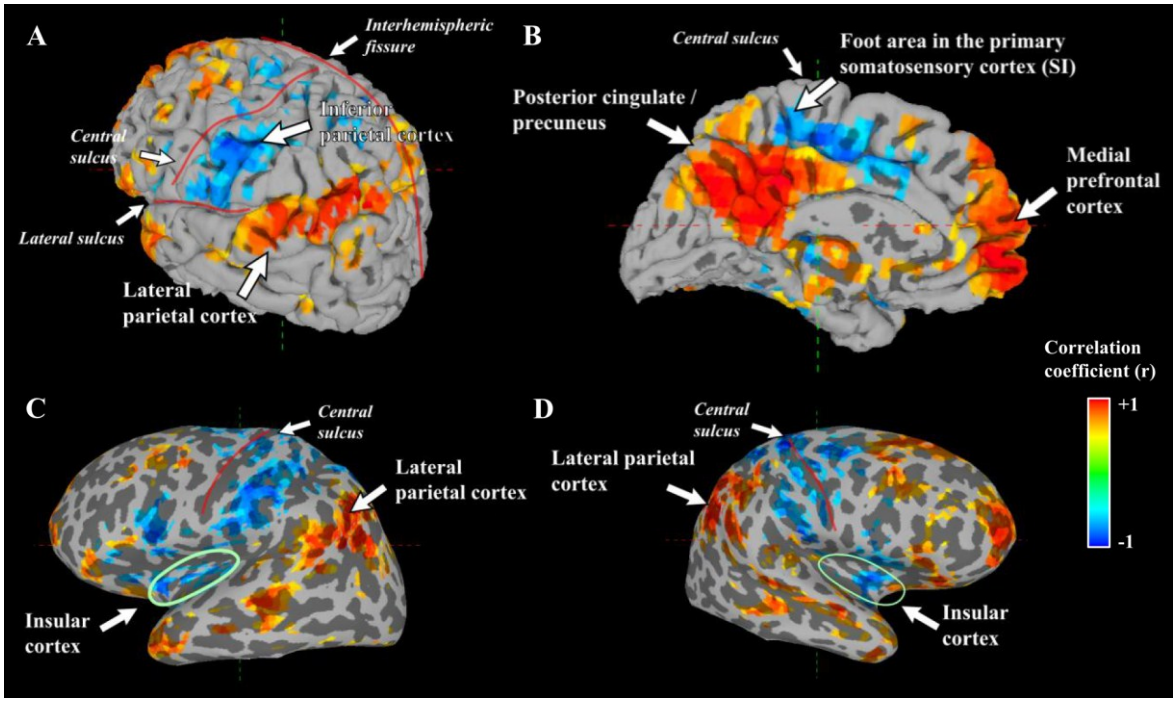

Figure 3.23 – Correlation map shown on the 3D model of the pial surface. Differently from results observed in Day1 resting state, in Day2 the correlation map **with seeds in the default-mode network** included both positive and negative correlation patterns. In particular, signals of pain network areas show significant anti-correlation with time series of areas of the default mode network. *A* and *B* show positive and negative correlations in the default mode network and the pain network. In *C* and *D*, pial surface is inflated to better visualize significant anticorrelation within the insular cortices of both hemispheres; light and dark greys represent respectively circumvolutions and sulci.

In conclusion, the correlation analyses detected that pain matrix and default-mode network were both active at rest in the brain of the patient with phantom limb pain, whereas the imagination tasks highlighted that the activity of both these networks was modulated by an attentional shifting. In particular, functional connectivity among the pain network at rest was higher when the patient referred a lower intensity of persistent pain, while the positive correlation pattern within prefrontal and parietal areas of the default-mode network was similar in the two subsequent days.

3.4 - Discussion

The investigation of pain perception in chronic conditions with functional magnetic resonance imaging (fMRI) has been challenged by technical and methodological reasons that make difficult to study persistent ongoing state and spontaneous fluctuations in blood-oxygen level dependent (BOLD) signals. The study presented in this thesis was aimed to understand which brain areas are involved in phantom limb pain, a specific chronic pain condition, and how these brain structures are related to different levels of persistent pain intensity. For this reason, data obtained from an experiment, which included both tasks execution and resting-state period, were analysed in two different ways. Firstly, a regression analysis was applied to identify increased or decreased activities associated with the performing of two opposite tasks, specifically a distraction task and a pain-imaginative task. Subsequently, the degree of correlation between BOLD signals from three regions of interest and all the other voxels of the brain was investigated in brain volumes acquired at rest, in order to identify brain regions that could be spatially separated but showed functional connection in their activities. The same experimental procedure, including tasks and resting state, was performed by the patient in two subsequent days, characterized by different levels of persistent pain intensity in the phantom right foot.

3.4.1 - Regression analysis

To begin with, data acquired in the two subsequent days were analysed using a general linear model (GLM) to identify the brain regions in which brain BOLD signal changed during the tasks relative to rest.

Considering statistically significant activations and deactivations the pain imaginative task was able to induce a greater modulation of brain activity when pain level at rest was low (Day1) than when was high (Day2).

Painful imagination

In Day 1, significant activations were found in areas that are commonly associated with pain processing and are described within the pain network (Bornhovd et al. 2002; Porro et al. 2004; Chen 2008). In particular, when the patient focused his attention on the pain in his phantom right limb and tried to imagine the sensation at the highest level, significant cortical activations were found in left primary somatosensory cortex (SI) and insula, as well as in regions of the frontal and parietal cortices that several authors have found to be involved in executive control and attention processing, such as lateral prefrontal cortex and right inferior parietal lobule

(Ingvar 1999; Bornhovd et al. 2002). Moreover, a significant deactivation was observed in the orbitofrontal cortex, a region that is known for its involvement in the activation of descending pain-inhibiting pathways (Wager et al. 2004). These changes in neuronal activity in specific pain-related areas but also in areas that have been demonstrated to be involved in cognitive and executive control of pain perception were not present in Day2. It should be notice that Day2 was characterized by a higher level of pain perception, that the patient rated as 80 in a range from 0 (no pain) to 100 (worst imaginable pain) and that this was the intensity normally perceived by the patient in those days in which the persistent pain was at the most intense levels (see figure 3.6 in 'Material and methods').

The lack of significant difference from rest in Day2 can therefore be explained by an ineffective modulation of brain activity by imaginative processes, which could not increase a pain level that was already high. On the contrary, in Day1, when the perceived pain was mild, the modulating effect of attention on ongoing pain processing was more effective in increasing pain intensity.

Distracting imagination

Despite of a different pattern of activation between Day1 and Day2 in attention task, the distraction task induced similar activations in both days. The GLM analysis of data acquired during the execution of the second type of task showed an extensive activation of cortical areas involved in the mechanisms of attention and pleasant imagination. Considering the significant modulation in the pain network, the results were specular to those observed with the pain imaginative task. In fact, the distraction task was effective in reducing the activation of pain-related areas, such as SI and insula, only with intense pain perception at rest (Day2), whereas it proved to be less effective with low pain (Day1).

The more interesting finding was the increased brain activity found mainly in the lateral parietal cortex and in the posterior cingulate cortex/precuneus. Extensive literature has demonstrated that these areas belong to the default-mode network, a network (figure 3.24) that showes an opposite relation with task-related changes (for a review, see Fox and Raichle, 2007). Interestingly, this network is commonly active at rest and deactivates during the execution of a task, whereas, in the case of phantom limb patient, it was activated during distraction task.

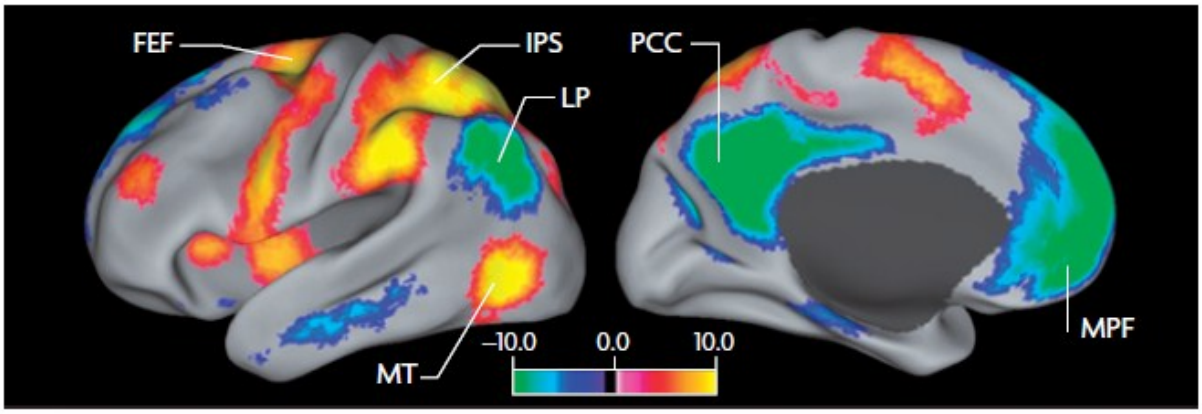

Figure 3.24 – Intrinsically defined anticorrelated networks in the human brain. The default-mode network is defined as an assemble of cortical areas that routinely de-activate during focused attention and working memory (cool colours). On the opposite, the fMRI signal in task-positive nodes, shown in warm colours, is significantly correlated with the execution of attention-demanding cognitive tasks. Task-positive and task-negative nodes are abbreviated as follows: IPS, intra-parietal sulcus; FEF, frontal eye field; MT, middle temporal area; PCC, posterior cingulate/precuneus; LP, lateral parietal cortex and MPF, medial prefrontal cortex. *From (Fox and Raichle 2007)*

During the distraction task, the patient was asked to think about something pleasant in order to move his attention away from the persistent pain and reduced in this way the harmful sensation. He was free to think whatever he wanted: the first day the patient referred that he had thought about an activity he liked doing before the amputation (skiing), in the second day, instead, he reported that he had imagined to drive a sport car. It is worth noting that, in both days, he remembered passed experiences that he personally enrolled, in other words, he performed introspective thought that involved the explicit memory. This task is generally thought to be associated with the activation of the default-mode network. In fact, the default mode network has been associated indirectly with the pattern of evoked activity that is observed with tasks involving self-judgments, autobiographical memory recall, moral dilemma, and prospective thinking, among others. In a more general context a common feature of these tasks is that they enhance subjects' attention toward themselves (Harrison et al. 2008).

The pattern of activations found in both days during the distraction tasks suggest that the distraction from persistent pain could move the brain state from a condition in which pain processing involved the most of the cognitive demand to a state generally described at rest, that is when the brain is active even in the absence of explicit input or output.

3.4.2 - Correlation analysis

Correlation analysis of fMRI data acquired at rest showed a pattern of brain areas whose signals were temporarily correlated and, possibly, functionally connected. In both days

functional connectivity was identified within brain regions of the default-mode network, whereas the time courses in these areas were differently correlated with key regions of the pain network, such as the insular cortex and the primary somatosensory cortex.

The default-mode network have been described by several authors when subjects did not perform any specific task and kept their mind free (Fox and Raichle 2007; Harrison et al. 2008). In particular, a network of lateral and midline cortical regions is often referred to as "default system" to connote greater activity at rest. The results reported in figure 3.7 and 3.8 support the hypothesis that the distraction task was able to interfere with the ongoing mechanisms of persistent pain sensation, shifting the patient's brain state toward a condition similar to that described in healthy subjects in the absence of specific input or output.

In fact, only in Day2, that is the day when the patient referred a much more intense pain sensation in the phantom limb, the signal pattern in the default-mode network was anticorrelated to the one in the pain matrix voxels. Interestingly, this pattern of anti-correlated BOLD signal included most of the commonly identified pain matrix areas, such as S1, the insular cortex and the inferior parietal cortex. In particular, the involvement of this latter cortical area received further support by a recent study in which the default-mode network at rest in normal control and patient affected by diabetic neuropathic pain was compared. In this case, the findings suggested that the brain of chronic pain patients differs from that of healthy subjects (figure 3.25) by showing a reduced default-mode network and an increased resting functional connectivity in some pain-related areas, such as the inferior parietal cortex (Cauda et al. 2009).

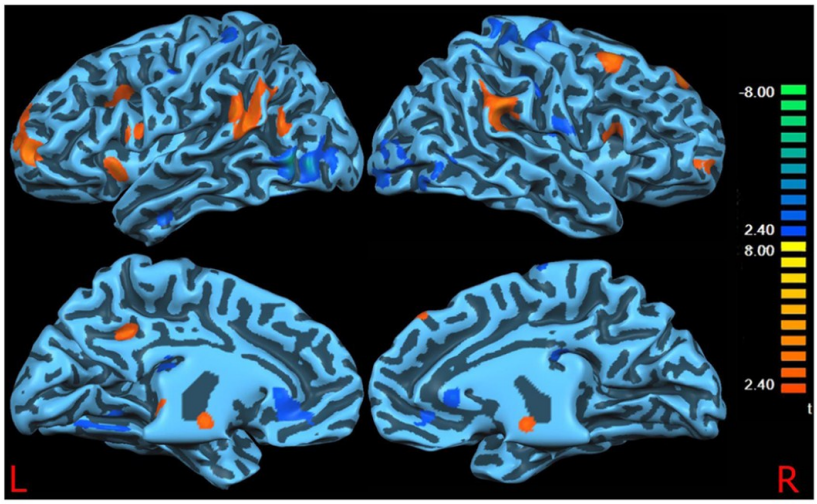

Figure 3.25 – DMN differences found in a study in which were compared resting-states between controls and patients with diabetic neuropathic pain. The blue foci indicate the areas that showed significantly less correlational activity in the pain group than in the healthy group. Vice versa the yellow/red foci indicate the areas that showed significantly more correlational activity in the pain group than in the healthy group. It should be notice the significant increased correlation in the inferior parietal cortex of patients. *From (Cauda et al. 2009)*

Among the cortical domains that showed a significant negative correlation with the nodes of the default-mode network, the observed synchronous activities in the insula are consistent with previous findings linking these region with processing of persistent pain (Apkarian et al. 2005) and emotional modulation of pain (Godinho et al. 2006). In particular, there is extensive evidence supporting the hypothesis that insula plays a prominent role in the evaluation of pain intensity and introspection in general (Critchley et al. 2004; Tagliazucchi et al. 2010). Moreover, insula plays a key role in regulation of emotional behaviours aimed to maintain optimal physiological balance in the body. The role of the insula in pain perception can be explained considering the behavioural relevance of pain as an 'exteroceptive' somatosensory system, but also as an 'interoceptive' system. Interoception is defined as the ability that the individual subject has to receive, and possibly bring to the level of consciousness, several inputs from its body (Panerai 2011). Several studies have highlighted the central role of the insula as an hub that not only receives inputs from the sympathetic and parasympathetic systems, but also from pain pathways, and sends them to other cortical areas (Craig, 2011, significance of the insula). According to this hypothesis, the integration of information in this brain area corresponds to what the subject recognizes as being its optimal or at least its present status, in other words its homeostatic being. Moreover, the internal representation of the 'normality' is used as comparative representation to identify changes in the optimal physiological state. When data do not fit, the destabilizing new input may be recognized as pain that drives appropriate behaviours, in an attempt to reach again the homeostasis.

In addition, since from early studies on pain, it has been demonstrated the association of the affective motivation of pain with anterior cingulate cortex (Ingvar 1999; Craig 2003a), which is considered the motor components of the limbic system that elaborates emotional responses. The hypothesis of the central contribution of cingulate cortex and insula in chronic pain processing is supported by several studies on brain activity in chronic pain which reported significant activation clusters in these two brain areas (Friebel et al. 2011).

The results found in the phantom limb patient support the hypotesis of the role of the insula as a key brain region for pain perception and its homeostatic purpose. In fact, the extent of voxels that showed synchronous fluctuations is larger when the pain sensation is higher. Furthermore, these findings suggest that the functional connectivity within the default-mode network could be negatively influenced by the activity of pain network areas.

These results are also in agreement with those found with regression analysis. In fact, default-mode network was found to be more active with the distraction task in both days, that is when the pain sensation was reduced by moving the attention away from the persistent sensation of pain, while keeping the attention on pain induced an increase in the activity of pain-related brain regions. A recent study have demonstrated how persistent pain disrupts the dynamics of the default-mode network, comparing default-mode network synchronous signals between normal subjects and chronic pain patients. Our findings suggest an opposite activation of default-mode network and pain matrix, in particular the insula, and that the extent of synchronous activation within the regions involved in pain processing are functionally related to the intensity of pain suffered by the patient.

4 - SENSORY AND COGNITIVE BAYESIAN INTEGRATION IN PAIN PERCEPTION AND PLACEBO ANALGESIA

4.1 - Introduction

Several factors can modulate the unpleasant sensation evoked by a noxious stimulus, influencing the sensory and cognitive processes that produce the conscious perception of pain. Anxiety state, previous experiences or verbal suggestions, to name just few of them, can influence perceptive mechanisms elaborating incoming noxious inputs, resulting in an increased or decreased pain perception. One of the best examples for such modulatory effect is the placebo analgesic effect. A placebo is an inert treatment, given as it has a real analgesic effect. In other words, the treatment is not active, but it is given in the same context of the real one, so that the contextual factors presented together with the placebo act on the neuronal nociceptive system reducing the pain perceived by the healthy subject or the patient. For this reason, through the study of the placebo analgesic effect, it is possible to investigate and understand how contextual factors can interact and influence perceptive mechanisms.

Different ways can be followed to investigate this interaction, in this thesis are reported results about the correctness of a theoretical model that try to integrate several aspects of pain perception processes. This model is based on the Bayesian decision theory and it is proposed as a theoretical and mathematical framework to reproduce the cognitive computation beneath the conscious perception of an noxious stimulus.

4.1.1 - A theoretic framework for modelling how the brain works

It is generally accepted that the experience of pain is not simply the passive transmission of noxious input along the sensory channels to the brain, driven by noxious stimulus characteristics. Analogous to well-known visual perceptions, such as ambiguous figures, the resultant pain experienced to the same sensory input can vary considerably. Along these lines, clinical and everyday life teaches us how cognitive and emotional variables such as attention, expectation, prior experiences, and mood shape our perception of pain (Bingel and Tracey 2008). In this sense, as the visual perception is based on the integration of different sources of information, in the same way, the pain perception system collects and integrates multiple pieces of information, that are often incomplete and ambiguous, to end up with a unique perception, with the aim of driving effective behaviors. Therefore, a complete knowledge of

all the perceptive systems comes from the investigation of the neuronal substrates that underlie to sensory and cognitive integration, but also from the comprehension of how they perform this integration and which are the. In particular, regarding the purpose of understanding the principles that govern the integrative process, in recent years the Bayesian inference has been adopted as a useful and valuable framework for a formal description of the computational processes involved not only in perception (Ernst and Bulthoff 2004; Beierholm et al. 2009), but also in sensorimotor control (Wolpert 2007), inductive learning and reasoning (Tenenbaum et al. 2006), classical Pavlovian conditioning (Courville et al. 2006) and decision-making (Lee 2008).

4.1.2 - Bayesian decision theory

When a perceptual representation of the world has to be generated in order to guide effective actions, the perceptive systems have to deal with ambiguous, incomplete and noisy information. For example, many factors contribute to limiting the reliability of visual information about the world, such as the mapping of 3D objects into a 2D image, neural noise introduced in early stages of sensory coding and structural constraints on neural representations and computations (Knill and Pouget 2004). To overcome this problem and end up with a robust and unique perception, the perceptive systems maximized and integrate multiple sources of sensory information (Ernst and Bulthoff 2004). Considering for example, the object in figure 4.1A, called the Necker Cube. The visual input that arrives to the central nervous system from the retina is ambiguous so that two stable percepts are both equally possible. However, the figure can be easily disambiguated by adding other elements to the scene, like shadows or a small bar that introduces an occlusion cue (figure 1.4B). If more than one perceptive systems are considered, this integration can also occur among different sensory information, such as visual and auditory information to detect the source of a sound (Knill and Pouget 2004; Beierholm et al. 2009) or visual and tactile inputs in the case of the perception of the dimension of an object (Ernst and Bulthoff 2004). Therefore, one of the key to robust perception is the combination and integration of multiple sources of sensory information.

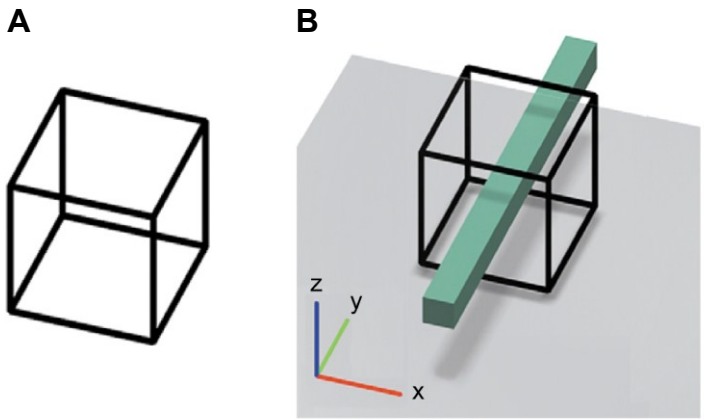

Figure 4.1 – The Necker Cube. The drawing object presented in *A* induces a bi-stable percept. However, disambiguation can be achieved by introducing an occlusion cue and a shadow (*B*). *Modified from (Ernst and Bulthoff 2004)*

It is however impossible to reconstruct the environment 'bottom-up' from the sensory information alone. Prior knowledge is needed to interpret ambiguous sensory information. Bayesian inference provides a formal way to describe such interactions and enables one to model the uncertainty about the world by combining prior knowledge (that might be unconscious) with observational, sensory evidence to infer the most probable interpretation of the environment (Ernst and Bulthoff 2004).

In this sense, information about the environment, available prior to encountering the given stimuli and, thus, not dependent on the incoming stimuli, can be integrated with the information carried by the incoming stimuli to generate the probability of each possible true state of the environment, according to the Bayes rule:

$$\underbrace{P(\theta|s)}_{\text{Posterior}} = \frac{\overbrace{P(s|\theta)}^{\text{Likelihood}} \; \overbrace{P(\theta)}^{\text{Prior}}}{P(s)}$$

where θ represents percepts and s the incoming stimuli.

However, the Bayes rule is not enough for describing the computational effect performed by the central nervous system. In fact, the Bayesian inference calculate a probability distribution of possible percepts given the incoming stimuli ($P(\theta|s)$), though daily life experience tells us that the brain come up with a unique and unambiguous representation of the surrounding world. Considering again the Necker Cube in figure 4.1A, although two percepts are equally probable, it is not possible to see two cubes at the same times. In other words, although more and more information, using different sources, can be collected to reduce the ambiguity in incoming stream of noisy sensory inputs, the brain has to pick a single solution from all the possibilities. Therefore a decision-making process has to be considered, in addition to the

sensory-estimation process, resulting in the definition of a Bayesian decision theory (figure 4.2) that can provide a useful and valuable tool to understand and interpret sensory and cognitive processes performed by the central nervous system (Geisler and Kersten 2002; Knill and Pouget 2004; Wolpert 2007).

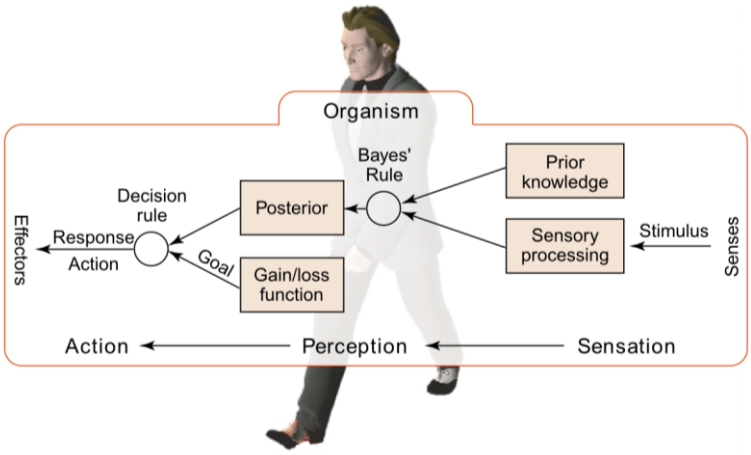

Figure 4.2 – Graphical representation of the Bayesian decision theory. descion The Necker Cube. T A complete model for the inferential process performed by the nervous system includes the integration of sensory information with prior knowledge and the decision-making phase. *Modified from (Ernst and Bulthoff 2004)*

4.1.3 - A Bayesian model of pain perception

The data presented in this part of the thesis are the results of two experiments performed with the aim of validating a theoretical model for the integration of noxious inputs with modulatory information to obtain pain perception. In particular, the model proposed is based on Bayesian decision theory and was tested comparing its predictions with data obtained from human subjects.

The underlying hypothesis is that, to come up with a useful and effective perception, the pain perceptive system has to integrate both noxious inputs coming from the peripheral nociceptors, with cognitive information derived from different sources, as demonstrated for other perceptive systems (see paragraphs 4.1.1 and 4.1.2). In the case of the model here described, these information come from the experimental paradigm applied in the study. Specifically, a classical condition procedure was used to induce a placebo analgesic effect in the healthy subjects: two noxious stimuli were associated with to visual cues presented on a PC screen, so that a high stimulus intensity was always applied together with a red cue, whereas a low level of pain was applied together with a green cue (see *Experimental procedure* in paragraphs 4.2.2 and 4.2.3 in 'Materials and methods'). Therefore, for what concern the Bayesian model, the conditioning session represents the basis for the esteem of prior distribution that enters in the Bayes rule together with the information about the stimulus. Furthermore, it constituted the

basis for the expectation generated by the visual cue presented during the testing session. A graphical representation of the model proposed is shown in figure 4.3.

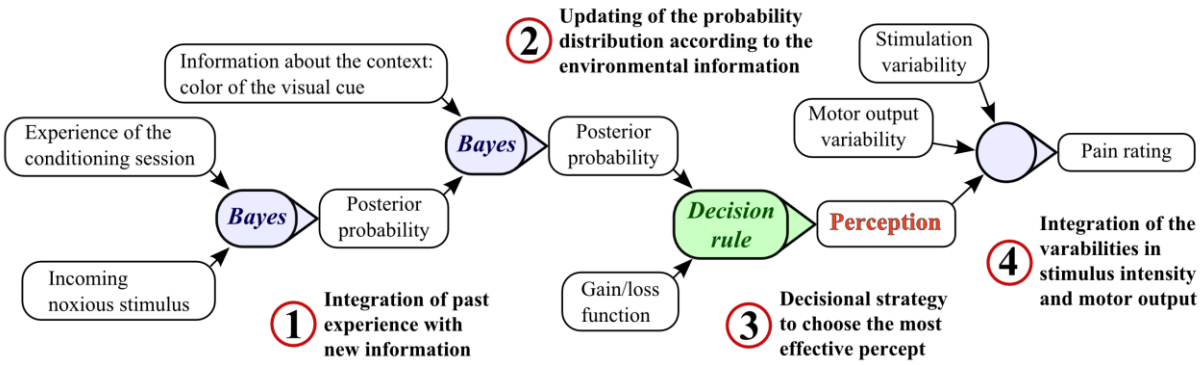

Figure 4.3 – Schematic representation of the proposed model, based on Bayesian decision theory. Different parts compose the Bayesian model. The first three parts represents the integrative processing performed by sensory pain system to produce a conscious sensation of pain. The last step adds two sources of variability that contributes to explain the variability found in pain ratings recorded in experiments with healthy volunteers

Given a firing rate of F_k, induced in nociceptors by a stimulus (S), the posterior probability of a possible pain perception (E_i) is $p(E_i|F_k,C_j,X)$, where C_j is the colour of the visual cue (where j may be red or green) presented together with the stimulus and X represents the knowledge derived from all the previous experiences of pain, that cannot be taken into account, but is added for formal correctness of the model. According to the Bayes rule, and assuming that frequency and cue are independent factors, the posterior probability distribution can be calculated as:

$$p(E_i|F_k C_j X) = \frac{p(E_i) * p(F_k|E_i X) * p(C_j|E_i X)}{p(F_k C_j|X)} \quad (1)$$

where $p(E_i)$ represents the prior probability, while $p(F_k|E_i X)$ and $p(C_j|E_i X)$ are the likelihood functions associated with the incoming firing rate and the visual cue presented. Therefore, the prior probability constitutes the prior knowledge about the possible experience of pain, while the two likelihood functions represent the knowledge about the current stimulation and, in particular, respectively the relationship between the current noxious and visual information and the possible pain perception.

These three terms can be estimated according to the following formulas:

$$p(E_i) = [p(E_i|S_1 I)p(S_1|I)+p(E_i|S_2 I)p(S_2|I)]*R + [p(E|B)*(1-R)] \quad (2)$$

$$p(C_j|E X) = \frac{p(C_j|X)*p(E|C_j X)}{p(E|X)} \quad (3)$$

$$p(F_k|E_i X) \sim N(\mu=1\rightarrow 10, \sigma^2=0.75) \quad (4)$$

More in details, it is assumed an arbitrary range from 1 to 10 to represent all the possible firing rates and that the relation between the noxious stimulus and the responses of the cutaneous nociceptors follows a normal distribution as expressed in the equation 4 (LaMotte and Campbell 1978; Torebjork et al. 1984).

Considering the prior probability $p(E_i)$, it depends on the knowledge acquired during the conditioning session due to the pain levels experienced, which are related to the stimulus intensities applied. However, it also depends, to a certain extent, on all the experiences of pain that the subject had before he or she underwent the experiment. For this reason, equation 2 can be subdivided in two parts: the first representing the knowledge acquired during the conditioning and the second representing the generic background (*B*) of the subject. Both these two parts contributed to the prior probability $p(E_i)$, but with different weights, according to the scaling factor R. Taking the two parts separately, the first can be calculated considering that, in the conditioning session, two equally probable stimuli are applied to the subjects and that these stimuli have a specific relation with the perception of pain. Therefore, in equation 2 the probabilities of each stimulus, $p(S_1|I)$ and $p(S_2|I)$, are multiplied by a normal distribution that links the stimulus intensity to the actual energy detected by the nociceptors.

This energy, in the absence of any manipulation (as in the conditioning stage), may be considered to have a direct and linear relationship with the esteem that the system makes of it and codes through the pain level (LaMotte and Campbell 1978; Torebjork et al. 1984). For this reason, $p(E_i)$ can be viewed as the prior probability of the possible energy levels in the experimental context.

$$p(E_i|S_m, I) \sim N(\mu=S_m, \sigma^2=25) \quad (5)$$

$$p(S_1|I)=p(C_r|I)=0.5 \quad \text{and} \quad p(S_2|I)=p(C_g|I)=0.5 \quad (6,7)$$

where *I* indicates the conditioning background, provided by the experimental experience and added for formal correctness of the model.

The second part or equation 2, concerning the background experience of the subject, can be modelled as:

$$p(E|B) \sim N(\mu=0, \sigma^2=400)+0.003 \quad (8)$$

in which, however, the negative values of the normal distribution are not considered because they would represent negative values of pain perception.

Together with the prior knowledge (equation 2) and the likelihood function of the firing rate (equation 4), two other terms complete the Bayes rule described in equation 1. These are the likelihood functions associated with the visual cue (C_j) and the denominator, which is simply a normalization factor.

The likelihood function of the stimulus energy, $p(C_j|E\ X)$, can be calculated applying again the Bayes rule. In fact, as shown in equation 3, it is equal to the probability associated with the visual cues C*j* presented during the conditioning (see equation 6 and equation 7), multiplied by the probability distribution of E given a visual cue: $p(E|C_j,X)$. In particular, $p(E|C_j,X)$ derives from the knowledge about pain perception and visual cue learnt during the conditioning session and can be obtained by one of the following equations according to the colour of the visual cue presented:

$$p(E|S_1X) = p(E|C_rX) = p(E|S_1I)*R + p(E|B)*(1-R) \quad (9)$$

$$p(E|S_2X) = p(E|C_gX) = p(E|S_2I)*R + p(E|B)*(1-R) \quad (10)$$

where R is the same weighting factor of equation 2, $p(E|S_1,I)$ and $p(E|S_2,I)$ are derived from equation 5, whereas $p(E|B)$ is the same probability of equation 5.

Finally, the denominator in equation 1 can be calculated as the joint probability of the visual cue C*j* and firing rate F_k:

$$p(F_kC_j|X) = \sum_i p(E_iF_k|C_jX) \quad (11)$$

where $p(E_i\ F_k|C_j\ X)$ derives from equation 4 and equations 9 or 10:

$$p(E_i, F_k|C_j, X) = p(F_k|E_i, X)*p(E_i|C_j, X) \quad (12)$$

The Bayesian framework allows to create a model that describes the relationship between the nociceptor activity elicited by the noxious stimulus, the coloured visual cue presented on the PC screen and the pain perception evoked in the subject. However, equation 1 describes this relationship as a distribution of probabilities associated to each possible pain experience, a situation that is far from what is actually reported from the subject. In fact, unique pain

perception is rated by the subject at the end of each stimulation. According to Bayesian decision theory, a decision rule has to be applied to the posterior probability distribution $p(E_i|F_k,C_j,X)$. Here we considered that the decision is made by choosing the maximally probable pain perception.

Applying this rule, the result of equation 1 becomes:

$$E_i = f(F_k C_j X) \qquad (13)$$

A final point is that equation 13 links a unique pain perception to the firing rate F_k and the visual cue C_j, however it does not consider two sources of variability that contribute to the outcome of real experiments, that is the pain rating through the visual analogue scale (VAS).

A first source of uncertainty is the variability both in the motor response of the subject that has to move an object (a potentiometer or a computer mouse) to rate the pain perceived, and in the coding of a pain level on a visual bar; the other is the variability in (14) onse of the sensory system to the noxious stimulus, in other words, the variability in the relationship between the stimulus intensity S_m and the firing rate F_k. The variability in the motor response and pain level coding can be modelled as a normal distribution centred on the pain percept:

$$p(V|F_k C_j X) \sim N(\mu = E_i, \sigma^2 = 5)$$

where V is the scoring reported by the subject on the VAS. The second source of variability can be calculated through this relation:

$$p(F_k|S_m X) = \frac{p(F_k S_m X)}{\sum_k p(F_k S_m X)} \qquad (15)$$

$$\text{where} \quad p(F_k S_m X) = \sum_i p(F_k|E_i X) * p(E_i|S_m X) \qquad (16)$$

that is obtained multiplying equation 4 by

$$p(E_i|S_m X) = p(E_i|S_m I) * R + p(E_i|B) * (1-R) \qquad (17)$$

Considering these sources of variability, the following formula is obtained:

$$p(V|S_m C_j X) = p(V|F_k C_j X) * p(F_k|S_m X) \qquad (18)$$

This equation describes the relationship between the variables considered in the placebo analgesia experiments and evaluated in this thesis. In particular, the experimental data expected from this formula were used to evaluate the predictions of the model about pain perception and its rating. All the equations described above are reported in figure 4.4, in which they are organized according to their relations.

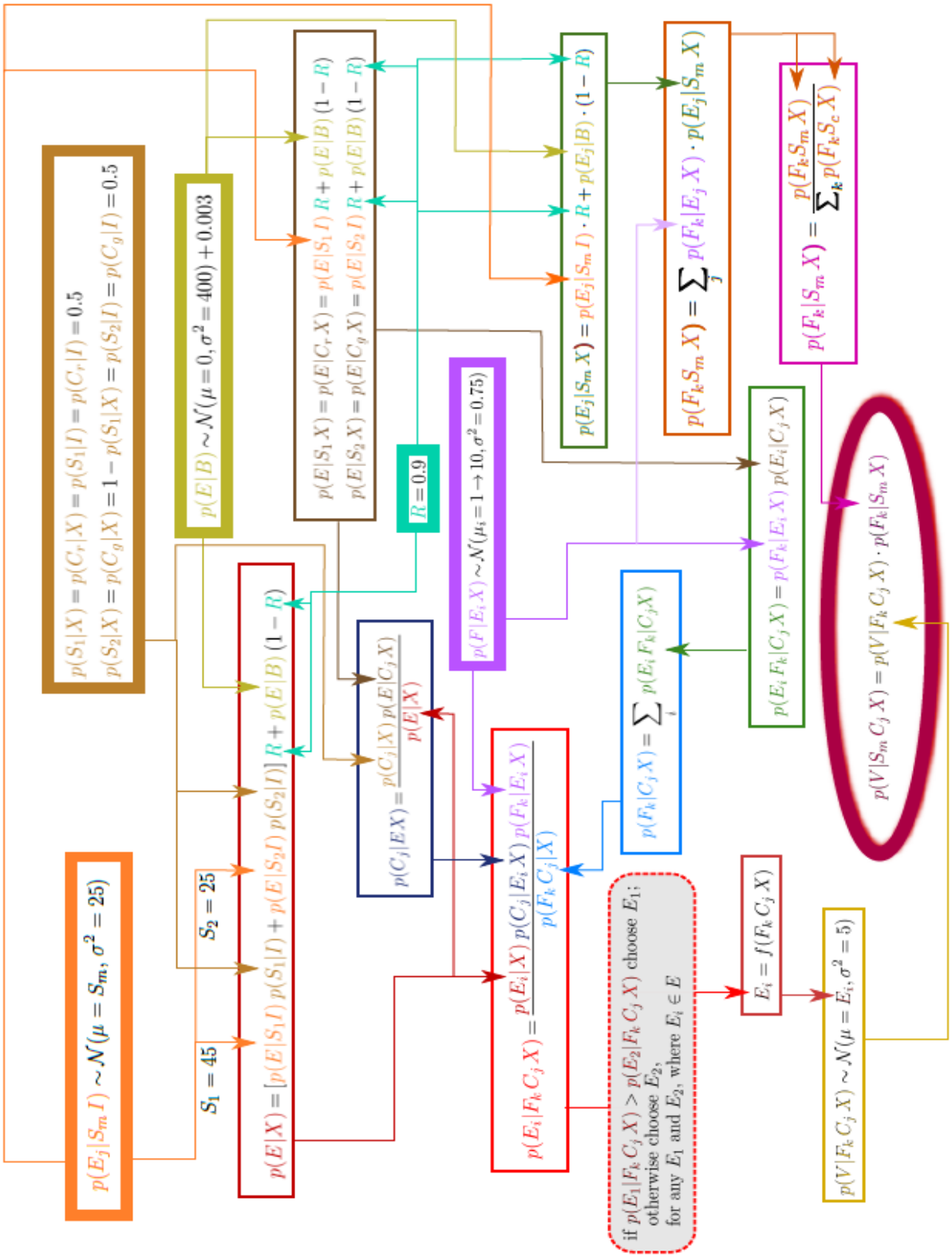

Figure 4.4 – Schematic representation of the equations that constitute the proposed model of pain perception. The variables taken into account are abbreviated as follows: S, stimulus intensity, specifically s1, high intensity and s2, low intensity; E, perceived intensity, what the system tries to estimate; F, nociceptor firing rate; C, visual cue, specifically Cr, red cue and Cg, green cue; B, generic background, provided by previous experience and the biological structure of the system; I, conditioning background, provided by the experimental experience during placebo conditioning; X, overall background, resulting from the experimental experience during the placebo conditioning procedure (I) and the generic background (B). The contribution of the conditioning session and the background knowledge are weighted by a scaling factor (R).

4.2 - Materials and methods

4.2.1 - Participants

A total of 44 healthy human volunteers were recruited by advertising at the University of Udine (Italy) subdivided in two groups for the two experiments described below. In particular the first experiment was performed by group of 31 subjects (mean age 21.50 ± 1.21; 19 female, 12 male), whereas a group of 13 subjects (mean age 23.13 ± 2.3; 7 female, 6 male) underwent the second experiment. Both groups were deceptively told that the experiment was aimed to evaluate a new protocol to be applied for Transcutaneous Electrical Nerve Stimulation (TENS), an analgesic technique already used as treatment for chronic pain conditions like chronic back pain. All the experimental procedures was conducted in conformances with the declaration of Helsinki and written informed consent was obtained from all participants.

The actual procedure surreptitiously performed in the experiment was a classical conditioning protocol to induce modulation in subjects' pain perception and was aimed to test the correctness of the proposed Bayesian model. For this reason, two experiments were conducted in order to evaluate different predictions made by the model, specifically the first experiment was aimed to assess the pain modulatory effect of previous experience, whereas in the second experiment the aim was to compare observed and expected data when conflicting pieces of information were presented to the subject. The classical conditioning protocol described by (Colloca and Benedetti 2006) was chosen for both Experiment1 and Experiment2; though, the two Experiments differed in respect to the stimulation protocol used in the testing sessions (see figure 4.5 and figure 4.7), and the type of painful stimuli applied: electrical stimuli in Experiment1 and pain-pressure stimuli in Experiment2.

In both the Experiments, the same description of the experiment was given to the subjects. In particular, the subjects were told that a painful stimulus would be applied, associated with a red or a green visual cue presented on a PC screen placed on a table in front of them. In particular, TENS would be applied as an analgesic treatment with green cue, whereas no treatment would be associated with red cue.

4.2.2 - Experiment 1

Experimental procedure

After collecting the informed consent and describing the sham experimental procedure, subjects set on a comfortable chair and were prepared for the experiment: they were asked to put one of their feet onto a wooden holder, two electrodes were placed on the back of the foot for painful electrical stimulation, while two electrodes for sham analgesic treatment were placed on the ankle. In particular, both paired of electrodes were placed on the right or left ankle of the subjects, in a random order among the group.

The experiment started with the determination of pain threshold (T) according to the method of the limits. Briefly, an ascending series of electric stimuli were delivered beginning from sub-tactile threshold until pain sensation was induced. After determination of T, an intensity able to induce a clearly detectable pain sensation was chosen, by continuing ascending series of pressure stimuli. Also, a lower painful intensity was chosen without subject's knowledge, for the purpose of inducing a sham analgesic effect as described below.

The experimental procedure was subdivided in two sessions (see figure 4.5). In the first session, classical conditioning was used to induce placebo effect in recruited subjects. For this purpose, the subjects were told that the same stimulus intensity would be applied, associated with a red or a green visual cue presented on a PC screen placed on a table in front of the subject. As state above, subjects were told that TENS would be applied as an analgesic treatment with green cue, whereas no treatment would be associated with red cue. Though, a lower painful stimulus was surreptitiously associated with the green cue, in order to make the subject experience the putative analgesic effect. The conditioning stimuli were delivered during two blocks, including 16 stimuli each (8 stimuli at the high intensity and 8 at the low intensity) each delivered after a 4-6 s presentation of the visual cue. Therefore, in this session 16 associations high-red and 16 low-green (8 for each block) were performed in random order. Following each stimulation, the subjects reported their perceived pain intensity according to a Visual Analogue Scale (VAS).

The testing session followed classical conditioning and was subdivided in two blocks of stimuli. Visual cues were presented on a PC screen and subjects reported perceived pain intensities according to a VAS. The first block of stimuli was aimed to test the predictions of the theoretical model about subjects' pain rating when no cue was presented, the latter was aimed to test for placebo effect. During the first block, 16 stimuli were applied paired with a

neutral blue cue in order to have an experimental condition similar to the conditioning session. Three levels of intensity were chosen for the test: 4 high-intensity stimuli, 4 low-intensity stimuli and 8 middle-intensity stimuli were delivered in random order (figure 4.5). The middle-intensity was chosen as the midpoint intensity between the other two. Finally, the placebo testing phase was similar to a block of the conditioning session: both red and green visual cues were presented, however only high intensity stimuli were used. Similarly to the conditioning blocks, 16 stimuli were delivered, randomly paired with the red (n=8) or green (n=8) cues (figure 4.5).

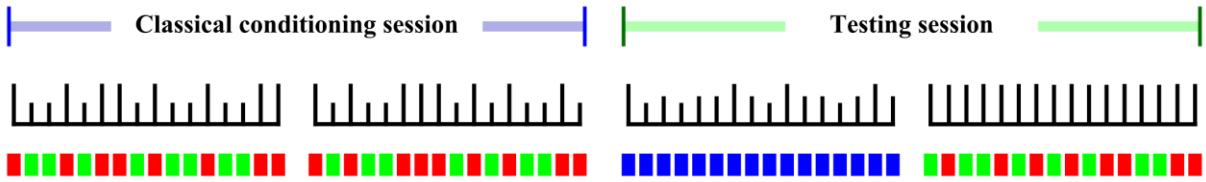

Figure 4.5 – Stimulation protocol used in Experiment2. The stimulation protocol applied in Experiment2 was constituted by four blocks of 16 stimuli each, two in the conditioning session and two in the testing session. Bars height indicates the stimulus intensities (low, medium, high), whereas the series of coloured rectangles below each block represent the visual cues associated with the stimuli.

Electrical pain stimulation

The electrical stimuli were squared pulses delivered by a constant current high voltage stimulator (DS7A model, Digitimer Ltd, Welwyn Garden City, England) with a duration of 200 µs. Two Ag/AgCl electrodes with foam and solid gel (ARBO, Germany; stimulation area = 2 cm^2) were placed on the back of the foot. Stimuli were delivered at the end of either a red or green visual cue presented on a PC screen (figure 4.6).

A toolbox developed with the software MATLAB 7.1-R14 (The MathWorks Inc., Natick, Massachusetts, USA) was used to present on the PC screen the visual cues, the VAS and to collect the pain ratings. All the data were stored on a computer for the subsequent analyses.

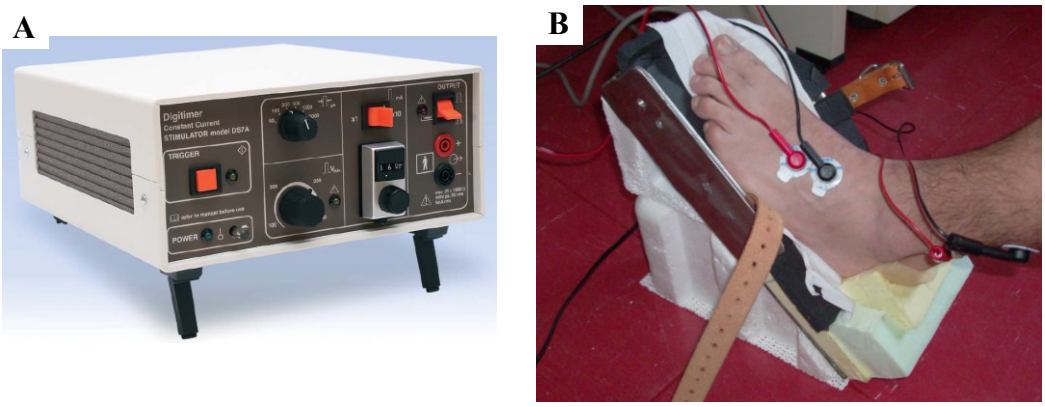

Figure 4.6 – Setup for the electrical pain stimulation. Electric pulses were delivered by a constant current stimulator (*A*), using two electrodes placed on the back of the subject's foot (*B*). Two other electrodes were placed on the ankle and were used to simulate the procedure for TENS (*B*).

4.2.3 - Experiment 2

Experimental procedure

The initial phases of the experimental procedure used in the second Experiment were similar to those described for the first Experiment. Briefly, after describing the experimental procedure, subjects set on a comfortable chair and were prepared for the experiment: they were asked to put one of their feet onto a wooden holder, in order to avoid large foot movements and therefore allow a more precise stimulation, and two electrodes for sham analgesic treatment were placed on the ankle. In particular, the electrodes were placed on the right or left ankle of the subjects, in a random order among the group.

Pain threshold was measured according to the method of the limits: an ascending series of pressure stimuli were delivered beginning from sub-tactile threshold until pain sensation was induced. Subsequently, an intensity able to induced a clearly detectable pain sensation was chosen, whereas a lower painful intensity was also chosen without subject's knowledge, for the purpose of inducing a sham analgesic effect as described below.

The experimental procedure was subdivided in two sessions (as shown in figure 4.7). In the first session, the same conditioning protocol described for Experiment1 was applied to induce a modulation of subject's pain perception. Two blocks of 16 stimuli each were delivered. The order of high and low intensity stimuli was randomly chosen. Each stimulus intensity was paired with a visual cue, according to the following associations: high-red, low-green. After each stimulus, the subject rated the perceived pain intensity by means of a VAS.

The testing session followed the classical conditioning one (figure 4.7) and comprised a single block of 16 stimuli, associated with visual cues presented on the PC screen; though, the intensity-cue associations was changed, without the subject's knowledge. In fact, a the mid intensity between the other two was applied with both the red and the green cues, in the same block with the high and low intensities used in the previous session that were still associated respectively the red and green cues. Therefore, in the testing session, 16 association were applied in a random order, specifically: 4 high-red, 4 low-green, 4 middle-red and 4 middle-green. As in the conditioning session, subjects were asked to rate their perception through VAS.

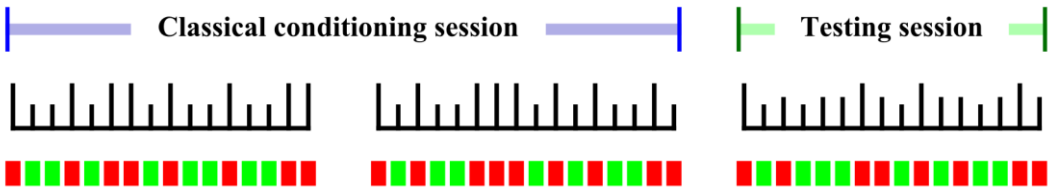

Figure 4.7 – Stimulation protocol used in Experiment1. The stimulation protocol applied in Experiment1 was constituted by three blocks of 16 stimuli each, two in the conditioning session and one in the testing session. Bars height indicates the stimulus intensities (low, medium, high), whereas the series of coloured rectangles below each block represent the visual cues associated with the stimuli.

Pressure pain stimulation

In the first Experiment mechanical noxious stimuli were delivered to the lateral ankle of the volunteers by mean of an custom-made manual pressure device (figure 4.8A), similar to those described in literature (see, for example, (Neziri et al. 2011). In particular, a plastic tipped probe (surface = 0.2 mm^2) was mounted on a load cell (AZ100, LUMAS Elettronica, Italy) allowing to continuously record the force applied to the foot (figure 4.8B) into an electric potential continuously recorded and digitalized by an amplifier (MP100A-CE, BIOPAC System Inc, Santa Barbara, California, USA).

The visual cues and the VAS were presented on the same PC screen. In particular, the VAS was a bar which length the subjects could increase or decrease in order to report their pain perception. This was done turning a potentiometer connected to the same amplifier used to record the load cell electric potential.

All the data were stored on a PC and subsequently used to obtained the pressure values applied during each stimulation and the pain rating.

The subjects placed their foot on a foot-holder to allow a stable position of the foot and therefore, a more precise stimulation. Three stimulus intensities were chosen by the operator during the preparatory phase, before starting the experiment; these intensities were subsequently delivered pushing a handle (figure 4.8B). The maximal pressure never went over 5000 N, in order to avoid subject's skin damage (Neziri et al. 2011).

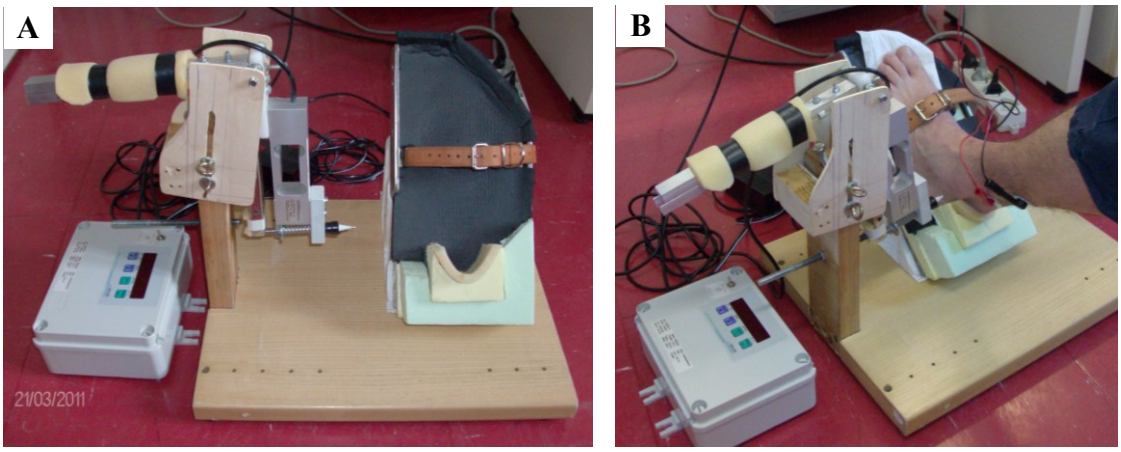

Figure 4.8 – Setup for the pressure pain stimulation. Pressure pain stimuli were delivered by a custom-made manual pressure algometer (*A*). A plastic tipped probe was used to apply painful stimuli on the lateral ankle of the subject, while a foot-holder allowed a stable position of the foot for a more precise stimulation (*B*). The operator chose could set the stimulus intensities by mean of a screw, whereas the stimuli were delivered with the yellow handle. Moreover, during all the experiment, the operator could check on a digital display the applied pressure. Finally, Two electrodes were placed just above the ankle and were used to simulate the procedure for TENS (*B*).

4.2.4 - Statistical analysis

In both the Experiments, statistical comparisons were performed by means of Wilcoxon rank-sum tests, carried out by using 'R' environment for statistical computing and graphics, freely available at http://www.R-project.org/ (Ihaka and Gentleman 1996). A probability value of 0.05 was accepted as the level of statistical significance in all the tests performed.

In Experiment2, a clusterization method was applied on the pain ratings of each subject when no cues were presented and a medium pain intensity was delivered. The data were clustered by the by the *k*-means method, which is basically aimed to partition the points into 2 groups such that the sum of squares from points to the assigned cluster centers is minimized. The algorithm was implemented in package 'stats' of the software 'R'.

4.3 - Results

In the Bayesian framework developed at our laboratory, pain perception results from an inferential process in which noxious stimulation, previous experiences and concurrent contextual information are integrated.

A summary of the mathematical steps followed to esteem the input to the Bayesian model and to calculate the model predictions is represented as a relational scheme in figure 4.4. Specifically, the model was applied to describe the outcomes expected in two experiments in which placebo analgesia was induced by classical conditioning.

To verify the predictions of such a model the predicted pain scoring where compared to the outcomes from two different experiments, performed on healthy volunteers (see 'Materials and methods').

In the following, the data predicted by the Bayesian model will be first desribed, then the data obtained in the experiments will be presented and compared with the predictions.

4.3.1 - Data expected according to the Bayesian model of pain perception

The model proposed represents the pain scoring reported by the subjects in terms of probability distributions. A visual analogue scale (VAS) was used by the subjects to evaluate their perception, therefore the model predicts the probability of any point on the scale, given a stimulus intensity and a visual cue. Figure 4.9 shows the probability distribution of the pain ratings, associated with a given noxious stimulus (in this case, with an arbitrary intensity of 35 on a 0-to-100 range).

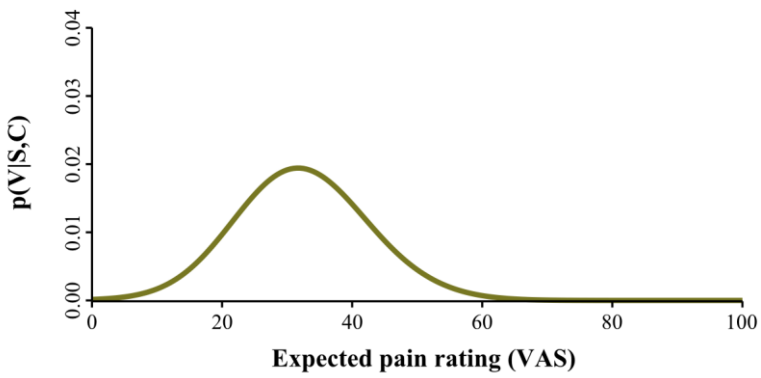

Figure 4.9 – Probability distribution of the pain ratings expected before conditioning session. The pain ratings expected before conditioning session are described in terms of probability distribution centred on the most probable pain perception evoked by a given stimulus intensity, which, in this case, was equal to 35 on an arbitrary 0-to-100 range.

The example in figure 4.9 describes the relation between the expected pain scoring and the stimulus intensity before any experimental manipulation.

After the conditioning session (which is used in experiments to induce an effective placebo effect, see 'Materials and methods'), the subject's knowledge about the possible noxious stimuli is updated and, thus, the probability distribution of pain scoring changes as shown in figure 4.10. This figure represents two cases in which a high or a low stimulus intensity are alternatively applied, without any visual cue, in order to show what the model predicts about the influence of pain perception after the conditioning session. The two curves are the probability distributions obtained for a stimulus of intensity similar to that used in figure 4.9 and another one at a higher intensity (45 on the same arbitrary scale of the first stimulus). A comparison with figure 4.9 highlights what the model describes when the previous experience modifies the knowledge of the subjects, adding new pieces of information regarding the experimental context and the painful stimuli that could be applied. In particular, the probability distributions change from a bell-shape curve to a double-peak curve, where the higher peaks represent the most probable pain rating reported in the testing session, after the conditioning phase.

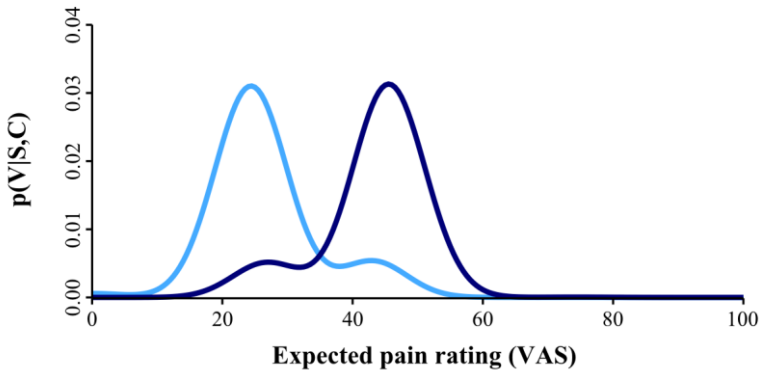

Figure 4.10 – Probability distribution of the pain ratings expected after conditioning, in the absence of the visual cues. The pain ratings expected after the conditioning session are presented for two stimulus intensities, similar to those applied during the conditioning session. In this case, however, no visual cues have been considered, in order to evaluate the model predictions about the changes induced by the conditioning procedure.

According to the model, the conditioning session changes the probability distribution due to the fact that two different pain intensity levels are experienced by the subjects in this phase. In other words, the stimulus-VAS relationship was modified because the subject learnt that only two pain intensities were possible. Therefore, the conditioning had the role of giving previous knowledge about the experimental context and which influence the possible and more probable intensities perceived during the test session, shaping the probability distributions that ruled the relationship between intensity of stimulus and perceived sensation. This means that the pain perceptive system would consider more probable a sensation similar to that felt during the conditioning phase and, therefore, would be biased towards these pain levels.

It is worthy of note that the probability distributions have now two peaks even if, in the case of a noxious stimulus similar to that applied during the conditioning session, one of the two is higher than the other. On the other hand, in the case of a stimulus with an intensity equal to the average of the high and the low intensities, the model predicts two peaks of equal height, and consequently two VAS values as the most probable pain ratings as shown in figure 4.11. This led to hypothesize that in such a condition the subjects would report two pain ratings with equal probability.

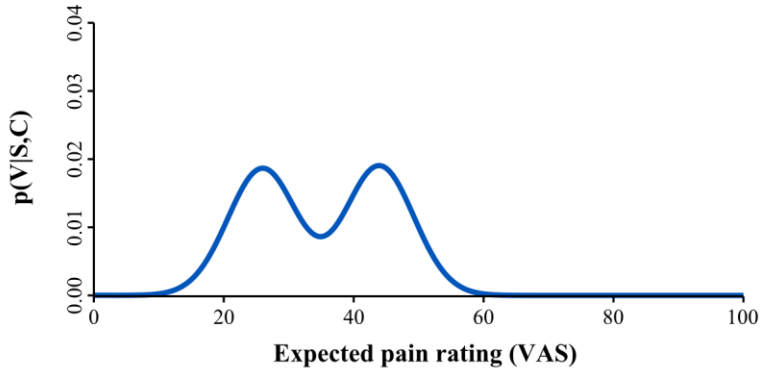

Figure 4.11 – Probability distribution of the pain ratings expected after conditioning without the visual cues and a medium-intensity stimulus. The curves represents the probability distribution of the subjects' pain ratings when a mid-intensity stimulus would be applied without any visual cue.

The model predictions were subsequently investigated in order to evaluate the cases in which visual cues were presented paired with noxious stimuli. In other words, after having assessed the data expected after the conditioning session, regarding the role of this phase in changing the subject's knowledge about the experimental procedure, the case in which the visual cues provided new pieces of information to the ongoing pain processing were considered. For this purpose, two conditions were taken into account: first, the model predictions about the placebo analgesic effect, induced by the experimental procedure, than what the model predictions about the condition in which a mid-intensity stimulation is applied together with the green or the red cue. Therefore, both these case involved the presentation of conflicting pieces of information, coming from the visual cues and the noxious stimulation.

The analgesic placebo effect was experimentally induced by pairing the noxius stimuli with a visual cue (see Materials and methods) that gave the subjects a clue about the possible noxious stimulus, thus shifting the expectation towards a low pain level, as previously experienced in the conditioning phase. As shown in figure 4.12, the model predicts that, when a high intensity stimulus is applied together with a green cue, the pain scoring is shifted toward the pain ratings reported for the stimulus intensity (low) associate with green cue in the conditioning phase, in agreement with the outcomes from experiments of classical conditioning of the placebo effect. Therefore, the model integrates two opposite sources of information: the incoming sensory data and the contextual information. These conflicting data

are weighted and integrates to produce a pain perception that is between those perceived in the conditioning session, correctly matching experimental results.

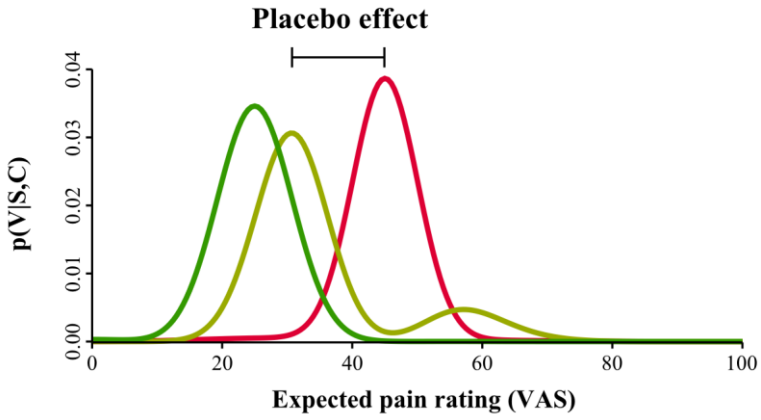

Figure 4.12 – Probability distribution of the pain ratings expected after the conditioning session, with the visual cues would be presented with the noxious stimuli. The model predicted an interaction between the information coming from the visual cue and the data regarding the noxious stimulation. In fact, when a high intensity stimulus would be applied together with the green cue, pain ratings would be expected to be shifted toward those observed during the conditioning session with green-low intensity stimulus.

To further test the predictions of the Bayesian model for stimuli applied after the conditioning session, the effect of a mid-intensity stimulus paired with a visual cues was considered in the model, and then experimentally verified. This experiment let evaluate if contextual information is combined with the knowledge acquired during the conditioning session and the nociceptive inputs according to Bayesian inference or following some different, maybe heuristic, strategy.

In a Bayesian framework, the probability distribution associated with the medium intensity is modified as a consequence of the conditioning procedure, according to the weighted integration of (1) the likelihood of the intensity given the colour of the visual cue, (2) the likelihood of the intensity given the nociceptor activity and (3) of the prior probability of the intensity given the experimental context. Figure 4.13 shows that the same stimulus intensity give rise to different probability distributions according to the cue and stimulus provided.

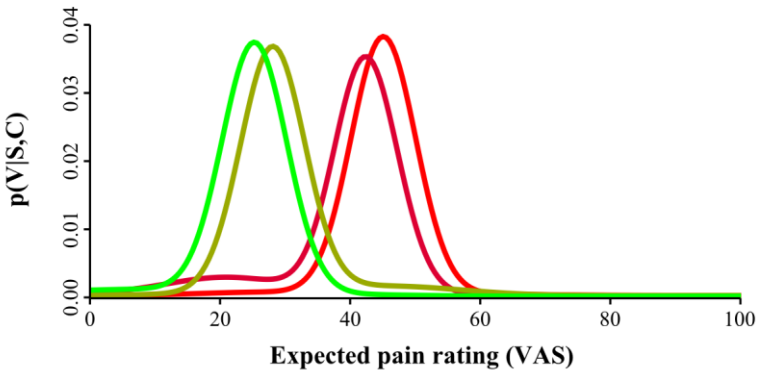

Figure 4.13 – Probability distribution expected after conditioning session, when contextual information are provided together with the noxious stimulus. The pain ratings expected in the testing session when a mid-intensity stimulus is paired to a visual cue. In this case, the model predicts that the information provided by the visual cue shifts the perception of pain towards those pain intensities perceived during the conditioning session in association with a specific visual cue. The probability distributions of the pain ratings reported after a low or high stimulus associated with the green or the red cue respectively, are also reported.

To summarize, the proposed model, based on Bayesian decision theory, was evaluated considering expected pain ratings in different experimental conditions. In order to assess whether the model predictions were in agreement with experimental data, two experiments were performed with healthy human volunteers.

1. The first experiment was aimed to acquire the data and test the following two predictions:
   - the placebo effect or, in other words, the differences between pain levels evoked by a high stimulus intensity associated with both visual cues (figure 4.12).
   - the pain perception evoked by an mean intensity chosen between the high and the low intensities used in the conditioning session, without the presentation of any visual cues (figure 4.11).

2. On the other hand, a second experiment was thought to verify whether the model correctly predicted the modulation of pain perception produced by the concurrent presentation of conflict pieces of information: a mid-intensity stimulus applied together with a visual cue indicating an high or low stimulus intensity (figure 4.13).

4.3.2 - Comparison between expected and observed data

The details of the experimental procedure used in the experiments are described in 'Materials and methods'. Briefly, the experimental procedure included a conditioning session in which two associations visual cue-stimulus intensity (green-low intensity; red-high intensity) were possible, with the same frequency. The subjects were actually told that an analgesic treatment would be used to reduce the painful perception evoked by the stimulus applied during the experiment; specifically the subjects knew that a single intensity level would be used and that the analgesic procedure would be active when a green visual cue was presented on a PC screen, whereas it would not be applied with the red cue. The efficacy of classical conditioning session in inducing the placebo analgesic effect was quantified in a subsequent session, evaluating the differences in the pain ratings reported for the high-intensity stimulation applied alternatively with the red or with the green cue.

A testing session followed the conditioning one. Although the conditioning procedure was the same, the stimulation protocols applied in this subsequent phase differed between the two experiments because they were planned to evaluate test specific predictions derived by the Bayesian model. In particular, different cue-stimulus associations were presented in the testing sessions. Therefore, the results of these experiments will be presented in separated paragraphs, according to the testing protocol applied and to the model predictions that were evaluated.

The placebo effect

The mean pain rating that subjects reported when both cues were associated with high-intensity stimuli is shown in figure 4.14A. In particular, the mean (±SD) pain observed for the red and the green cues were respectively 22.4(±16.3) and 31.1(±18.4) and the statistical analysis with Wilcoxon signed-rank test showed a significant mean percentage difference of 31.8% (±19.5), indicating that the conditioning session was effective in inducing the placebo analgesic effect in the group of subjects (figure 4.14B). Finally, figure 4.14C shows the probability densities predicted by the Bayesian decision model in the upper panel, while 100 random samples from the distributions are presented in the lower panel. These graphs are shown to facilitate the comparison between data observed and predicted, in order to evaluate the correctness of model predictions.

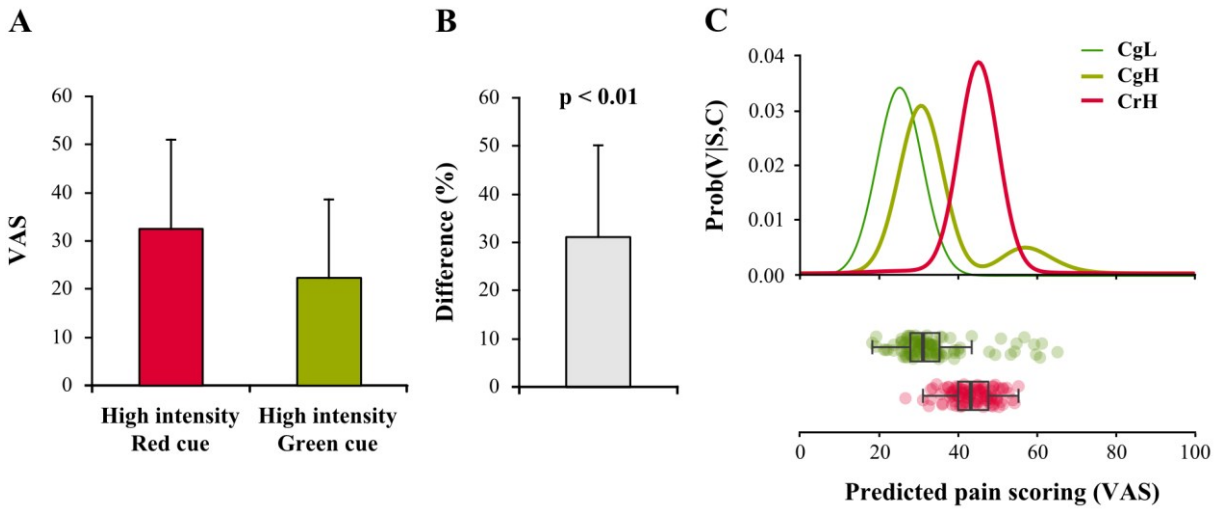

Figure 4.14 – Placebo analgesic effect in the group of healthy volunteers. The conditioning session was able to induce a placebo pain reduction, observable in *A* comparing the mean pain scoring with the two cue-stimulus associations, whereas in *B* it is expressed as the mean difference between red cue- and green cue-associated pain reports. In both panel, bars represent standard deviations. Model-predicted posterior probability densities and 100 random samples from each conditions are presented in *C*; boxplots represents the sample minimum, lower quartile, median, upper quartile and sample maximum. Samples outside the boxplot are considered as outlier. Stimulus-cue associations are abbreviated as follows: CgL, green-low; CgH, green-high; CrH, red-high.

Role of past experience in shaping the pain perceived with a noxious stimulus

The model predicts that the conditioning phase changes the relationship between the noxious stimulus and the evoked pain perception, as shown in figures 4.10 and 4.11. To test whether the data expected form the model were in agreement with those measured in an experimental setting, a block of stimuli was designed so that half of the pulses were delivered at an intensity equal to the average intensity of those chosen for the conditioning session, while, in the other half, the pulses were applied at the same high and low intensities used in the conditioning session (see figure 4.5 in 'Materials and methods'). The key point is that, in the test block, no visual cue was presented to the subject, in order to measure the pain perception in absence of contextual information about the analgesic procedure.

The results are shown in figure 4.15A. The mean (±SD) of the pain ratings reported by the subjects for the low, medium and high pain intensities was respectively 11.7 (±9.2), 24.3 (±15.4) and 33.11 (±17.6), that is they were proportional to the pain intensity. However, the computation of the mean scoring did not allow to evaluate possible multimodal clustering of the pain ratings, which is, instead, the prediction of the Bayesian model (see figure 4.11). Figure 4.15B shows the data recorded from three representative subjects, while the probability distributions provided by the model and 100 random samples are presented in figure 4.15C. Considering single pain ratings, it was noticed that some subjects reported a pain sensation

distributed around a medium value (right panel in figure 4.15B), between the higher and the lower ones. Though, other subjects reported pain sensation grouped around two values, one similar to the VAS scoring reported with the high-intensity stimulus and one similar to the rating with the low-intensity stimulus (see left and middle panel in figure 4.15B), supporting the validity of the Bayesian approach.

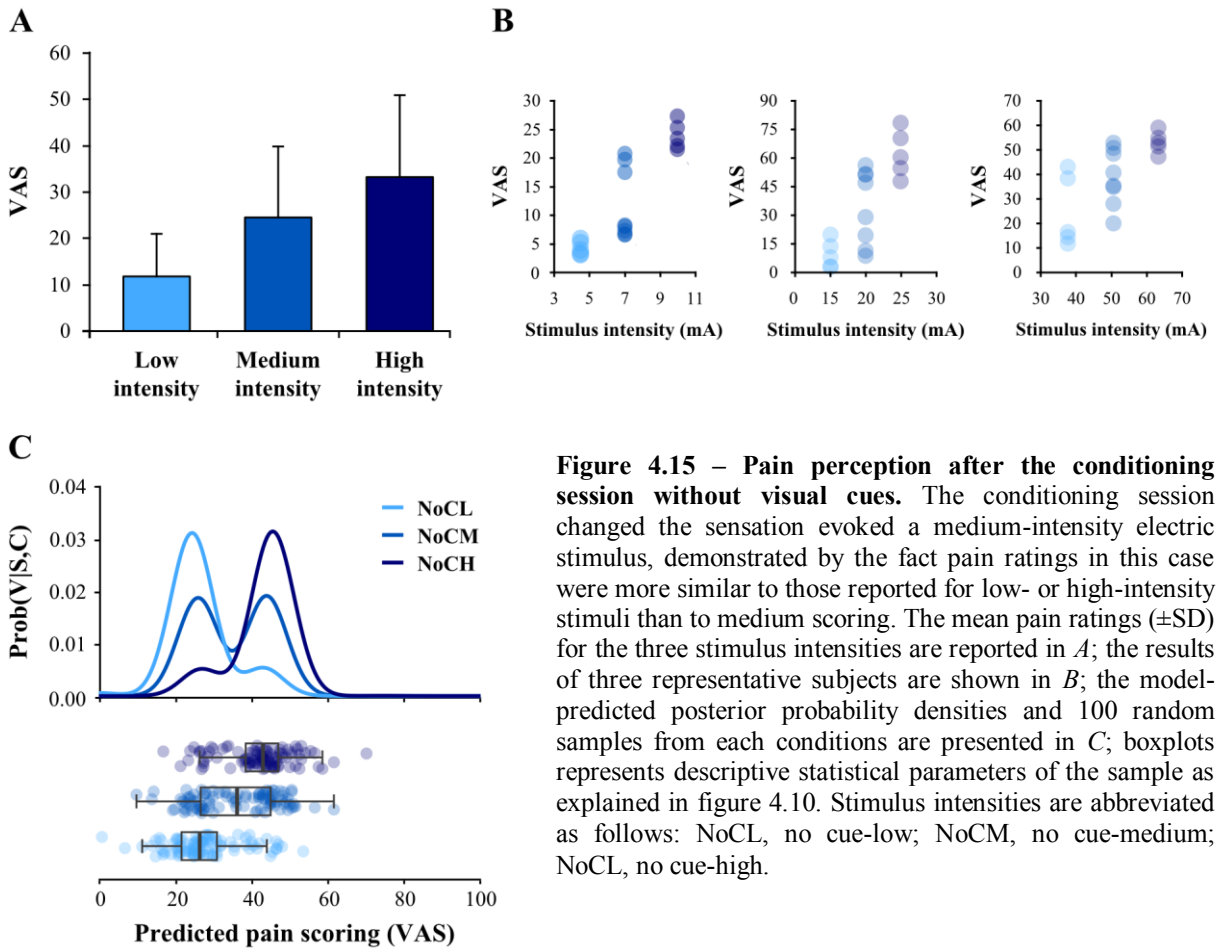

Figure 4.15 – Pain perception after the conditioning session without visual cues. The conditioning session changed the sensation evoked a medium-intensity electric stimulus, demonstrated by the fact pain ratings in this case were more similar to those reported for low- or high-intensity stimuli than to medium scoring. The mean pain ratings (±SD) for the three stimulus intensities are reported in *A*; the results of three representative subjects are shown in *B*; the model-predicted posterior probability densities and 100 random samples from each conditions are presented in *C*; boxplots represents descriptive statistical parameters of the sample as explained in figure 4.10. Stimulus intensities are abbreviated as follows: NoCL, no cue-low; NoCM, no cue-medium; NoCL, no cue-high.

As we have seen, not all the subjects reported grouped pain sensations (figure 4.15B, right panel) and the model predictions were investigated in order to evaluate whether it could explain the differences found within the group of subjects. We hypothesised that the effectiveness of the conditioning procedure could be a relevant factor related to such a behaviour. As previously explained (see paragraph 4.2.2), the model integrates different pieces of information that differentially contribute to the perception of pain evoked by a noxious stimulus. In the experimental context, a source of information was the past experience of pain that depended on two factors: the previous experiences that subjects had had in their lives and the knowledge acquired during the conditioning session, about the possible pain levels in the experiment and their associations with visual cues that, in this case, informed the subjects

about the analgesic treatment. The degree of efficacy of the conditioning session and how the pain sensory system weighted the knowledge acquired during this phase determined the pain perceived in the testing session and therefore the scoring on the visual analogue scale. To simulate this effect in shaping the prior knowledge at hand before the test stage, in the esteem of the input to the model these two sources of previous knowledge are weighted according to the scaling factor R, before entering in the Bayes rule as prior probability (see figure 4.4). Therefore, the role of conditioning was assessed considering different values for the scaling factor R. The results are presented in figure 4.16: the panel A shows the expected VAS reports (100 samples) from five probability distributions shown in panel C, each one computed considering a different value of the parameter R. Figure 4.16B represents the single pain scoring reported by the subjects when the mid-intensity stimulus was applied (these data were the same data considered in figure 4.15). In this case, the amount of pain reduction induced by placebo was considered with the purpose of evaluating the efficacy of conditioning or, in other words, the degree of influence that the experience made in the experiment before the testing session had on the perceived pain. It is possible to observe that the referred pain perceptions were divided in two groups for higher amount of placebo response (figure 4.16B).

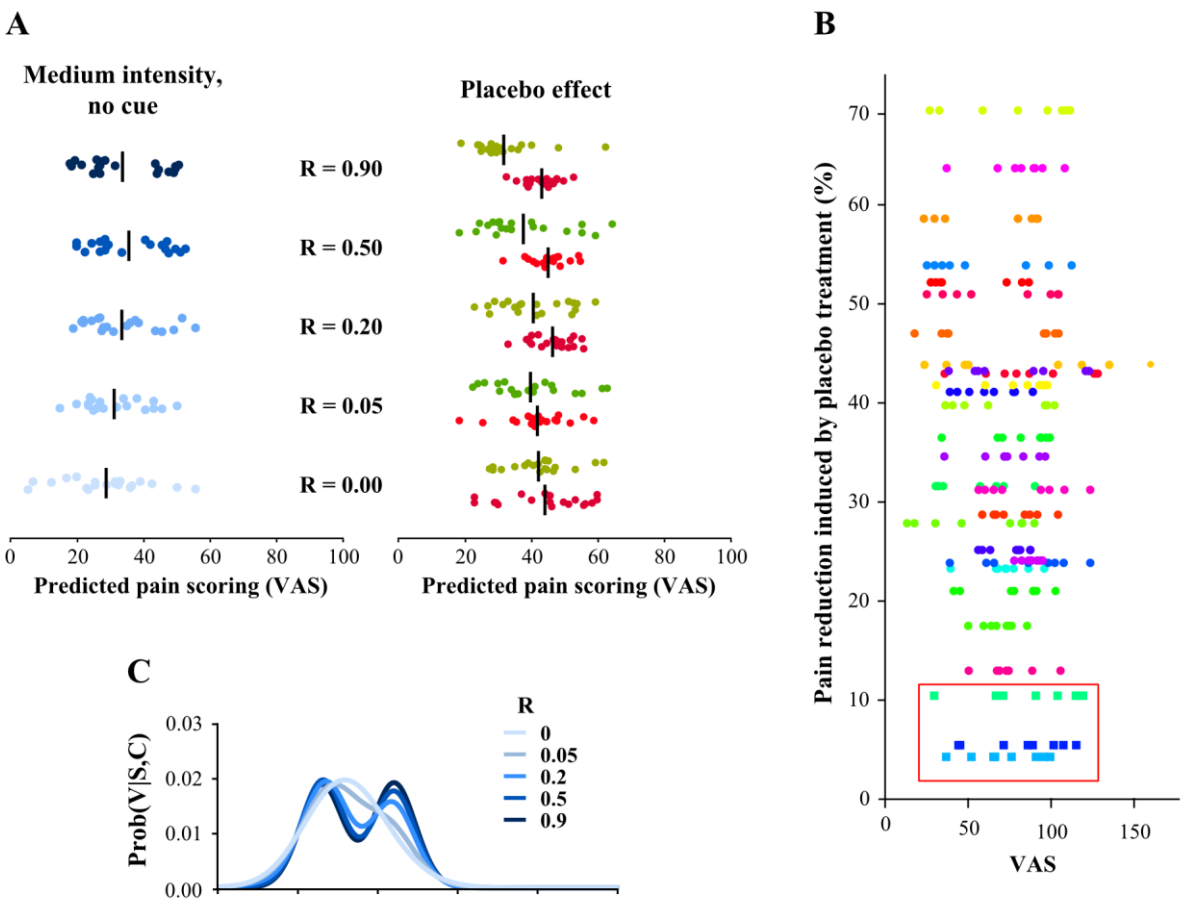

Figure 4.16 – Relationship between the efficacy of conditioning session and the pain reports for medium intensity noxious stimuli. The efficacy of conditioning session is important to determine the pain sensation perceive in the test session. In *A*, 20 samples from probability distributions with weights for conditioning session are shown: the left panel represents samples from the medium intensity-no cue condition, in the right panel expected data for placebo effect are presented. In the group of subjects, the efficacy of conditioning was assessed considering the amount of placebo effect in each subject. In *B* the relation between the placebo response of each subject and the VAS rating for medium stimulus intensity is represented. To allow comparison, VAS data of each subject was scaled to the mean report for subject's high stimulus. The placebo response was not statistically significant in three subjects (red box). In *C*, the five probability distributions (medium intensity stimulus, no cue) are presented, each one obtained with a different value for the parameter R that indicates the weight given in the model, to the conditioning session.

The modulatory effect of conflicting pieces of information

To address how the adding of new pieces of information could change the perception of the subject and whether the model predictions were in agreement with the data recorded from human volunteers in this condition, a second experiment was performed. This experiment was subdivided in two phases as the previous one: the same intensity-cue associations were maintained in the first two blocks of stimuli (conditioning session), while the test session consisted in a block of stimuli delivered at high, medium and low intensities. However, in this case the subjects were told when the analgesic treatment would be active, presenting the coloured visual cues on the PC screen. In particular, the high intensity stimulus was always associated with the red cue as well as the low intensity with the green cue. In the case of

medium intensity, half of the noxious stimuli were associated with red cue, whereas the other half with the green cue (for details see 'Materials and methods' and figure 4.7). Thus, in the second experiment the stimulation procedure was similar to that used in the previous experiment, expect for the presentation of the visual cues with the noxious stimuli, when mid-intensity was applied.

The results of this experiment are presented in figure 4.17. As expected, the subjects reported higher mean pain scoring with the association red-high intensity than with green-low intensity (29.9±13.1 and 6.6±7.4 respectively) and the mean percentage difference of 77.5%(±18.5) was statistically significant ($p<0.001$). In the case of the intermediate intensity, the observed pain ratings were different according to the colour of the cue: although the stimulus intensity was always the same, the volunteers referred to perceived a higher pain intensity when the red cue was presented (23.6±17.1) then when the green cue was displayed on the PC screen (11.5±13.1); a mean percentage difference of 51.4% (±31.0) was found to be statistically significant ($p<0.01$). Moreover, the differences between the pain rating reported for different intensities associated with the same cue were considered. In the case of the green cue, the mean percentage difference between VAS rating with low and medium intensities was equal to 41.7% (±26.5) and it was statistically significant ($p<0.001$); on the contrary, no significant difference was observed between the two intensities associated with red cue.

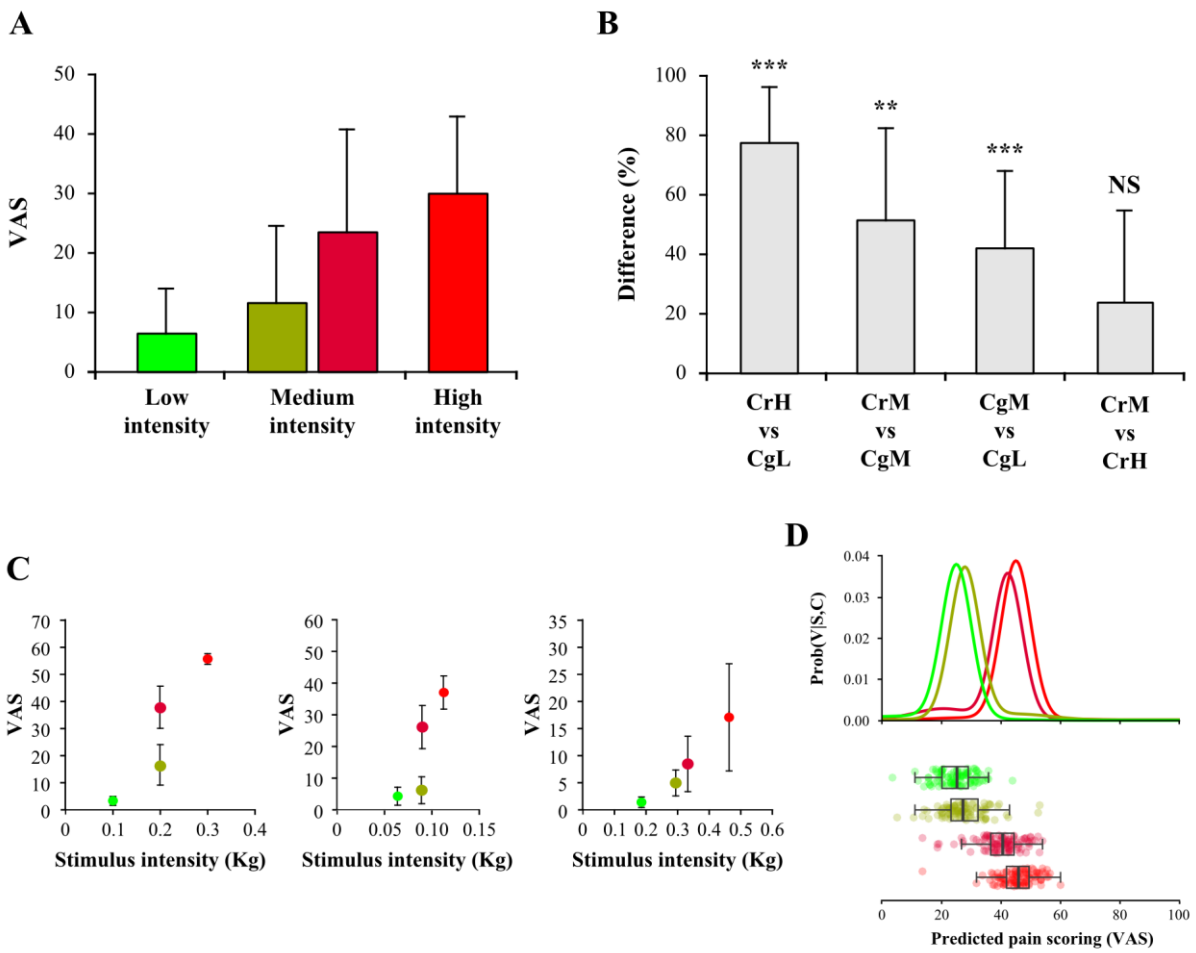

Figure 4.17 – Pain sensation referred when contrasting inputs were given to the subject. In the second experiment, three stimulus intensities (low, medium and high) were used in the test session, as in the first experiment. However, in this case, a new piece of information was added through the presentation of visual cues: the presence or absence of the sham analgesic treatment. The mean pain ratings for the four associations (low-green, medium-green, medium-red and high-red) are shown in *A*, while the mean differences are shown in *B*. The results of three representative subjects are shown in *C*. To compare observed and expected results, model predicted probability distributions and data are presented in *D*. Boxplots represents descriptive statistical parameters of the sample as explained in figure 4.10. Stimulus-cue associations are abbreviated as follows: CgL, green-low; CgM, green-medium; CrM, red-medium; CrH, red-high. Statistic significance in the mean differences is indicated as follows: **, $p<0.01$; ***, $p<0.001$; NS, not significant.

These results demonstrated that, when two contrasting inputs were handled by the pain perceptive system, the system weighed both sources of information, resulting in a pain percept that was not determined exclusively by the nociceptive information but was biased by the contextual information. The same results were predicted by the Bayesian decision model as it is possible to observe comparing figure 4.17A and figure 4.17C.

To conclude, the findings presented here demonstrated the consistency between the data expected according to the Bayesian model and the VAS pain ratings reported in experimental settings. The model predictions were compared with data recorded in two experiments which involved a classical conditioning session to induced modulation of pain perception

Specifically, in the first experiment, a testing session was used to evaluate the placebo effect and the pain-modulating effect of the knowledge acquired during conditioning, whereas the weights given by the pain sensory system to contrasting pieces of information were investigated in the latter. In both cases, the predicted data were in good agreement with the data observed, supporting the hypothesis that the integration performed by the brain to produce the pain perception can be described in terms of inferential statistics and that Bayesian decision theory represents a valuable formal framework for describing and understanding this integrative process.

4.4 - Discussion

In recent years, the view of pain as a hard-wired system in which noxious input are passively transmitted along sensory channels to the brain has changed into a description of pain as an multifactor process, driven by noxious stimulus and many other factors. Clinical and everyday life teaches us how cognitive and emotional variables such as attention, expectation, prior experiences, and mood (figure 4.18) shape our perception of pain (Bingel and Tracey 2008). So pain can be considered as the result of an integrative process initiated by an incoming noxious stimulation, but subsequently modulated by other factors in order to create a reliable perception and guide effective actions and behaviours. The pain experience evoked by the same sensory input can vary considerably, and we suggest that it resembles what happens with well-known visual perceptions, such as ambiguous figures (Ernst and Bulthoff 2004).

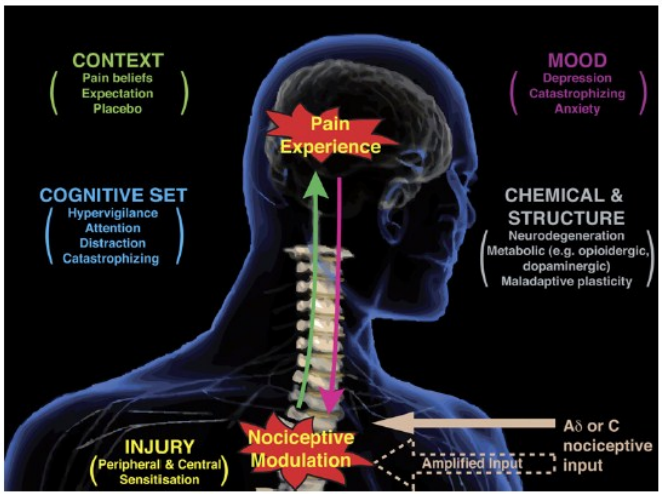

Figure 4.18 – Schematic illustrating the main factors that influence nociceptive inputs and affect pain perception. *From (Tracey and Mantyh 2007)*

How the pieces of information derived from these factors are integrated within the central nervous system and which computational and logical steps and strategies are followed by the nervous system are mainly unknown. Therefore, together with the investigation of the neuronal substrates that are involved in this integrative process, in recent years the development of formal model to explain and predict observations of psychophysics phenomena has become of great interest in many fields of cognitive neuroscience (Lee 2008). In particular, Bayesian methods have been recognized as one of the most complete and coherent available way to describe perception, and several examples have been proposed for describing visual perception (Geisler and Kersten 2002; Ernst and Bulthoff 2004), multisensory integration (Rowland et al. 2007; Beierholm et al. 2009), sensorimotor control (Wolpert 2007) and learning (Courville et al. 2006; Tenenbaum et al. 2006).

In the study described in this thesis, a theoretical model describing the integrative processes underlying the perception of pain was evaluated. The model, developed in our laboratory, is

based on Bayesian decision theory and its correctness was supported by the results of here described, which show the agreement between the prediction of the model and the data recorded from healthy human subjects in two different experiments. In particular, the model was built to optimally integrate past experience with different sensory and cognitive information involved in the perception of pain. As demonstrated by other authors, a classical conditioning procedure, in which pain perceptions of different intensities were associated with different visual cues, is effective in inducing a significant placebo effect, proving to be a valuable tool for studying the placebo effect in an experimental environment (see for example (Colloca and Benedetti 2006). Among all the several factors that modulate the experience of pain, the conditioning session acts as a prior positive experience that plays a key role in maximizing the placebo response in the subsequent testing session. Considering the experimental procedure used in the experiments from the point of view of the Bayesian decision theory, the conditioning session is essential to shape the prior knowledge (prior probability) that enters the Bayes rule:

$$\underbrace{P(\theta|s)}_{\text{Posterior}} = \frac{\overbrace{P(s|\theta)}^{\text{Likelihood}} \; \overbrace{P(\theta)}^{\text{Prior}}}{P(s)}$$

Testing sessions followed the conditioning ones in our experiments. They were designed to evaluated the response of the subjects under different conditions, for which the model made testable predictions.

The data recorded in the group of subjects demonstrated that the conditioning procedure applied in the experiments here described was effective in inducing the analgesic placebo effect (see figure 4.14). Moreover, the Bayesian model was effective in explaining the placebo effect observed in the subjects (see figure 4.12 and figure 4.14C). It should be notice that the model was not developed with the specific intent of obtaining the placebo effect, but to describe the integrative process performed by the pain perceptive system in a formal framework. In fact, the Bayesian model was developed considering the pieces of information that the sensory-cognitive system integrated to produce the pain sensation, without the specific goal of predicting the analgesic placebo effect. Therefore, the agreement between the data obtained from the experiment and those predicted by the model should be considered a first confirmation of its value in explain the pain perceptive processing.

To further investigate the validity of the model, the ability of the Bayesian model to predict subjects' responses was tested, considering two different stimulus intensity-visual cue associations. Two testing protocols different from that applied for evaluating the placebo effect were considered. Also in these cases, the model makes prediction in agreement with the pain ratings reported by the subjects in the experimental conditions. In the first case (mid-intensity stimuli with no cue, see figure 4.15) the model correctly predicted the role of the conditioning session in shaping the pain scoring of mid-intensity stimuli. Moreover, the model seems to be able also to explain the variability in the subjects responses, and this on the basis of the different weights that the subjects could give to the conditioning session and the pain perceived in it (figure 4.16).

Finally, in the second case, another source of information was added, specifically one of the two visual cues was presented together with mid-intensity stimuli, in order to assess whether the subjects integrated concurrent conflicting pieces of information in the same way as predicted by the model, that is following Bayesian inference rules. In this case, in fact, the noxious sensory inputs were in contrast with the visual contextual information that indicated an expected higher (with the red cue) or lower (with the green cue) pain perception. Given these factors, the model predicted that the subjects pain scoring would be biased toward the VAS scoring reported during the conditioning session after the presentation of the cues (figure 4.13). The data expected were correctly replicated by the results reported in the experiment (figure 4.17), further supporting the consistency of the model developed.

Together with the results about the placebo responses, these latter cases gave further support to the correctness of the model proposed, because its predictive validity was demonstrated in two conditions that were different from what assessed in the experimental procedures commonly applied for investigating placebo analgesia.

The model proposed was not intended to be definitive; however, its application in the experiments presented showed, was that even with this simple formulation, such a Bayesian approach may provide useful information not available in previous analyses, and can lead to the design of knew experiments.

To conclude, the experiments presented here agree and support the Bayesian model of pain perception. Such a theoretical approach not only fits experimental data, but it seems to be useful both because it makes knew predictions, which may lead to new experiments and insights in pain perception, and also because it may be used in explaining, at a computational level, the integrative processes performed by the nervous system and the logic underlying pain

perception. This would hopefully contribute in the understanding of pain both in healthy subjects and in patients.

5 - MODULATION OF PAIN PERCEPTION AFTER EXHAUSTIVE EXERCISE AND INFLUENCE OF FITNESS STATUS

5.1 - Introduction

Exercise is an important and low-cost method to maintain or increase a good status of health and many studies have demonstrated its efficacy as preventing factor of several pathologies such as cardiocirculatory diseases (Larun et al. 2006; Bender et al. 2007). Furthermore, a constant and middle-intensity exercise is able to produce a physical and mental state of well-being, that includes sedation, anxiolysis and analgesia (Dietrich and McDaniel 2004; Bender et al. 2007). In particular, the effect on pain perception and the mechanisms of its modulation are two of the most interesting, in the light of possible therapeutic application of physical activity as nonpharmacological treatment for several diseases (Edmonds et al. 2004; Whiteside et al. 2004; Hoffman et al. 2005; Hauser et al. 2010; Stagg et al. 2011).

In the last few decades, many studies have investigated the relationship between physical exercise and pain perception, showing how the exercise can modulate pain perception inducing an hypoalgesic state called exercise-induced analgesia (Koltyn et al. 1996; Koltyn and Arbogast 1998; Koltyn 2000). These studies focused their attention on exercise intensity and duration as well as the type of exercise, even if in most of the cases findings were obtained in experiments on aerobic exercises at treadmill or cycle ergometer (Koltyn 2002; Koltyn and Umeda 2007). In this context, several authors have tried to define whether there is a direct correlation between the amount of analgesia and the intensity of the exercise or its duration, using subject's pain scoring or pain threshold and tolerance as parameters to assess modification in pain perception (Koltyn 2002). Considering exercise intensity, some work has suggested that intensities of over 70% of maximal aerobic capacity are required, and that pain inhibition increases with increasing intensities above this level. For example, Hoffman and colleagues (Hoffman et al. 2004) focused their attention on maximal oxygen uptake (VO_{2max}), as a possible parameter to measure the analgesic-effect threshold. They showed that aerobic exercise of 30 minutes at 75% of VO_{2max} resulted in a significant exercise-induced analgesia, instead 30 minutes of exercise at 50% of VO_{2max} or 10 minutes at 75% of VO_{2max} were inadequate to induce the effect (figure 5.1). Therefore, their findings support the hypothesis of an intensity threshold (>50%-70% of VO_{2max}) for exercise-induced modulation of pain

sensation, suggesting in addition a duration threshold (>8-10min) for eliciting a significant hypoalgesic effect and suggested that there may be not only a threshold for intensity but also for its duration.

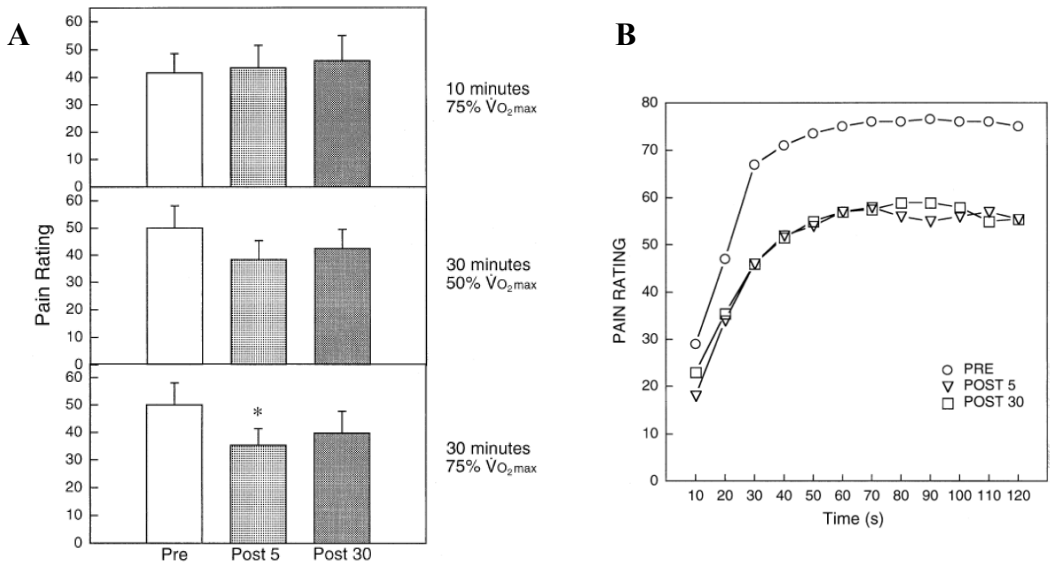

Figure 5.1 – Pain-pressure thresholds are influenced by physical exercise. A threshold for both exercise intensity and duration has been suggested, in fact Hoffman and collaborator (2004) have demonstrated that an analgesic effect was observed only in the group of healthy subjects that performed an exercise at 75%VO_{2max} and for 30 minutes on treadmill. No significant effects were observed in the other two groups (*A*). Moreover, reduced pain ratings were measured 5 minutes after the exercise, while the scoring was slight or not different from baseline values after 30 minutes. In *A* mean pain ratings for the three groups of subjects at different time points are reported, while an example of ratings for a single subject at each 10-second interval before, 5 minutes after and 30 minutes after exercising for 30 minutes at 75%VO_{2max} is shown in *B*. Significant differences are indicated as *, $p<0.05$. *Modified from (Hoffman et al. 2004)*

However, the duration and the intensity of exercise required to elicit the exercise-induced hypoalgesia still remains not fully clarified. For example, the same authors that investigated pain ratings with mechanical stimuli, failed to observe influences of thirty minutes of aerobic exercise at 75% of VO_{2max} on sensory threshold, pain threshold, or pain ratings to hot or cold stimuli, suggesting that exercise-induced analgesia is dependent on the type of painful stimulus used.

Despite many studies have demonstrated that physical activity acts reducing pain sensation, data from pathological conditions such as fibromyalgia and chronic fatigue syndrome suggest a possible relation between fatigue and pain. In fact, subjects affected by these pathologies often report enhanced responses to painful stimuli (Whiteside et al. 2004; Meeus et al. 2010), together with fatigue (Mourao et al. 2010; Sarzi-Puttini et al. 2011). These observations together with finding in healthy subjects (Svensson et al. 2001; Torisu et al. 2007) led to hypothesize an interaction between pain and fatigue, such that fatigue may enhance pain. A

recent study have revealed that fatigue enhanced the hyperalgesic state in an animal model chronic widespread pain (Yokoyama et al. 2007). In fact, mechanical sensitivity, measured as the number of withdrawal response to mechanical stimulation of the paw with von Frey filaments, were significantly enhanced in mice that were forced to run on a wheel and therefore were fatigued. Further investigation about the neurobiological mechanisms that could be responsible for this effect have revealed the involvement of structures of the central nervous system, such as brainstem nuclei and pain-sensitizing descending pathways (Sluka and Rasmussen 2010; Sluka et al. 2011). In the context of exercise-induced modulation in pain perception and analgesic effect, these findings suggest that physical activity could differentially act on a balance between opposite pain-modulating mechanisms (Heinricher et al. 2009), in relation with the type and/or the intensity of the exercise. The investigation of the relation between exercise intensity and the effect on pain sensation and the underlying neurobiological mechanisms acquires particular relevance, in the light of the clinical importance of exercise-induced analgesia as a possible therapeutic treatment for diseases often associated to chronic pain, such as, for example diabetes (Hassett and Williams 2011; Shankarappa et al. 2011; Stagg et al. 2011).

5.2 - Materials and methods

5.2.1 - Subjects

Fifteen cyclists (age, mean ± S.D. = 29.7 ± 10.1 years) and fifteen untrained volunteers (age, mean ± S.D. = 23.5 ± 6.3 years) entered the study. All subjects were healthy males with no known neurological and cardiovascular diseases and were not familiar with the protocol before participation in the study. The two groups of subjects were characterized by main biometric and metabolic parameters and values related to exercise performance: age, weight, height, maximal oxygen uptake (VO_{2max}), anaerobic threshold in percentage of VO_{2max} (AT%), maximal workload reached at the end of the incremental activity ($Load_{max}$), total time of exercise (t_{tot}) and the ratio between the time of exercise performed above the anaerobic threshold and the total time (T_{overAT}/T_{tot}).

All participants completed the same experimental protocol, which comprised the measurement of pressure-pain threshold before, immediately after and 15 minutes after an exhaustive exercise on a cycle ergometer.

5.2.2 - Experimental procedure

The experimental protocol applied in this study included the measurement of pressure-pain threshold three times: before, immediately after and 15 minutes after a graded exercise to exhaustion on cycle ergometer (see figure 5.2).

The participants sat comfortably on a chair and were instructed about the experimental procedure familiarized with the pressure-pain test device. Increasing pressure stimuli were then applied in order to calculate the pain threshold (Pre), as described below. This was followed by the maximal exercise test that generally lasted for about 12 or 16 minutes depending on the subject's training level. Immediately after performing the exercise, the participants underwent a second pain testing (Post0), then the subjects waited at rest for 15 minutes and the pain threshold was measured again (Post15).

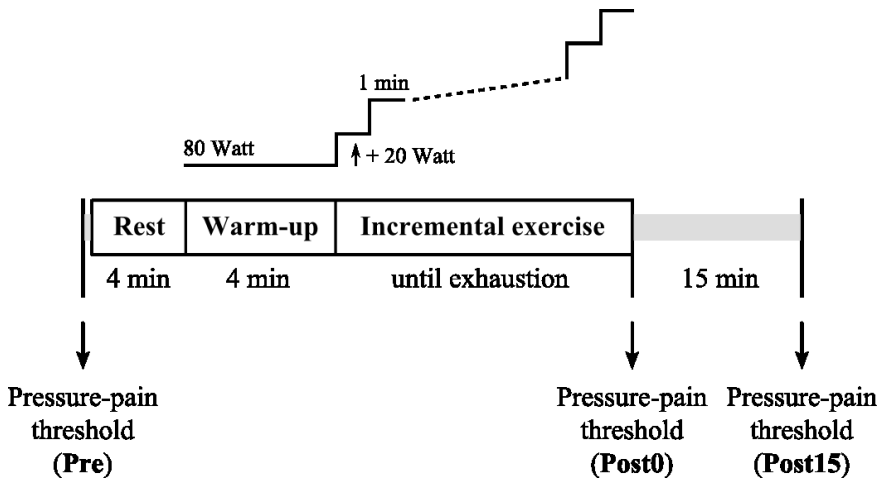

Figure 5.2 - Experimental paradigm applied to test whether modulation of pain sensation could be induced by exhaustive exercise.

5.2.3 - Pressure-pain testing

The pressure-pain custom-made device (figure 5.3) previously described (see paragraph 4.2.2 - *Pressure pain stimulation*) was here used to measure pain threshold in the recruited subjects. The device was made of a plastic tipped probe (surface = 4mm^2) mounted on a force transducer (AZ100, LUMAS elettronica, Italy). As described in Naziri and collaborators (2010), the probe was applied on the back of subject's hand and the force was increased at a rate of 1 N/s, until the subject detected the pain threshold, defined as the point at which the pressure sensation turned to pain. The subjects were instructed to press a button when this point was reached. Eight stimuli were applied and the pain threshold was calculated as the mean of seven trials (the first trial was excluded because the subjects generally overestimated the threshold). The stimulation site was marked after the measurements, in order to be able to apply the stimuli on the same site after the exercise.

Figure 5.3 – Pressure pain device. The pressure-pain device described for placebo-induced analgesia experiments was here used to measure pressure-pain thresholds on the back of subjects' hands.

5.2.4 - Graded exercise and physiological measurements

The exhaustive exercise consisted in a graded continuous protocol with stages performed on a cycle ergometer (Excalibur, Lode, Nederland), while ventilation and oxygen and carbon dioxide concentrations of the inhaled and exhaled air were measured breath-by-breath (figure 5.4). After the recording of metabolic parameters at rest, the subject begins the incremental exercise with 4-minute warming-up stage performed at workload of 80 Watt. Subsequently, the workload was increased by 20 Watt every minute. The exercise continued until the subject was unable to perform the workload.

Pulmonary ventilation, oxygen uptake, carbon dioxide output and respiratory frequency were measured on a breath-by-breath basis by means of a metabolic unit (Quark b^2, Cosmed, Italy). Expiratory flow measurements were performed by a turbine flow meter calibrated before each experiment by a 3 L syringe at three different flow rates. Calibration of O_2 and CO_2 analyzers was performed before each experiment by utilizing gas mixtures of known composition.

Maximal oxygen uptake (VO_{2max}) and aerobic threshold (AT) were calculated from recorded data in order to assess the physical fitness of the subjects recruited for this study.

Figure 5.4 – The subjects performed the exhaustive exercise on a cycle ergometer. Both groups of subjects performed an incremental exercise until exhaustion, during which ventilation and oxygen and carbon dioxide concentrations were measured breath-by-breath.

5.2.5 - Statistical analysis

The main focuses of the study were to examine for changes in pain perception as a result of exhaustive exercise and to investigate whether this change was different between trained cyclist and untrained subjects.

Age, weight, height, VO_{2max}, AT%, $Load_{max}$, t_{tot} and T_{overAT}/T_{tot} were compared with independent-samples t-tests.

A repeated-measures ANOVA was used to analyse differences in pain threshold, with 'Time' and 'Group' as, respectively, within-subject and between-subjects factors. In particular main effects for these two factors were investigated, whereas their interaction ('Time × Group') was considered in order to assess whether the effect of exercise was different for the trained and untrained subjects. Analysis of variance was followed by post hoc comparisons, with Bonferroni's correction of significance level.

Exercise-induced pain changes were evaluated according to the following formulas:

$$\text{PrePost\%} = \frac{\text{threshold}_{\text{post}} - \text{threshold}_{\text{pre}}}{\text{threshold}_{\text{pre}}} * 100$$

$$\text{PrePost15\%} = \frac{\text{threshold}_{\text{post15}} - \text{threshold}_{\text{pre}}}{\text{threshold}_{\text{pre}}} * 100$$

The normal distribution of the variables taken into account in this study was tested with a Shapiro-Wilk test.

Pairwise correlations between PrePost0 or PrePost15 and VO_{2max}, $Load_{max}$, AT% and T_{overAT}/T_{tot} were assessed computing the Spearman's rho correlation coefficient whose statistical significance was tested with non-parametric test.

Data are reported as means ± standard deviation (SD) within the text and displayed as mean ± SD in the figures; a probability value of 0.05 was accepted as the level of statistical significance in all the tests performed.

5.3 - Results

The study presented in this thesis tested in healthy human subjects the hypothesis that exercise, in particular at high intensity, can produce an hyperalgesic state, in addition to the analgesic effect previously described in literature (Boecker et al. 2008). Moreover, the study was aimed to address whether the effect of exhaustive exercise could be different in subjects with different fitness status.

5.3.1 - Effect of exhaustive exercise on pain thresholds

To investigate the relationship between fatiguing physical exercise and pain perception, two groups of healthy male subjects were recruited: one included trained cyclists, the other included untrained volunteers as comparison group to test for differences due to fitness status. All participants completed the same experimental protocol, which comprised the measurement of pressure-pain threshold before, immediately after and 15 minutes after an exhaustive exercise on a cycle ergometer. Specifically, the exercise consisted the incremental exercise with workloads increased every minute until the subject was unable to perform the workload. During all the performance, ventilation and concentrations of oxygen and carbon dioxide in the inhaled and exhaled air were measured breath-by-breath.

Table 5.1 summarizes the main characteristics of the two groups of subjects. It is possible to notice that the trained and untrained subjects were significantly different only in parameters indicating their fitness status, such as maximal oxygen uptake or maximal workload reached at the end of the exercise.

Table 5.1 – Main characteristics of the two groups of recruited subjects.

	Untrained group	**Trained group**	*P-value summary*
Age (year)	24.6 ± 7.6	29.7 ± 10.1	*NS*
Weight (Kg)	77.9 ± 9.5	73.1 ± 7.2	*NS*
Height (cm)	182.0 ± 6.0	180.4 ± 6.8	*NS*
Maximal workload (Watt)	296.3 ± 43.3	385.3 ± 48.1	***
Maximal oxygen uptake (ml/min/kg)	41.5 ± 5.8	55.2 ± 8.2	***
Anaerobic threshold (% of maximal oxygen uptake)	83.4 ± 2.9	85.8 ± 3.6	*
Total time of exercise (sec)	708.1 ± 139.2	956.7 ± 126.8	***
Time above anaerobic threshold (sec)	275.6 ± 58.2	316.0 ± 86.5	*NS*
T_{overAT}/T_{tot}	39.3 ± 6.8	32.8 ± 5.9	**

For each parameter, mean values ± SD are reported. Significant differences between the groups are indicated as follows: NS, $p > 0.05$; *, $p<0.05$; **, $p<0.01$; ***, $p< 0.001$

Mean pain thresholds before, immediately after and 15 minutes after the exhaustive exercise are shown in figure 5.5. Specifically, mean thresholds (±SD) of untrained group were 5.9N

(±31), 7.1N (±4.2) and 4.2N (±2.4) respectively before, immediately after and 15 minutes after the fatiguing physical activity, whereas at the same time points, cyclists' mean thresholds were respectively 3.7N (±2.0), 5.3N (±3.1) and 3.9N (±2.3). Repeated-measures ANOVA was used to assess differences between and within the two groups. Significant differences for the within-subject factor 'Time' ($p<0.01$), but not for the main effect of the between-subject factor 'Group' ($p=0.153$), were detected, meaning that there was no overall difference in pain thresholds between the two groups, while thresholds changed significantly among time points. Moreover, the 'Time × Group' interaction was found significant ($p=0.015$). Considering the significant effects of both 'Time' and 'Time × Group' interaction, the results indicated that different pain thresholds were recorded at different time points and, furthermore, that this effect was different in trained and untrained subjects. For this reasons, post-hoc comparisons were performed within the groups to investigates whether physical exercise had different effects in trained and untrained group.

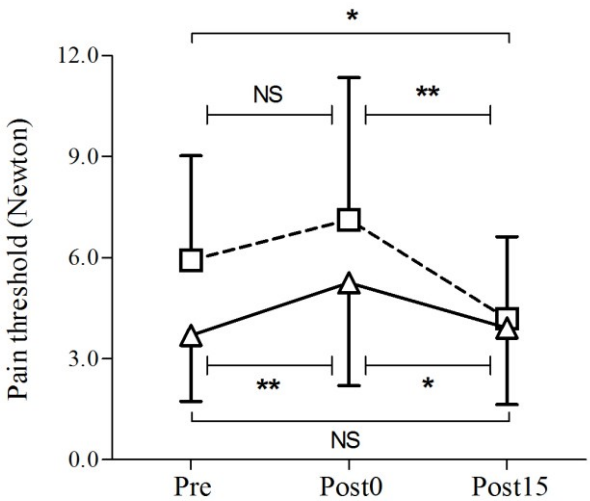

Figure 5.5 – Mean pain thresholds measured in trained (Δ) and untrained (□)subjects, before (Pre), immediately after (Post0) and 15 minutes after (Post15) an exhaustive exercise. Standard deviations are indicated as error bars. Significant differences are indicated as follows: NS, $p> 0.05$; *, $p<0.05$; **, $p<0.01$.

As indicated in figure 5.5, testing for within-group differences in time points revealed significant differences between thresholds recorded before and immediately after the exercise in untrained subjects (mean difference=-1.5, $p=0.02$), while in trained subjects pain threshold measured at time points Post0 was significantly different both from Pre and Post15 values (mean differences of 1.6 and -1.3, $p=0.04$ and $p=0.3$, respectively). To better understand the effect of physical activity on pain perception, mean differences were calculated as percentage of the pre-exercise values. In particular, positive variations can be considered as reduction in pain perception (analgesic effect), whereas negative values indicated an increased harmful sensation (hyperalgesic effect). As shown in figure 5.6, trained and untrained subjects differed in the hyper- or analgesic effects induced by the incremental exercise. Specifically, in

untrained subjects an analgesic effect was observed immediately after the exercise (mean difference=18.2% ± 32.5), whereas a significant hyperalgesic effect was observed after 15 minutes (mean difference=-30.1% ± 21.0). On the other hand, in trained volunteers, only a significant analgesic effect was observed immediately after the exercise, but not 15 minutes later (mean differences of 45.2% ± 41.4 and 9.6% ± 46.0, respectively).

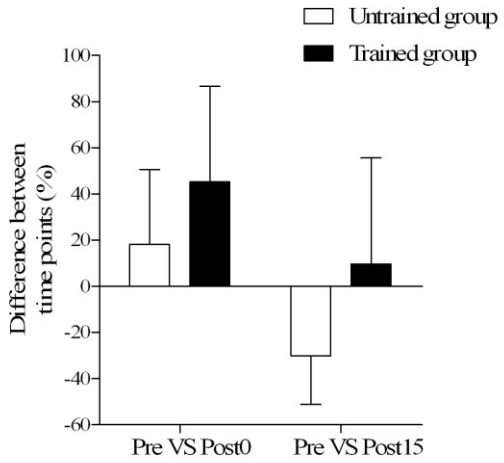

Figure 5.6 – Mean exercise-induced differences immediately after (Pre Vs Post0) and 15 minutes (Pre Vs Post15) after exhaustive physical activity, expressed as percentage of pre-exercise values. Standard deviations are indicated as error bars.

5.3.2 - Correlations between pain-modulating effects and exercise parameters

The results reported in the previous section demonstrate that physical exercise was able to modulate pain perception in healthy volunteers and that these changes were different according to the fitness level of the subjects. To further investigate this relationships, correlation analyses were performed considering the pain-modulating effect observed immediately after the exercise (PrePost0) and 15 minutes later (PrePost15), in relation to four parameters that described the training level of the recruited subjects: maximal oxygen uptake (VO_2max), maximal workload reached at the end of the incremental activity ($Load_{max}$), the anaerobic threshold measured as percentage of VO_2max (AT%) and the ratio between the time of exercise performed above the anaerobic threshold and the total time (T_{overAT}/T_{tot}), expressed in percentage. In particular, PrePost0 and Prepost15 were calculated as the difference in percentage between Pre and, respectively, Post0 and Post15, relative to Pre time point (see the formulas in Material and methods). Also in this case, all the variables considered were tested for normality. Shapiro-Wilk test revealed that the two variables accounting for the effect of physical activity were significantly different from a Gaussian distribution ($p=0.018$, $p<0.001$ respectively), therefore non-parametric tests were used for assessing significance and Spearman's rho correlation coefficients were calculated.

The graphs in figure 5.7 represent the relation among the variables considered for correlation analysis. In each case the Spearman's rho correlation coefficient was computed and tested

whether it was significantly different from zero; these data are reported in table 5.2. Among all the considered couples, significant negative correlations were found between the time spent over the threshold and the pain-modulating effect investigated both immediately after the incremental exercise and the 15 minutes later (Spearman's rho = -0.56 and -0.61 respectively; $p<0.01$ and $p<0.001$ respectively). This findings indicated that longer was the time of exercise that the subject spent in anaerobic condition, less analgesic effect (or greater hyperalgesic effect) was induced by the exhaustive physical subjects.

Furthermore, a significant positive correlation was detected between maximal workload reached at the end of the exercise and the pain-modulating effect evaluated 15 minutes later, but not immediately after (Spearman's rho=0.42, p=0.018), therefore suggesting that the subjects able to pedal at higher workload were those in which a pain-inhibiting mechanisms had a greater effect.

No other significant correlations were found between PrePost0 or PrePost15 and the measured variables.

Table 5.2 – Spearman's rho correlation coefficients and their statistical significance.

	PrePost0		**PrePost15**		**VO_{2max}**		**AT_{perc}**		**T_{overAT}/T_{tot}**	
	Rho	*p-value*	Rho	*p-value*	Rho	*p-value*	Rho	*p-value*	Rho	*p-value*
$Load_{max}$	0.28	*NS*	0.42	*	0.75	***	0.22	*NS*	-0.44	*
VO_{2max}	0.06	*NS*	0.28	*NS*			0.13	*NS*	-0.35	*NS*
AT_{perc}	0.18	*NS*	0.09	*NS*					-0.45	*
T_{overAT}/T_{tot}	-0.56	**	-0.61	***						

Four variables were taken into account to characterized the fitness level of each subject: VO_2max, maximal oxygen uptake; T_{overAT}/T_{tot}, ratio between the time of exercise performed above the anaerobic threshold and the total time; $Load_{max}$, maximal workload reached at the end of the incremental and AT%, anaerobic threshold measured as percentage of VO_2max. PrePost0 and PrePost15 indicate the pain-modulating effects respectively evaluated immediately after the fatiguing exercise and 15 minutes. later. Significant differences between the groups are indicated as follows: NS, $p> 0.05$; * $p<0.05$; ** $p<0.01$; ***, $p< 0.01$

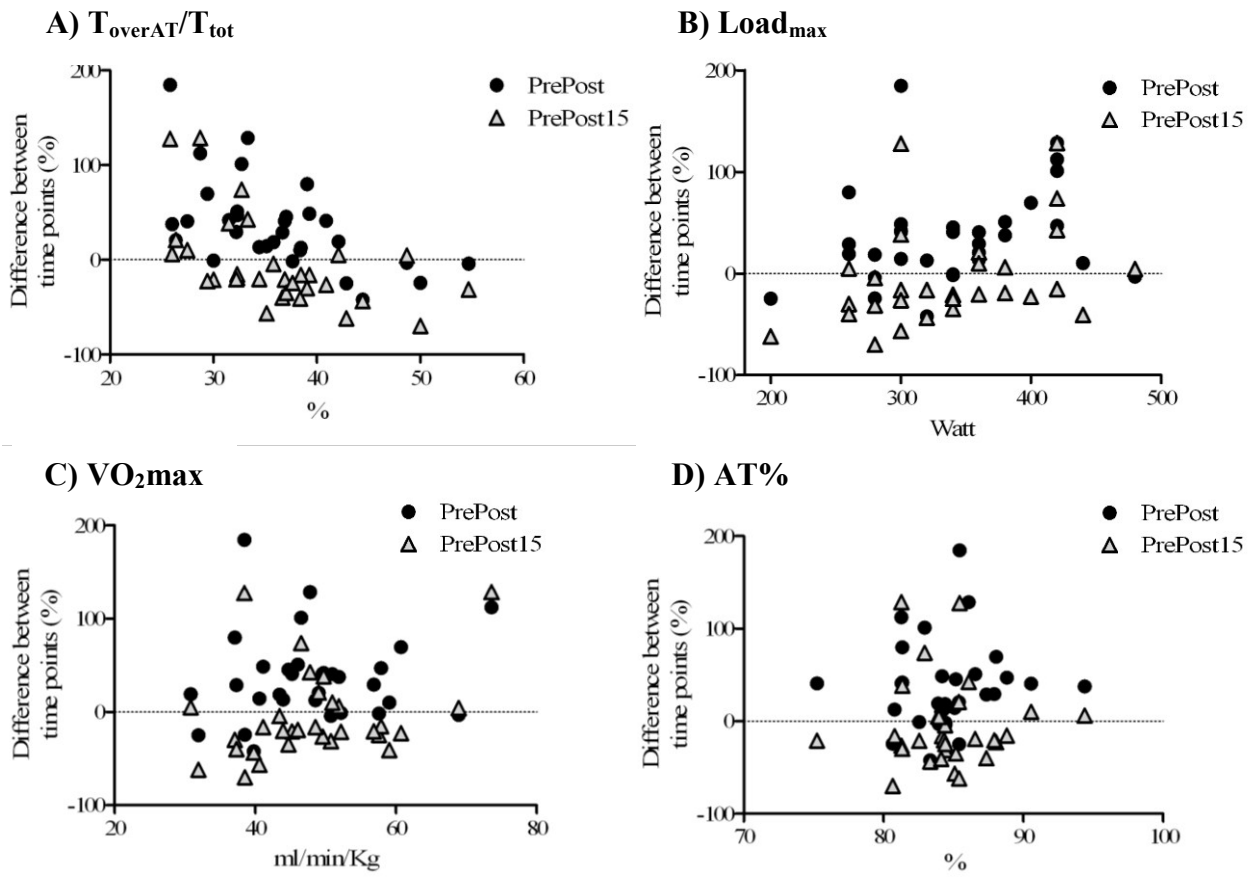

Figure 5.7 – Relationships between pain-modulating effects and fitness level parameters. PrePost0 and PrePost15 indicate the differences in percentage between pain threshold recorded before the exhaustive exercise and, respectively, immediately after or 15 minutes after the exercise. Fitness level parameters are abbreviated as follows: VO_2max, maximal oxygen uptake; T_{overAT}/T_{tot}, ratio between the time of exercise performed above the anaerobic threshold and the total time; $Load_{max}$, maximal workload reached at the end of the incremental and AT%, anaerobic threshold measured as percentage of VO_2max.

5.4 - Discussion

The present study was aimed to investigate the effect of an exhaustive exercise on the perception of pain whether the fitness level could influence the pain-modulating effect. For this purpose, 30 healthy male volunteers were recruited and subdivided in two groups, depending on their fitness level. Both groups performed an exhausting exercise on a cycle ergometer and pain-pressure threshold were measured before and after the this exercise. Two main findings were obtained analysing the data recorded in the study. Firstly, fatiguing physical activity induced modulation in pain perception, highlighted by changes in pressure-pain threshold measured immediately after exercise and 15 minutes later. Secondly, pain modulation is different in trained and untrained groups: trained subjects showed a significant hypoalgesic effect after the exercise, while thresholds values were similar to pre-exercise values after 15 minutes; on the other hand, in the case of untrained volunteers, thresholds increased after the exercise (however the difference was found not significant) and, more interestingly, they were significantly decreased 15 minutes later. All together these results are in agreement with the hypothesis that physical activity has different effects according to the fitness level, in particular when the exercise is performed at intensity levels able to exhausts the metabolic/muscular capacity of the subject.

The exercise-induced analgesia, observed immediately after the exercise, have been demonstrated for different exercise types, performed at different intensity or for different duration (Hoffman et al. 2007). This effect is part of a wider spectrum of psychophysiological modulation that is induced by physical activity and that is commonly known as runner's high (Boecker et al. 2008). In fact physical activity, in particular endurance training, there are numerous reports in the popular and scientific press regarding a state of euphoria, for example while running, usually characterized by stress reduction, anxiolysis, mood elevation and reduced pain perception (Boecker et al. 2008). However, there are no consistent findings about the different amount of exercise-induced analgesia in trained and untrained subjects that underwent the same exhausting exercise, as observed in this study.

Little is currently known about the mechanisms mediating the decrease in pain perception upon physical exercise and whether the training level can influence this modulatory effect. Some studies have suggested that reduced pain perception is not similar in trained and untrained subjects. For example, the results reported by Hoffman and colleagues (Bender et al. 2007) about the amount of analgesic effect in ultra-marathon runners supports this hypothesis. The authors described an hypoalgesic effect in the faster runners, but not in the slower

participants, and discussed this finding suggesting that only in trained, and therefore faster, participants the antinociceptive mechanisms were activated. For the slower runners, instead, the exercise was too intensive and stressful to "exhaust" the systems responsible for exercise-induced analgesia (figure 5.8).

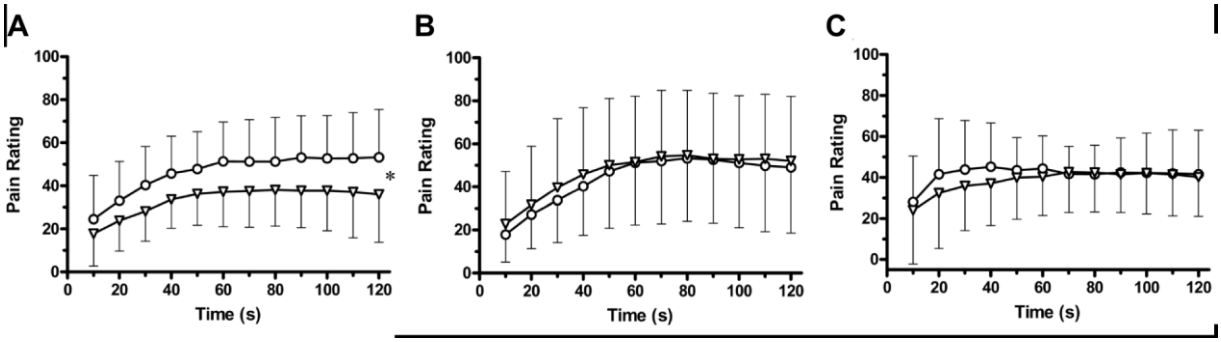

Figure 5.8 – Pain ratings in ultra-marathon runners. After running a 100-mile marathon, only the faster runners (*A*) showed a significant (*, $p < 0.05$) reduction in pain perception, measured as a difference between pain ratings measured with a pain-pressure device on the nondominant hand before (○) and after (∇) the competition. On the contrary, slower runner (*B*) and control subjects that did not participated to the race (*C*) showed similar pre- and post-race pain scoring. Mean pain ratings and SD are reported in each graph. *Modified from (Hoffman et al. 2007)*

The most widely accepted neuronal mechanism behind the exercise-induced analgesia involves the endogenous opiates and the 'endorphin hypothesis' (Morgan 1985) is the most accredited one. Several findings have demonstrated that blood concentration of endogenous opiates increases even three times during the physical activity and remain stable for minutes after exercise (Hoffman et al. 2007); moreover antagonist of opioid receptors such as naloxone, can reverse the analgesic effect of exercise (Schwarz and Kindermann 1992).

A significant contribution toward the understanding of how the endogenous opiates are related to the effect of physical activity in human came from a study performed by Boecker and collaborators (2008). In this work the authors studied with positron emission tomography (PET) the opioid receptor availability in trained male athletes, comparing the changes of nonselective opioid binding before and after running. They demonstrated the endogenous release of central opioids preferentially in brain regions belonging to the frontolimbic circuits that are known to play a key role in emotional and pain perception processing (figure 5.9). Moreover, the degree of affective modulation, evaluated with visual analog scale euphoria ratings, was found to be significantly correlated with the amount of endogenous opiates release in brain structures such as orbitofrontal, anterior cingulate and insular cortices (Boecker et al. 2008).

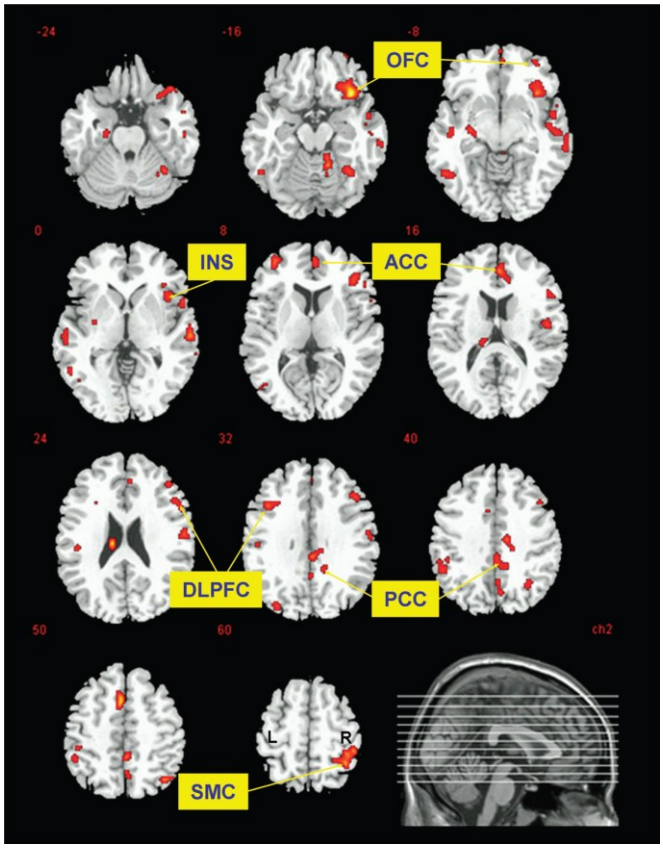

Figure 5.9 – Brain areas showing significant reduction in opioid receptor availability, evaluated with PET. After 2h of running, trained male runners showed a decreased binding of a nonselective opioid ligand in key frontolimbic areas, involved in affective and pain processing. The areas with significant lower receptor availability are abbreviated as follows: ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; INS, insula; OFC, orbitofrontal cortex; PCC, posterior cingulate cortex, SMC, sensorimotor cortex. *From (Boecker et al. 2008)*

Investigating in deeper details the relationship between the exercise intensity and the opiates release, many authors have suggested that the release of these substances could be linked with the perception of fatigue and tolerance to lactic acid (Dietrich and McDaniel 2004). In fact, measuring the blood level of lactic acid before, during and after an aerobic exercise, it was shown that the lactic acid concentration increased during the exercise and remained stable for few minutes after the ending. Interestingly, this trend significantly correlated with the amount of analgesic effect reported by each subjects involved in the experiment (Ring et al. 2008). Moreover, exercises involving anaerobic components increased β-endorphin and lactate levels more than pure aerobic exercises (figure 5.10), in fact endogenous opiates concentration significantly increased only for exercise intensities exceeding the 75% of the maximal oxygen uptake (Meyer et al. 2000).

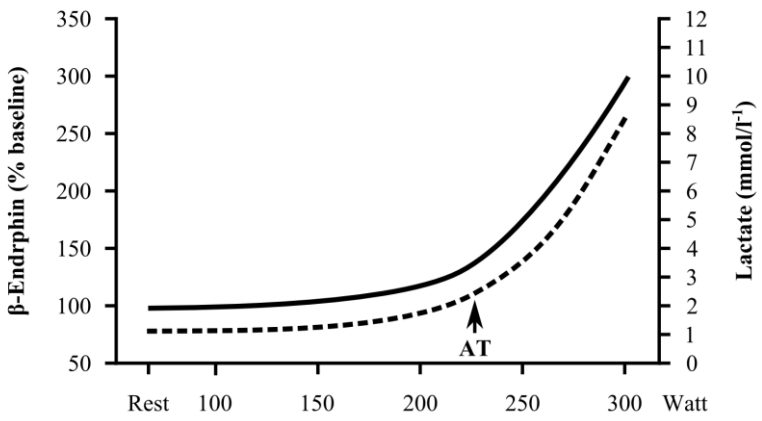

Figure 5.10 – Relation between plasma levels of β-endorphin and lactate. The amount of β-endorphin release depends on exercise intensity, in particular a significant increase of this endogenous opioids is observed for intensity values above anaerobic threshold (IAT), when the concentration of this peptide is elevated in parallel to lactate. *Schematic drawing from (Schwarz and Kindermann 1990)*

Taken all together, these results support the hypothesis that, when the intensity or the duration of the physical activity increased over a given threshold, the endogenous opiates system is activated and modulates the painful sensation caused by the accumulation of lactic acid. The release of substances, like β-endorphin or enkephalin, allows to better tolerate the stress associated with accumulation of lactic acid during an exercise performed at medium or high intensities, contributing to increase the performance.

Although the involvement of the endogenous opiates system in the exercise-induced analgesia has received extensive support both in human and in animal experiments, several studies seem to indicate that there could be multiple analgesic systems, including opioid and non-opioid systems. For example, some authors described the role of endocannabinoids (Koltyn and Umeda 2006) or the involvement of arterial baroreceptors, revealing a possible interaction between the pain modulation and the cardiovascular systems (Mense and Schiltenwolf 2010). In particular, Koltyn and Umeda (Sluka et al. 2011) proposed that baroreceptors could act as sensors that trigger the analgesic systems.

For what concerns pain thresholds recorded 15 minutes after the exhaustive exercise, the untrained subjects showed a significant amount of hyperalgesic effect, whereas the trained cyclists did not. This result suggests that the fitness level plays an important role in the effect produced by fatigue on pain perception and it could be related with the fact that the untrained subjects were much less prepared to very intense exercise, therefore they over-exercised, inducing a protective reaction characterized by an hyperalgesic state, whereas trained subjects were to high intensities and associated negative effects such as increase in lactic acid level, muscular damage, high workload for the heart and circulatory system. In fact, there is a

growing body of evidence that demonstrate that how stressful events can induce and hyperalgesic state, possibly aimed to increase vigilance to prevent potential harm (Martenson et al. 2009). In the context of the presented results, two recent studies have investigated how muscle fatigue can influence pain in animal models. Yokoyama and colleagues have recently demonstrated that fatigue enhanced the hyperalgesic state induced in mice by intramuscular injections of acidic saline (Yokoyama et al. 2007), while Sluka and co-workers started to identify the neuronal mechanisms that could be involved in the enhanced pain response after fatiguing running (Sluka and Rasmussen 2010).

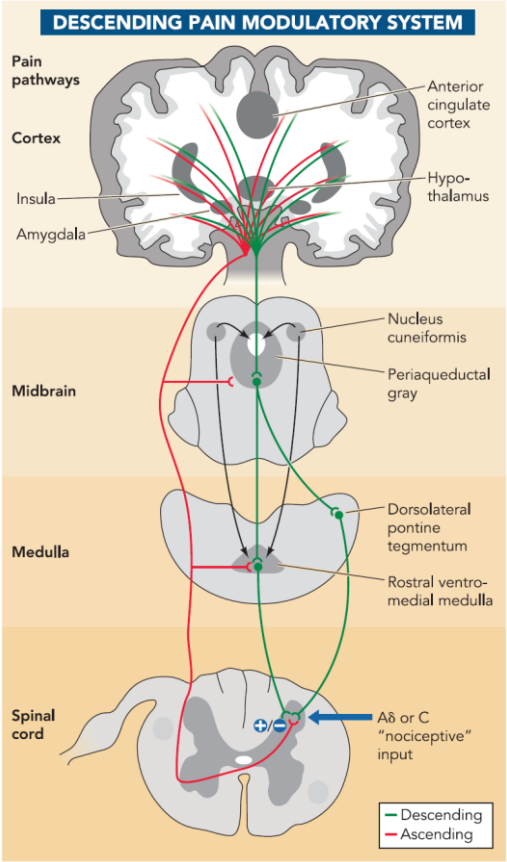

Figure 5.11 – The descending pain modulatory system. Several regions within the brain are involved in pain descending pathways that end in the spinal cord, determining both an inhibition (-) and a facilitation (+) of nociceptive ascending transmission. *From (Bingel and Tracey 2008)*

Considering that a fatiguing exercise could by perceived as a stressful event by untrained subjects, the findings presented in this study add new pieces of information about the possible effect of stress on pain perception. Despite many studies demonstrated that stress induced an hypoalgesic state (Butler and Finn 2009), recent studies have revealed a sensitization effect on pain perception, suggesting the hypothesis that stress acts on a balance between pain-inhibiting and pain-facilitating neuronal mechanisms (figure 5.11). In other words, a stressful event or situation can induce either an analgesic or an hyperalgesic state, depending on the context of the event/situation, as in the case of different fitness levels. In fact, although we did not directly investigate the neurophysiological processes underling the results presented in this article, it is possible to speculate that these mechanisms could be involved in the effects produced by the physical exercise and that the balance between inhibiting and facilitating processes could be influenced differentially whether the subject is naïve in respect to the exhaustive exercise or he/she is well trained.

From a behavioural point of view, it is possible to speculate the functional significance of these opposite pain modulating effects, in particular in subjects that are not accustomed to this type of exercise: taking a competition as an example of stressful physical exercise, the

analgesia induced during the race could be aimed to increase the tolerance to lactic acid and muscular pain in order to increase the athlete's performance during the race; however, at the end of the competition, the analgesic state could be no more useful and therefore it is substituted by an hyperalgesic state that induces the athlete to stay at rest, in order to allow restorative processes to occur in the organism. In other words, the hyperalgesic state could be thought as the price that the athlete has to pay for the increase in performance allowed by the analgesic state. This may not occur in trained athletes because their organism is much more accustomed to, thanks to the preparation the physical but also mental training.

For what concern the role of training in the differences between sedentary volunteers and trained cyclists, there are two possible explanation. The physical training could act directly on the mechanisms controlling pain perception, reducing, in trained subjects, the effect of pain-facilitating processes that are, on the other hand, triggered by stressful exhaustive exercise in untrained volunteers. In this sense, training can have an effect both on the descending pathways that control nociception and at more cognitive and motivational levels (Wiech et al. 2008), changing how the subject perceived the exhaustive exercise. In fact, the trained subjects could perceived the exhaustive exercise not as a stressful and potentially harmful activity, but as an activity which they were more prepared, resulting in the involvement of mechanisms related to anticipation (Porro et al. 2002; Porro et al. 2003) and/or in the release of pain-inhibiting substances (Fields 2004).

On the other hand, physical training could act indirectly on the pain perceptive system. In fact, because cyclists were trained, they were able to perform the exercise for longer periods, resulting in a greater activation of the analgesic mechanisms. In this sense, analgesic and hyperalgesic processes would be the same in both groups, but the overall resulting effect was pain sensitization in untrained subjects, because the analgesic mechanisms had less time to contrast the pain-facilitating processes. However, it should be noticed that a relation has been demonstrated between the plasma concentration of lactate and endogenous opioids, in particular β-endorphin (see figure 5.10), and that the analgesic effect has been observed for intensities above the anaerobic threshold. In this case, it is worthy of note the fact that the time of exercise spent above the anaerobic threshold was found to be not statistically significant different between the two groups of subjects (see table 5.1).

The differences found between trained and untrained healthy volunteers also suggest that pain perception could indicate how the organism would respond to an intensive exercise, leading to speculate that information given by the pain perceptive system could be related to the general

state of the organism, an hypothesis that has been proposed by authors who considered pain as an homeostatic emotion (see for example, (Craig 2003a; b; Panerai 2011)). In this context, the different responses of pain perceptive system to exercise could possibly indicate how the organisms will face or respond to an high-intense activity or in situation in which the organism's capacities are overreached as, for example, in overtraining syndrome (figure 5.12), a complex sport phenomenon described as a physical, behavioral, and emotional condition that occurs when the volume and intensity of an exercise exceeds the athlete's recovery capacity (Urhausen and Kindermann 2002; Halson and Jeukendrup 2004; Purvis et al. 2010).

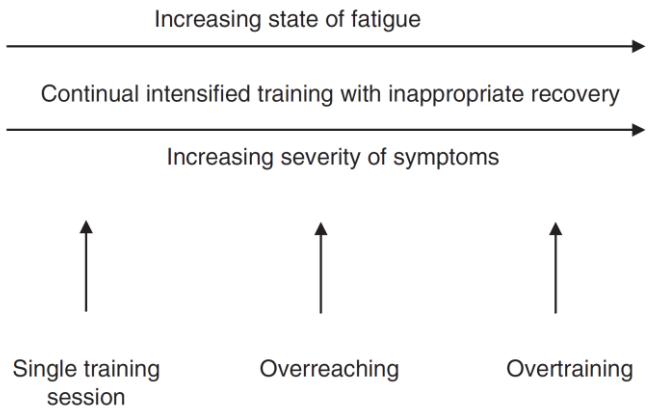

Figure 5.12 – The overtraining continuum. *From (Halson and Jeukendrup 2004)*

Finally, the results found in this study have clinical implication in relation to the effect of exercise on the development and persistence of disease-associated acute/chronic pain (Shankarappa et al. 2011) and to the possibilities offered by physical activity as a non-pharmacological treatment of pathological conditions characterized by pain (Busch et al. 2008; Hauser et al. 2010; Hassett and Williams 2011; Sarzi-Puttini et al. 2011) or where the its psychophysical effects can improve the symptoms of neurological and psychological disorder such as anxiety and depression (Larun et al. 2006).

6 - CONCLUSIONS

The experience of pain has always been of great interest for neuroscientists, for its role as an essential mechanism for the survival organisms and its inherently relationship with disease. The reasoning about pain and how nociceptive information are collected and elaborated has been an issue in the many religious and philosophic argumentation about the human being and the surrounding world since ancient time (Pearl, 2011). However, only in the last few centuries, the anatomical and functional neurobiological basis of pain perception has been revealed. In particular, the neuronal mechanisms of the nociceptive transmission in the peripheral nervous system have been described with molecular resolution, while, on the other hand, the central processes that elaborate the sensory nociceptive information into a conscious perception of pain are far from being completely known.

In recent years, modern neuroimaging techniques have allowed to shed light in the complex mechanism that underlies the pain processing in the brain. These methods have revealed a large distributed network, instead of a single area, involved in the complex, subjective experience of pain. This network, usually called "the pain matrix", includes both cortical areas, like the primary and secondary somatosensory, insular, anterior cingulate and prefrontal cortices, and subcortical structures, such as the brainstem and the amygdalae. However, this assembly does not constitute a stand-alone and static entity but rather a substrate that is significantly and actively modulated. In fact, a variety of factors, such as attention, expectation, mood and injury, contribute in shaping the individual perception of pain.

The three studies that have been presented in this thesis were aimed to investigate different aspects that are involved in the perception of pain and can modulate it. The first study described the brain areas involved in the conscious perception of pain evoked in a pathological condition and how the activity of these areas could be modulated by attention tasks. In the second study, the correctness of a theoretical model based on Bayesian decision theory was demonstrated by the agreement between the data predicted by the model and those observed in two experiments with healthy subjects. In particular, the model provided a formal description of the integrative processing that has been hypothesized to be at the basis of the pain experiences reported by the subjects in a conditioning-induced placebo analgesia experiment. Finally, in the last study, the pain modulatory effect of a fatiguing exercise, was assessed in

subjects with different fitness level. Specifically, modification in pain threshold were evaluated in subjects that were either trained or untrained to perform an exhaustive exercise and therefore responded in different ways to the stressful events.

The results observed in the three studies contributed to shed light on different aspects of pain perception and its intrinsic variability.

In the first study presented, the pain in the phantom limb of a patient, suffering from phantom limb syndrome, was investigated as a specific case of neuropathic pain. Specifically, the patient referred spontaneous pain in the missing right foot and the study was aimed to investigate the neuronal activity in pain-related areas associated with the pathological condition or during the modulation of the abnormal perception. The subjects performed an fMRI experiment which included the acquiring of functional data during the execution of attention tasks and 5 minutes of rest. Both a distractive and an attention on pain tasks were performed in order to modulate the activity of neuronal substrates Moreover, because the intensity of the persistent pain was different in subsequent days, the same experimental protocol was performed when the patient referred a high intensity of pain and a low intensity of pain.

The results observed demonstrated how the attention on pain, in particular the imagination of a maximal intensity of pain in the missing limb, can modulate the activity of several brain areas, commonly found to be included in the pain matrix, such as the primary somatosensory cortex and the insula, modulation that was reflected in an increased perception of pain in the missing limb. Moreover, the involvement of prefrontal cortex was also observed, suggesting the role of higher-order executive areas in the control of the ongoing pain-processing. Interestingly, these results were observed only in the day in which the patient referred a low intensity of pain, suggesting a relation between the activity of these brain areas and the intensity of spontaneous pain. Specifically, when the pain was referred at a high intensity, the imagination of a maximal intensity of pain failed to modulate the activity in brain domains that were already active, like the primary somatosensory cortex, or that have been demonstrated, by other authors, to be involve in cognitive modulation of pain, like the prefrontal cortex. On the other hand, a distractive task, that consisted in the imagination of pleasant situations, was able to reduce the persistent pain and to induce a significant deactivation of areas normally included the network of pain, especially in the day when the patient referred a low pain scoring. Moreover, the distractive task induced significant bilateral activations in the lateral parietal cortex and in a medial region, comprising the posterior cingulate cortex and the precuneus. These areas have

been commonly found to be active at rest in healthy subjects, therefore suggesting that in the chronic condition that was suffered by the patient, the distractive task shifted the patient's brain state toward a condition similar to that described in the absence of specific input or output in healthy subjects.

The brain activity of the patient with phantom limb pain was also investigated in a resting-state condition, in order to define the network of areas activated in the chronic condition. Together with functional assembly of areas commonly observed in normal condition and referred to as default-mode network, the activity among areas belonging to the pain network were observed to be functionally correlated in the resting condition. Specifically, the insula, the primary somatosensory and the inferior parietal lobule showed an increased functional connectivity when the spontaneous pain was rated at high level, supporting the hypothesis that these brain areas had a key role in mechanism involved in the perception of chronic pain, in particular, in the perception of pain at high levels.

Chronic pain, in particular neuropathic pain, is an abnormal perception of pain not evoked by a direct stimulation of nociceptors, but induced by an alternated peripheral and/or central processing of nociceptive information. However, also in normal condition, the pain perceptive system can be influenced by factors like expectation that therefore modulate the conscious experience of pain. The placebo analgesia is one of the best example of this modulation induced by psychosocial context that surrounds a noxious stimulation can induce a influence the pain perception. In recent years, several studies have highlighted the neurobiological basis and the mechanisms involved in the placebo analgesic effect, demonstrating the key role of expectation and classical conditioning. The context of understanding how this factors are processed and integrated with the noxious information to generate the final pain percept, the second study evaluated the correctness of a theoretical model based on the Bayesian decision theory. The model was a formal description of the inferential process that is hypothesized to be responsible for pain modulation. The comparison between the pain ratings predicted by the model and those reported by healthy subjects in two distinct experiments demonstrated the correctness of the model in describing the inferential processes involved in pain perception. The experiments included a classical conditioning session for inducing an effective placebo analgesic effect, furthermore the subsequent testing session were designed on the basis of the considered model predictions. Specifically, the role of pieces of information derived by the previous experience and the integration of conflicting contextual information administered with the noxious stimulation were separately examined in the experiments. The results demonstrated that the proposed model was correctly developed to consider these distinct

sources of information. The model was not intended to be definitive, but it correctness demonstrated the possibility to apply Bayesian decision theory in pain research, in the same way as it has been applied in other fields of neuroscience research, such as visual perception, multisensory integration, sensory motor control or learning. Furthermore, the second study described in this thesis showed how a model based on Bayesian inferential statistic represents a useful formal framework to describe different processes involved in the modulation of pain due to factors such as conditioning or concurrent contextual information.

One of the most interesting pain-modulating factors is the physical exercise. Physical activity can induce an analgesic effect when performed at medium-high levels or if it is performed for a long interval of time. However, when performed intense levels, so that exhausts the energetic capacity of the athlete, it can induce an opposite effect in subjects who are not prepared for performing an exercise at exhausting levels. In the third study presented here, changes in the perception of pain, evaluated as significant differences in the pain threshold measured before and after a fatiguing exercise, were observed both in trained and untrained healthy subjects. However, a significant analgesic effect, lasting for less than 15 minutes, was observed after the exercise in the group of athletes, while, on the contrary, it was not observed in the group of untrained volunteers; instead, an hyperalgesic effect was found in this group. Furthermore, these results were further supported by correlation analyses between metabolic parameters accounting for the fitness status of the subjects and the differences in pain thresholds measured before and after the exercise.

The third study provided, on one hand, a better description of physical exercise as a pain-modulating factor, giving new insights on the effects that it can induce when performed at high intensities; on the other hand, the study further demonstrated that the modulation of pain perception depends on the psycho-biological context in which the noxious stimulation is applied. In fact, the different results observed in trained and untrained subjects suggests that the effect on pain relayed on neurobiological factors but also on the different perception that the subjects had of the exercise.

To conclude, the results reported in this thesis demonstrated, from different points of view, how pain perception can be modulated, which factors can induce this modulation and how their interactions with sensory information can described and which brain areas are involved in the case of chronic pain. All together, these findings support the hypothesis that describes pain a vital mechanism for the organism and that the inherent variability observed in pain

perception derives from the necessity of adapt the percept in according to the context in which the noxious stimulation is applied, in order to drive effective behaviours.

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9 - LIST OF PUBLICATIONS

Papers

1. Anchisi D. and **Zanon M.**

   Sensory and cognitive Bayesian integration in pain perception and placebo analgesia

   Article in preparation

2. **Zanon M.**, Grainer A., Megighian A., Reggiani C., Anchisi D.

   Modulation of pain perception after exhaustive exercise and influence of training status

   Article in preparation

3. Busan P., **Zanon M.**, Vinciati F., Monti F., Pizzolato G., Battaglini P. P.

   Transcranial magnetic stimulation and preparation of visually-guided reaching movements

   Submitted to Frontiers in Neuroengineering

4. **Zanon M.**, Busan P., Monti F., Pizzolato G., Battaglini P. P.

   Cortical connections between dorsal and ventral visual streams in humans: Evidence by TMS/EEG co-registration

   Brain Topogr. 2010 Jan;22(4):307-17. Epub 2009 Jun 5.

Conference proceedings

1. **Zanon M**., Gremese M., Anchisi D.

   Evaluation of a Bayesian model of pain modulation and placebo effect

   Poster presented by Z. M. at "8th IBRO World Congress on Neuroscience" – July 2011, Firenze (Italia)

2. **Zanon M**. , Gremese M. , Anchisi D.

   Evaluation of a Bayesian model of pain modulation and placebo effect

   Poster presented by Z. M. at "SiNAPSA Neuroscience Conference '11" – September 2011, Ljubljana (Slovenia)

3. Grainer A., **Zanon M.**, Anchisi D., Megighian A., Paoli A., Angeli A., Agnolet A., Gremese M., Reggiani C.

   Variations in pain perception after an exhaustive exercise

   Oral communication (by G. A.) at "16th Annual Congress of the European College of Sport Science" – August 2011, Liverpool (GB)

4. Grainer A., **Zanon M.**, Anchisi D., Megighian A., Paoli A., Angeli A., Agnolet A., Gremese M., Reggiani C.

   Variations in pain perception after an exhaustive exercise

   Poster presented by G. A. at "III Congresso Annuale Società Italiana di Scienze Motorie e Sportive" – October 2011, Verona (Italia)

5. Busan P., Guerra V., Della Mora A., Jarmolowska J., **Zanon M.**, Monti F., Pizzolato G., Battaglini P. P.

   Cortical connections investigated by magnetic stimulation of the parieto-occipital cortex: a TMS/EEG co-registration study

   Poster presented by B. P. at "SiNAPSA Neuroscience Conference '11" – September 2011, Ljubljana (Slovenia)

6. Busan P., Guerra V., Della Mora A., Jarmolowska J., **Zanon M.**, Monti F., Pizzolato G., Battaglini P. P.

   The stream of cortical connections elicited by magnetic stimulation of the parieto-occipital cortex in humans: a TMS/EEG co-registration study

   Poster presented by B. P. at "Workshop on Concepts, Actions and Objects" – May 2011, Rovereto (Italy)

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