The dynamic dimension of the emotional experience assessed during painful stimulation and in the resting-state using functional magnetic resonance imaging

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Department of Clinical Sciences
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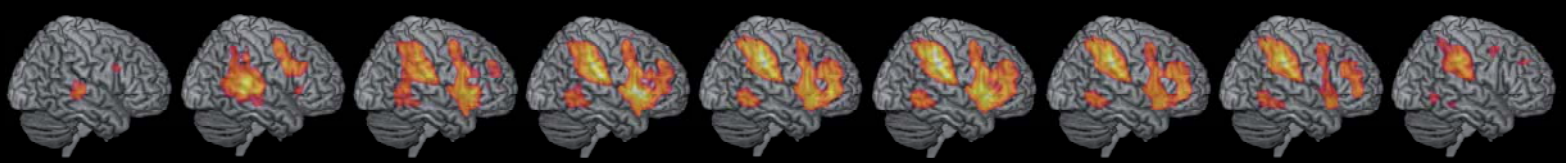

Department of Clinical Sciences, School of Medicine
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Thesis presented by Marina López-Solà to obtain the grade of PhD
by the University of Barcelona

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Medical PhD Program

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Dr. Jesús Pujol Nuez and Prof. Dr. Julio Vallejo Ruiloba certify that they have supervised and guided the PhD thesis entitled "The dynamic dimension of the emotional experience assessed during painful stimulation and in the resting-state using functional magnetic resonance imaging", presented by Marina López-Solà. They hereby assert that this PhD thesis fulfils the requirements to be defended.

Jesús Pujol Nuez

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A mi familia, que me dio el sentimiento.
A la Mare, por haber sido refugio incondicional.
Por la ilusión que habría tenido.

A tu, Jesús, per un viatge poderós i inoblidable.

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"... perque tot és molt difícil abans de ser senzill"
Marie Curie

"Todo hombre puede ser, si se lo propone, escultor de su propio cerebro"
Santiago Ramón y Cajal

"No olvidemos que las emociones son los capitanes de nuestras vidas y las obedecemos sin siquiera darnos cuenta"
Vincent Van Gogh

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López-Solà M, Pujol J, Hernández-Ribas R, Harrison BJ, Contreras-Rodríguez O, Soriano-Mas C, Deus J, Ortiz H, Menchón JM, Vallejo J, Cardoner N. Functional connectivity alterations in the mood depression state identified from changes in brain anatomy. **Biological Psychiatry. Under review.**

López-Solà M, Pujol J, Hernández-Ribas R, Harrison BJ, Contreras-Rodríguez O, Soriano-Mas C, Deus J, Ortiz H, Menchón JM, Vallejo J, Cardoner N. Effects of duloxetine treatment on brain response to painful stimulation in major depressive disorder. **Neuropsychopharmachology. Accepted for publication, 21st June 2010. In press. IF: 6.993.**

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1. Introduction

1.1. The scientific study of emotions

1.1.1. Relevance and complexity of the study of emotions

The study of emotion is one of the most complex subjects in psychology and neurosciences, and has a crucial role in the understanding of animal and human behavior in both normal and psychopathological states. Numerous definitions of "emotion" have been proposed over the last century suggesting that such a phenomenon is essentially broad and complex. An emotional experience or an emotional state is conceived as an inner state characterized mainly by two components, one referring to the bodily state (physical sensation) and the other as a conscious feeling (Kandle et al, 2000; Plutchik, 2001). The internal experience of emotion is highly subjective and often confusing and difficult to measure. Consequently, the study of emotion as an inner state (of the brain/mind) has been traditionally eluded by behaviourism, which was mostly interested in the observable (emotional) instinctive or conditioned reactions to environmental evoking stimuli (Watson and Morgan, 1917; Watson and Rayner, 1920). Additionally, psychoanalytic views have shown that emotions may be repressed, inhibited or unconscious and therefore mostly unavailable to introspection (Freud, 1916-1918/1968). Descriptive reports of emotional experiences, albeit informative and necessary to the understanding of underlying brain processes, are prone to ambiguity as they constitute interpretations, at times very difficult to express, of underlying and complex states of the mind (i.e., of the brain), and may not necessarily reflect emotion processing itself. Additionally, language structure and use may significantly constrain the description of the mixture between feelings and the emotional states that actually constitute the experience of interest (Plutchik and Kellerman, 1980; Ortony and Turner, 1990).

The study of emotion has, therefore, always constituted a scientific challenge. Such a challenge should be considered of special relevance taking into account the basic and primary role of emotions in guiding human behavior (and influencing cognitive processes), self-environment relationships and in constructing self-identity and personality. Moreover, the neuroscientific study of emotion has contributed to the understanding of the pathophysiology of a wide variety of psychiatric conditions in which a crucial emotional imbalance is at the core of the disturbance. The acquisition of

knowledge in this field will help to guide a selection of tailored treatment strategies in order to achieve greater efficiency in medical and psychological interventions.

When neuro-scientifically studying any aspect of human emotion, it may be relevant to consider that it is an intrinsically complex and diverse matter (e.g., note the difference between joy and fear) and that it may reflect the final outcome of a variety of underlying brain systems and processes with specific properties (LeDoux, 1996). For this reason, basic, simple and robust experimental contexts and conditions of study, albeit less ambitious, are of special interest to avoid confusion and gain a more modest but highly significant knowledge as to such a relevant domain in neural sciences.

1.1.2. The legacy from History

Several theoretical perspectives of the study of emotion have been developed through history. René Descartes is one of the first in proposing a conceptualization of emotion in his work entitled "Les Passions de L'âme" (Descartes, 1649/1972). Descartes focused on the study of emotion mainly considering the description of subjective experience. He considered that in order to discover the nature of "passions" (the word he used to describe emotions) it was not necessary to observe anything, but rather just to feel and reflect. From his perspective, it was not possible to study emotion from observable behavior, but it was accessible through introspection and internal observation. His theory made important contributions regarding the factors that generate passions (emotions), the number of primary passions and their effect and control through the will.

Many centuries later, Charles Darwin (1859; 1872/1965) eliminated the strict separation Descartes had made between animals and humans in terms of their experience of emotion, and he initiated the conceptualization of emotions as a basic element of survival and evolution, much as it is conceived in the present day. He suggested a possible innate nature for the expression and recognition of basic (fundamental) emotions in animals and humans, which worked across species and cultures (including anger, fear, surprise and sadness). He also suggested that animal emotions are homologues for human emotions on the basis of his extensive comparison and analysis of sketches and photographs of animals and people in different emotional states to

reveal cross-species similarities (see figure 1). Darwin greatly influenced the study of emotion as he established the basis for the study of emotional behavior in a biological and evolutionary context, and he was also the first to study and describe the range of emotional expressions (Darwin, 1872), which was latter to influence Ekman's theory of facial expression of emotions (Ekman, 1973). His work provides evidence of the continuity between the emotional expression of animals and that of humans.

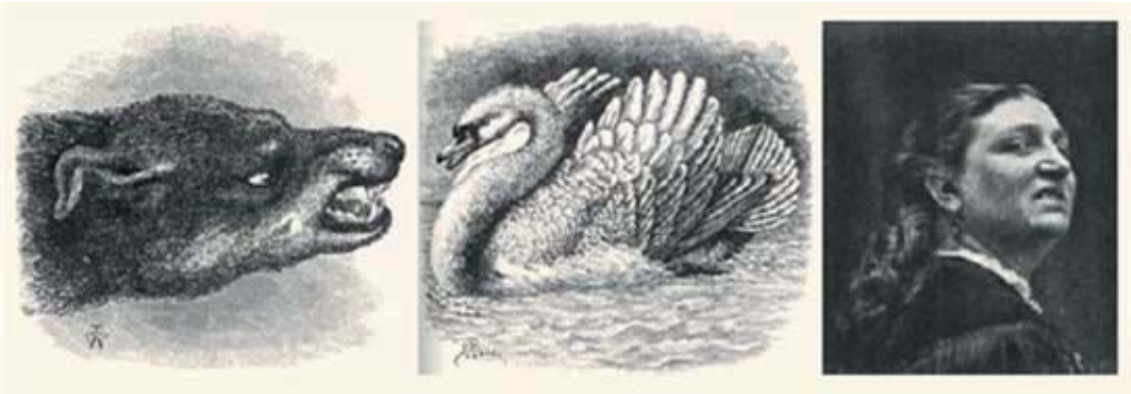


Figure 1. Drawings and photographs used by Darwin in *The Expression of the Emotions in Man and Animals* to illustrate cross-species similarities in emotion expression (anger/aggression in this case).

William James, considered the father of scientific psychology, provided a relevant contribution to the conceptualization of emotion by introducing the relevance of visceral and bodily state changes to emotion perception and also emphasized the importance of introspection to analyze the emotional experience (James, 1884). Carl G. Lange introduced the importance of physiologic changes of muscles and the vasomotor apparatus to emotion perception (Lange, 1885/1922). So, James and Lange stressed the contribution of physiologic changes of the body (autonomic function evidenced in visceral, somatic and motor bodily changes) to emotion perception (figure 2). In original words, James elucidated his concept as: *"...My theory ... is that the bodily changes follow directly the perception of the exciting fact, and that our feeling of the same changes as they occur is the emotion. Common sense says, we lose our fortune, are sorry and weep; we meet a bear, are frightened and run; we are insulted by a rival, are angry and strike. The hypothesis here to be defended says that this order of sequence is incorrect ... and that the more rational statement is that we feel sorry because we cry, angry because we strike, afraid because we tremble ... Without the bodily states following on the perception, the latter would be purely cognitive in form, pale, colorless, destitute of emotional warmth. We might then see the bear, and judge it best to run, receive the insult and deem it right to strike, but we should not actually feel afraid or angry."* (In Ellsworth, 1994).

Not long afterwards, Walter B. Cannon had a relevant role in introducing the study of the central nervous system as a crucial element to emotion-related processes (Cannon, 1927; 1931). Philip Bard (1928; 1934a;b) together with Cannon, defended a theory of emotion based on diencephalic-cortical processes, giving special relevance to the thalamus and the hypothalamus. The Cannon-Bard theory stated that, when people face an event that affects them, the nervous impulse travels to the thalamus where the message divides and projects to the cortex to originate subjective experiences such as fear, rage, sadness, joy, etc. and to the hypothalamus to determine the peripheral neuro-vegetative changes. On the basis of their animal lesion studies, Cannon and Bard were the first scientists to strongly emphasize the contribution of the central nervous system to emotion and to the homeosthatic processes associated to emotion (figure 2). His work was positioned mainly against the existence of such crucial influence from bodily changes to emotion perception that had previously been suggested by the James-Lange theory, arguing that the total separation of the viscera from the central nervous system does not considerably alter emotional experience. The main contribution of the Cannon-Bard theory was perhaps the fact that it acknowledged the brain's leading role as the generator of emotional experiences and responses and their pioneering investigation applying animal experimentation to the area of emotion, thus providing the study of emotion with the scientific-experimental conception it still enjoys today.

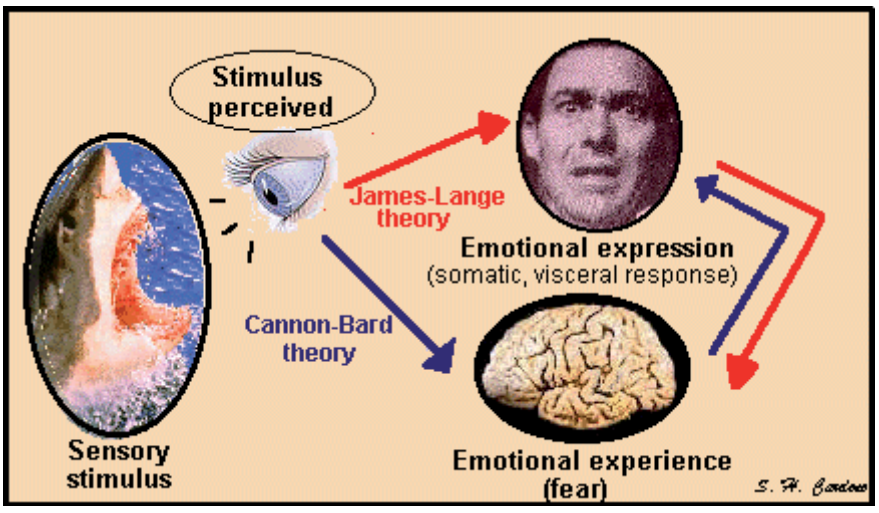
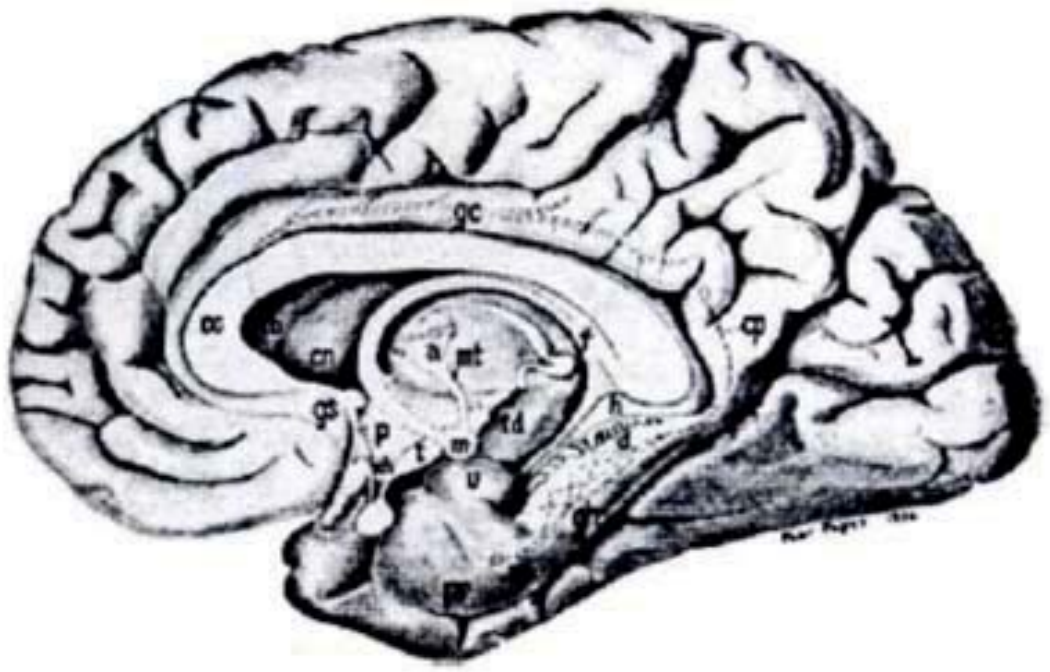


Figure 2. Comparison of the James-Lange and Cannon-Bard theories on emotions. According to the James-Lange theory (red arrows), the man perceives the frightening animal and reacts with physical (neurovegetative) manifestations. As a consequence of such unpleasant physical reaction, he develops fear. In the Cannon-Bard theory (blue arrows), the frightening stimulus leads, first, to the feeling of fear which, then, brings about the physical response. http://www.cerebromente.org.br/n05/mente/limbic_i.htm

The essential problem of the Cannon-Bard theory was to consider the thalamus as the initial "center" for emotions. Soon enough, however, in 1937, the neuroanatomist James

Papez would demonstrate that emotion is not a function of any specific brain center but of a circuit involving several interconnected medial structures, which was thereafter referred to as the "Papez circuit" (figure 3). This circuit included most of the regions Paul Broca (1878) earlier described as "le grand lobe limbique". Specifically, in Papez's work (1937) entitled *"A proposed mechanism of Emotion",* it is proposed on the basis of cumulative evidence that: *"... the hypothalamus, the anterior thalamic nuclei, the gyrus cinguli, the hippocampus, and their interconnections constitute a harmonious mechanism which may elaborate the functions of central emotion, as well as participate in emotional expression. This is an attempt to allocate specific organic units to a larger organization dealing with a complex regulatory process. The evidence presented is mostly concordant and suggestive of such a mechanism as a unit within the larger architectural mosaic of the brain".* Papez believed that the experience of emotion was primarily determined by the cingulate cortex and, secondly, by other cortical areas. Emotional expression was thought to be governed by the hypothalamus.



Abbreviations

a	anterior nucleus	h	hippocampus nudus
ab	angular bundle	m	mamillary body
cn	caudate nucleus	mt	mamillothalamic tract
cc	corpus callosum	p	pars optica hypothalami
cp	cingulum posterius	pr	pyriform area
d	gyrus dentatus	sb	subcallosal bundle
f	fornix	t	tuber cinereum
gc	gyrus cinguli	td	tractus mamillotegmentalis
gh	gyrus hippocampi	th	tractus hypophyseus
gs	gyrus subcallosus	u	uncus

Figure 3. The papez circuit. From Papez JW. A proposed mechanism of emotion (1937; Reprint, 1995).

More recently, the neurologist Paul MacLean (1952) created the expression "limbic system" (figure 4) accepting the essential bases of Papez's proposal. MacLean viewed the brain as a triune architecture (MacLean, 1973). The first part is the evolutionarily ancient reptilian brain (mainly the basal ganglia complex), which he saw as the seat of primitive emotions such as fear and aggression. The second part is the 'old' mammalian brain (which he originally called the 'visceral brain'), which augments primitive reptilian emotional responses such as fear and also elaborates the social emotions. This brain system includes many of the components of the Papez circuit (the thalamus, hypothalamus, hippocampus and cingulate cortex) along with important additional structures, in particular the amygdala and parts of the frontal cortex. Finally, the 'new'

mammalian brain consists mostly of the neocortex, which interfaces emotion with cognition and exerts top–down control over the emotional responses that are driven by other systems.

MacLean's limbic system concept survives to the current days as the dominant conceptualization of the 'emotional brain' and neuro-scientific view of emotion, and the structures that he identified as important for their functional roles in emotion perception have been the focus of much of the research in affective neuroscience to date (see figure 5 for a representation of the MacLean's limbic system theory).

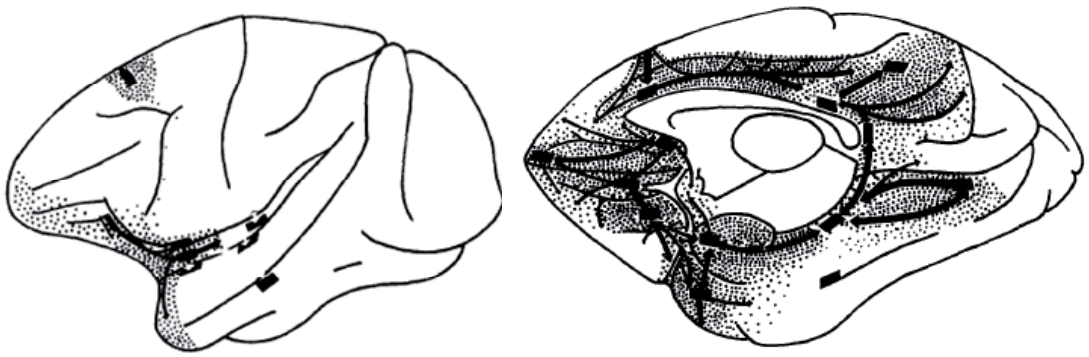


Figure 4. A representation of the limbic and extralimbic cortex in the macaque. Arrows indicate anatomical connections. From MacLean, 1952.

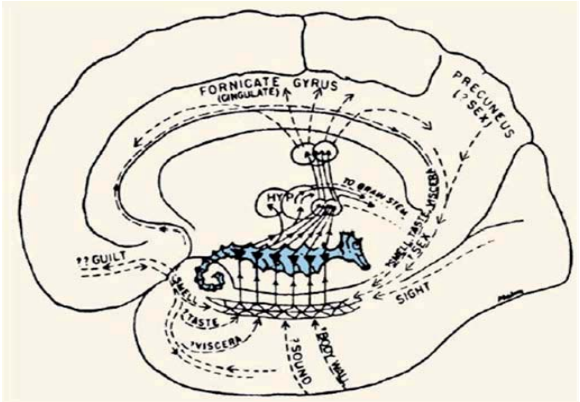


Figure 5. MacLean's limbic system theory of the functional neuroanatomy of emotion. According to MacLean, the hippocampus (illustrated here as a seahorse) received sensory inputs from the outside world as well as information from the internal bodily environment (viscera and body wall). Emotional experience was a function of integrating these internal and external information streams. HYP, hypothalamus. From MacLean, 1949.

Table 1 presents major historical milestones to the study of emotion from the neuro-scientific perspective, which have relevantly contributed to the major conceptualizations of brain emotion processing during the past 50 years (that will be presented in the following epigraph).

Table 1 – Key scientific milestones in the scientific study of emotion in the 19th and 20th centuries (adapted from Dalgleish et al, 2004; 2009)

1868 - Harlow describes the effects of prefrontal cortex damage to **Phineas Gage**
1872 - Charles **Darwin** publishes ***The expression of emotions in man and animals***
1878 – **Broca** outlines the architecture of the le **grand lobe limbique**
1884/5 - **James and Lange** independently propose their bodily **theory of emotion**
1912 - **Mills** first puts forward a **right hemisphere** hypothesis of emotion
1931 - The **Cannon-Bard theory** of emotion is outlined
1937 - **Kluver and Bucy** publish their work on temporal lobectomy
1937 - **Papez** outlines his theory of emotion
1943 - **Hess and Bruger** describe their earlier work on single cell recording in the **hypothalamus**
1949 - **MacLean** proposes his **tripartite 'limbic' model** of emotion
1956 - **Weiskrantz** describes the effects of **amygdala ablation in monkeys**
1959 - **Schneirla** outlines an **approach-withdrawal** model of emotion
1962 - **Schachter and Singer** describe experiments indicating the importance of **cognitive factors** in determining the nature of emotion experience
1970/1971 – **Pribram and Nauta** propose early versions of the somatic marker hypothesis
1980 - **Zajonc** argues the case for emotion in the absence of cognition
1982 - **Lazarus** argues the case for emotions requiring cognition
1983 - **Ekman** and colleagues propose that different basic emotions can be distinguished autonomically
1986 - **LeDoux** proposes multiple **amygdala** pathways for **fear conditioning**
1991 - **Damasio** outlines his **somatic marker** hypothesis
1994 - **Adolphs** et al. describe impaired **recognition of emotion in facial expressions** following bilateral damage to the human **amygdala**
1995 - **Bechara** et al. show that the amygdala is necessary for fear conditioning but not for explicit memory of the conditioning experience
1996 - **Cahill** et al. reveal how the **amygdala** is important in the consolidation of **emotional memories**
1997 - **Phillips** and colleagues reveal the involvement of the **insula in disgust**.

1.1.3. Significant conceptualizations of brain emotion processing during the past 50 years

The tradition of interoceptive/somatic/autonomic influences on emotion perception. A modified version of the James–Lange theory has gained influence in the lastest conceptualizations of emotion brain processing. Such a view suggests that bodily signals interact with other forms of information to build the final emotional experience. Schachter and Singer (1962) provided important information when they demonstrated that similar patterns of bodily arousal could be experienced as anger or happiness as a function of the social and cognitive context in which they occur. Such perspective on the interaction of bodily information and cognition to generate emotional experience has become one of the most influential conceptualizations (Mandler, 1975).

Damasio and colleagues have continued the tradition of promoting a key role for bodily feedback in emotion, implicating the prefrontal cortex (PFC, especially the ventromedial PFC), with their presentation of the somatic marker hypothesis

(Damasio et al, 1991; Damasio, 1994; 1996; 1997), which builds in previous work by Nauta (1971), who used the term 'interoceptive' markers instead of somatic markers, and by Pribram (1970), who used the phrase 'feelings as monitors', all reflecting the original ideas of James and Lange. Somatic markers, i.e., physiological reactions of the autonomic nervous system, provide a signal to the cortex indicating the importance of current events that have given rise to emotion-related consequences in the past, therefore helping to guide future behavior on the basis of relevant emotion sensations that may not even be accessible to consciousness. Neuroimaging studies have also provided relevant data on the subject (mainly from the work based on interoceptive awareness, Critchley et al, 2004; Craig, 2003; 2004), which will be commented on in following epigraphs.

The right brain and emotion. Right-left hemispheric asymmetries in emotion processing. Another influential view of the brain's emotion processing was the so-called "right hemisphere hypothesis", which was substantially developed in Davidson's work (1984a;b) from an original idea by Mills (1912) that was extended by Sackeim and Gur (1978a;b) and others (Schwartz et al, 1975; 1979). In summary, this hypothesis emphasized a specialized role of the right hemisphere in all aspects of emotion processing, with specifically relevant roles in the perception and expression of emotion (Adolphs et al, 1996).

Davidson's valence asymmetry model is related to the right-hemisphere hypothesis, emphasizing, though, the differential contributions of the left and right hemispheres to positive and negative emotions, respectively (Davidson, 1984a;b). In the same direction, Schneirla (1959) proposed that emotions may be separated into two major groups, those related to approach and those related to withdrawal behavior, which would be mediated by left and right hemispheres and particularly left and right frontal lobes, respectively. Other common terms for the same idea are "behavioral activation" and "behavioral inhibition" systems, approach and withdrawal systems, and appetitive and aversive systems. Finally, Rolls proposed a dual-system approach that conceptualizes emotions in terms of states elicited by positive (rewarding) and negative (punishing) instrumental reinforcers (Rolls, 1990; 1999).

Functional specialization within emotion brain systems. Regional specialization of distinct brain regions and networks in emotion processing has also been partially documented to date. Lesion studies have been relevant in informing as to such regional specialization, although widely increasing and compelling evidence has been mainly provided by means of functional neuroimaging, and especially fMRI studies, which have grown exponentially over the past fifteen years. For example, a wealth of studies have suggested a particularly relevant role for the amygdala in the processing of fear and fear conditioning through the last decades, with a remarkable contribution from work by LeDoux and Adolphs and their collaborators (Adolphs, 2002; 2008; Adolphs et al, 1994; 1999; Bechara et al, 1995; Calder et al, 1996; LeDoux, 2003; Scott et al, 1997; Young et al, 1995), the insula has been particularly involved in the processing of disgust (Phillips et al, 1997) and the basal ganglia and ventral medial orbitofrontal regions in the processing of reward and its anticipation (Kringelbach and Rolls, 2004; Peterson, 2005). A complete overview of new insights from such studies is presented in later sections of the present introduction (see epigraph 1.3.2.).

1.2. Measurable markers of emotion before functional neuroimaging

The objective measure of emotion has traditionally been a complex matter in psychology and affective neuroscience. From the psychological perspective, a wide variety of techniques have been proposed to assess subjective experiences and impressions of emotional perception, such as adjective lists, interviews, self-registries, questionnaires, scales and inventories (Aebischer and Wallbott, 1986; Pinillos, 1985; Plutchik, 1989; Wallbott and Scherer, 1989; Zuckerman and Lubin, 1965). There are a variety of lists to register affective states, with each selecting a number of words corresponding to diverse and very different emotional experiences (see from Plutchik, 1989: Plutchik 1966, Izard 1972; Howard 1977; Curran and Cattell, 1975; Clyde, 1963), such as happiness, pleasantness, fear, anger, interest, disgust, sadness, surprise, affliction, guilt, embarrassment, contention, concentration, depression, optimism, cooperation, fatigue, activation, aggressiveness, tiredness, etc. Some scales enquire whether or not a subject usually feels or has felt a particular emotion during a specified period and with what level of intensity on the basis of various ranges (1-3 points; 1-5 points; 1-10 points). Additionally, there are semi-open questionnaires (e.g., Wallbott and Scherer, 1989) for a more complete description of the emotional situation, including

questions asking where a particular situation occurred, how long it lasted, what exactly happened, how long the feeling persisted and when the situation came to an end. In this open questionnaire there are also questions about the personal description of the emotional reaction and a measure of its intensity using a numerical rating scale from 0 to 9 and questions as to the degree of the exerted control over the elicited emotional state.

Self-report measurements of emotional experiences, although clearly valuable in informing as to the description subjects make of their own state, have important disadvantages, as the measure may be contaminated by many variables that do not depend on the emotional experience *per se*, but on the subjective, conscious, reflexive and effortful assessment made by subjects, who are also constrained by the verbal description they are able to provide.

In part, this is why other, more objectively-based and less contaminated, measures have been developed to register the autonomic sympathetic component of emotion, which normally occurs when the evoked emotional response is sufficiently intense (Ekman et al, 1982; 1983; Kreibig, 2010; Scherer, 2005). Such techniques include registering heart rate and blood pressure increases (electrocardiography or oxy-pulsimeter), pupil dilatation, electro-dermal resistance (conductance), muscle contraction (electromyography), respiration changes (pneumatic ventilation registering), and tremor (Levenson, 1994). Almost all psychophysiological signals generate electrical, infrared or mechanical changes that can be recorded from the surface of the skin.

Other objective encoding techniques used in the psychological environment have been based on registering and analyzing the sonorous (Pittam and Scherer, 1993; Scherer, 1986) and non-verbal expression of emotions (including corporal, gesture, and facial expressions related to tension changes in a variety of muscles in the head [Ekman, 1982; Ekman and O'Sullivan, 1991]).

Finally, and prior to the huge development of neuroimaging, which will be commented on later, another very relevant group of emotion-related registering techniques developed addressed to measure signals directly obtained from the central nervous system, such as electroencephalography, magnetoencephalography or evoked potentials (Cohen, 1972; Niedermeyer and Lopes da Silva, 2004; Nunez and Srinivasan, 1981; Regan, 1979). Such techniques have the advantage of a very high (in the order of

milliseconds) temporal resolution. Their core problem lies in the incapacity to precisely localize the brain source generating the registered electrical signal.

All such measurements, despite providing relevant information as to how the emotional response is reflected in each particular domain, pose problems related to the reliability of the measure (noisy data) and the validity (mostly ecological validity) of some experimental approaches (e.g. the artificiality of the experimental situation where the data are recorded).

1.3. Functional neuroimaging advances to emotion research

The last decades have been especially relevant for the understanding of brain physiology underlying emotional experiences thanks to the revolution of neuroimaging techniques. The contribution of neuroimaging to the conceptualization of emotional experiences and affective states as dynamic states of the brain, involving parallel and integrated activity within relevant brain interconnected systems, has been revolutionary in improving our understanding of both normal emotion processing and the pathophysiology of affective disorders.

Single-photon emission tomography (SPECT), positron emission tomography (PET) and, later, functional magnetic resonance imaging (fMRI) and Near-infrared spectroscopy (NIRS), especially PET and fMRI, completely changed the context for the neurobiological study of emotion in humans (Andreasen, 1988; Brown et al, 2007; Goodwin, 1996; Otte and Halsband, 2006; Phelps and Mazziotta, 1985). In particular, in terms of the whole brain, it was possible to characterize in-vivo the specific contribution of distinct regions and functional systems to the generation, maintenance and regulation of distinct emotional responses and affective states.

PET studies, although important for providing absolute quantitative measurements of brain metabolic activity and blood flow during distinct emotional states, have two particularly relevant disadvantages in terms of the study of emotion: i) its invasive nature as it involves administering intravenous injections to infuse the radiotracer and, in some cases, arterial blood sampling and ii) the low temporal resolution due to signal averaging requirements of approximately 1 min (thus hindering the assessment of most

of the brain regional dynamics contributing to emotion perception). Other general limitations of the technique are related to the need for a nearby cyclotron facility to prepare radioactive tracers (problematic in terms of multiple repetition of the procedure due to the cumulative effects of radioactivity) and the high cost and feasibility for large and representative group studies.

1.3.1. Functional magnetic resonance imaging

Functional magnetic resonance imaging (fMRI) has made significant advances, in comparison to PET, in a variety of issues that will be discussed later with special relevance to the study of brain emotion responses. Considering the relevance of this technique and its use in the four studies that form part of the present PhD thesis, a special mention will be made as to its basic principles, measurements of neural brain activity and activity changes and analysis procedures, prior to explaining its contribution to the study of brain systems underlying and serving the construction of emotional experiences.

1.3.1.1. fMRI advantages for assessing brain emotion circuits

Most fMRI experiments measure *blood oxygenation level dependent* (BOLD) contrast - an endogenous haemodynamic signal reflecting blood oxygenation changes linked to neuronal activity (Ogawa et al, 1990). BOLD fMRI is therefore an *indirect* measure of neuronal function, which is generally considered to reflect synaptic input and local processing in neuronal ensembles as opposed to neuronal spiking activity *per se*. In conventional applications, BOLD fMRI has a temporal resolution in the order of seconds (1 to 3 s) and a spatial resolution in the order of cubic millimeters (cubes of tissue 3 to 5 mm on each side) when covering the whole brain. Largely due to its non-invasive nature and its good spatio-temporal resolution, adequate for assessing regional responses to behaviorally-relevant events or brain states, but also as a result of advances in the acquisition, design and statistical analysis of brain mapping experiments, BOLD fMRI has become, over the past 15 years, the main research tool in human affective neuroscience (Logothetis, 2008).

Two early discoveries of special relevance to BOLD fMRI are *(i)* that deoxygenated hemoglobin is paramagnetic and *(ii)* that there is an oxygenation dependence of the

transverse relaxation time of water protons in whole blood at high magnetic field strengths (≈/> 1.5 Tesla). This led Ogawa and colleagues (1990) to investigate whether altering blood oxygenation levels would influence the visibility of blood vessels on T_2*-weighted MR images. When increasing the relative concentration of deoxygenated hemoglobin in blood, they observed reduced T_2*-weighted signal intensity in local vasculature on gradient-echo images. Ogawa and colleagues went on to suggest that their observation of "blood-oxygenation-level dependent (BOLD) contrast" might potentially be used to investigate neuronal activity, albeit indirectly, through changes in blood flow and tissue oxygenation.

As introduced above, BOLD signal is inversely proportional to the concentration of deoxygenated hemoglobin, which is influenced by three physiological parameters: cerebral blood volume (CBV), cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen consumption (CMRO2). Signal increases reported in BOLD fMRI experiments are related to the fact that neuronal activity increases regional CBF -and glucose utilization (CMRglu)- to a much larger extent than CMRO2. The net effect of neuronal excitation is therefore to decrease the concentration of deoxygenated hemoglobin, which in turn increases BOLD signal strength. It is now understood that the characteristic BOLD signal changes observed in fMRI studies reflect the summation of these competing events (CBF, CMRO2 and CBV), resulting in a complex response function that is controlled by several parameters (Buxton, 2004). In other words, the BOLD signal does not reflect a single physiological process, but rather represents the combined effects of CBF, CBV, and CMRO2.

The first fMRI studies of the brain in humans were reported in 1991-1992 and involved sensory-related activation of the visual and motor cortices (Belliveau et al, 1991; 1992; Kwong et al, 1992; Ogawa et al, 1992; 1993). This work confirmed that MRI could be used to investigate regional changes in brain activity similar to functional brain mapping studies at the time with PET imaging (see Figure 6). Since these initial studies, the growth of BOLD fMRI in neuroscience applications has been extraordinary, initially by providing a non-invasive and improved brain mapping alternative to PET imaging, but subsequently assuming its own unique role in cognitive and affective neuroscience research.

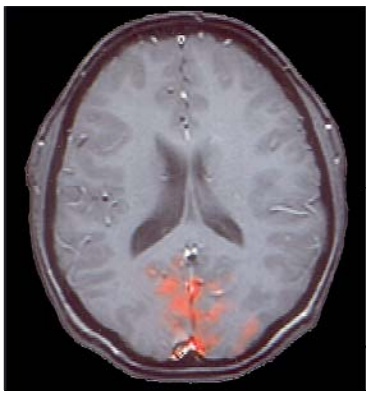


Figure 6. An early BOLD fMRI study of visual cortex activation in a single human subject. This study was performed in October 1992 at the Magnetic Resonance Centre of Pedralbes in Barcelona, Spain (gradient-echo sequence at 1.5 T, GE Signa), single-slice acquisition, 96 x 64 pixel matrix; round surface coil; TR=7 s. The subject was stimulated with an 8 Hz visual flicker in a blocked-design experiment that compared 4 blocks of visual stimulation alternating with 4 blocks of darkness. Nine images were acquired per block (image courtesy of J. Pujol).

1.3.1.2. Experimental designs of interest to the studies of the thesis

The most typical fMRI experiment consists of the acquisition of a time-series of T2*-weighted images of the whole-brain while subjects are exposed to an alternatively varying "control"-"activation" conditions. The goal of this approach is to evoke significant changes (either above or below the control condition) in blood flow and oxygenation within a given region or functional network associated with the 'task-on' state. In practice, the magnitude of task-related changes in fMRI studies is small (up to ± 5%, but usually less) in comparison to the total image intensity and variability across time due to various sources of physical (MR system) and physiological noise. Careful experimental design and the use of post-processing methods for maximizing the detection of activation in the BOLD time-series is therefore a critical feature of fMRI studies (Ch. 8 to 13, Huettel et al, 2004). One common approach is to take advantage of the summed signal as a way of minimizing the influence of noise in fMRI experiments. The idea here is that the BOLD response summed over several trials will reduce the influence of random noise sources as a result of averaging.

Another approach that has recently been introduced in fMRI studies due to advances in the analysis domain involves acquiring a time-series of T2 *-weighted images of the whole-brain during specific sustained (several minutes) states, such as passive or active resting, sleep, anesthesia and several affective and symptomatic states (in psychiatric/neurological disorders). This unconstrained design allows us to study the functional organization within multiple brain networks (pattern of coherent dynamic fluctuations of BOLD signal across brain regions gathered in the form of networks) during a specific state of the brain and mind, and to detect variations in brain

functional connectivity across distinct subjective states, which may correlate with distinct measures of emotion perception.

1.3.1.3. fMRI statistical analysis approaches relevant to the studies of the thesis

Classic model driven activation analysis. The majority of fMRI studies to date have adopted a conventional voxel-based mapping approach based on extensions of the general linear model for time-series analysis (Huettel et al, 2004; Friston et al, 2007). The basic premise behind such approaches is that the observed fMRI data may be accounted for by a combination of several experimental (or model) parameters (factors) and uncorrelated (or independently distributed) noise. Given the high number of statistical tests performed (voxel by voxel) some correction factor for multiple comparisons will generally be applied, leading to the generation of statistically thresholded 'activation' maps related to the experiment at hand. This may be performed for the whole brain or for specific regions of interest.

Multivariate data-driven fMRI connectivity analysis. Other techniques based on multivariate analyses such as independent component analysis (ICA) may also be used to investigate which areas of the brain are 'activated' by a task in fMRI studies or to decompose whole brain fMRI data into independent networks of brain regions (spatial components) involving voxels (region units) following similar temporal dynamics of common neuronal (or non-neuronal, i.e. physiological and MRI noise sources) origin (McKeown et al, 2003; Calhoun et al, 2009). Results are presented as a set of spatial maps with their associated time courses. These techniques, as opposed to the general linear model approach, are data-driven and do not require the specification of experimental models *a priori,* and may therefore be very useful in the context of emotion-related activation paradigms as they may provide relevant new (and *a priori* unpredictable) information as to the response dynamics within distinct region networks involved in different aspects of emotional processing.

Finite impulse response analysis approach. Another statistical analysis approach useful in characterizing the temporal evolution of brain activations across a representative activation cycle so as to graphically represent (using a movie display) the evolution of whole-brain responses during an emotional task on a "real-time" basis, is the finite

impulse response (FIR) analysis approach (Dale and Buckner, 1997). Specifically, it involves modeling fMRI time-series using as many boxcar regressors as the number of consecutive fMRI scans covering the activation cycle and then obtaining a "contrast" image representing whole-brain activations occurring at each specific scan. Finally, the concatenation of the obtained "contrast" brain images will appear as the scan-by-scan evolution of brain activity (which may be used to generate movies of real-time brain activity changes in response to an event of interest). Such an approach will be of special relevance to the study of complex brain responses integrating different functional units such as those underlying emotional experiences.

Seed-based functional connectivity analysis. Finally, another useful approach for the assessment of state-dependent functional connectivity (i.e., temporal co-oscillation of BOLD signal between separate brain regions representing brain patterns of synchronized neural activity) within specific networks using an *a priori* selected region of interest ("*seed*"), is the so-called *seed-based analysis* (Harrison et al, 2009). In this approach, the time course of each selected region of interest is used as a regressor to be correlated with the time course of all the voxels throughout the brain. In addition to the signal of interest (seed), estimates of white matter, CSF, and global brain signal fluctuations are derived, to be included as non-interest nuisance variables in the linear regression analyses. These nuisance signals are typically adjusted for in resting-state functional connectivity studies as they reflect global signal fluctuations of non-neuronal origin (e.g., physiological artifacts associated with variables such as cardiac and respiratory cycles, CSF motion, and scanner drift; Fox and Raichle, 2007).

1.3.2. Contribution of functional neuroimaging to the understanding of brain emotion systems

Functional neuroimaging studies of emotion attempt to disentangle the neural basis of emotion perception and the neural circuitry serving its various forms. Neuroimaging studies have used very distinct forms of elicitor stimuli or emotional induction methods (visual, painful somatosensory, auditory, autobiographical recall/imagery) and very different emotions and emotional contexts of study (recognition and visualization of facial expressions of emotion [fear, happy, sad, disgust, surprise], fear conditioning and responses, visualization of aversive non-facial pictures, love and attachment, vocal emotional processing, music, pain and empathy for pain, regret, moral dilemma

situations, sad mood states, expectations of reward and punishment, craving states and craving-related activation to drug cues, among others) (for relevant examples of emotion processing brain networks in a variety of contexts see: Fisher et al, 2006; Harrison et al, 2008a;b; Peterson, 2005; Pujol et al, 2009; Singer et al, 2004; For comprehensive reviews and meta-analysis on brain emotion processing, see: Fisher et al, 2006; Fujiwara et al, 2009; Fusar-Poli et al, 2009; Kalisch, 2009; Knutson and Greer, 2008; Kober et al, 2008; LaBar and Cabeza, 2006; Lee and Siegle, 2009; Mechias et al, 2010; Murphy et al, 2003; Ochsner and Gross, 2005; Peterson, 2005; Phan et al, 2002; 2004; Phillips et al, 2003a; Schirmer and Kotz, 2006; Sommer et al, 2009; Vignemont and Singer, 2006; Vytal et al, 2009; Wilson et al, 2004). Such data sets, taken as a whole, have shown a complex, extensive and partially specialized (as to emotion types and stimulation modalities) picture of the brain systems involved in the elicitation, maintenance and regulation of affective responses and states (see figure 7 for relevant and different examples of emotion brain responses and figure 8 and 9 for different types of meta-analysis on emotion processing and its various aspects).

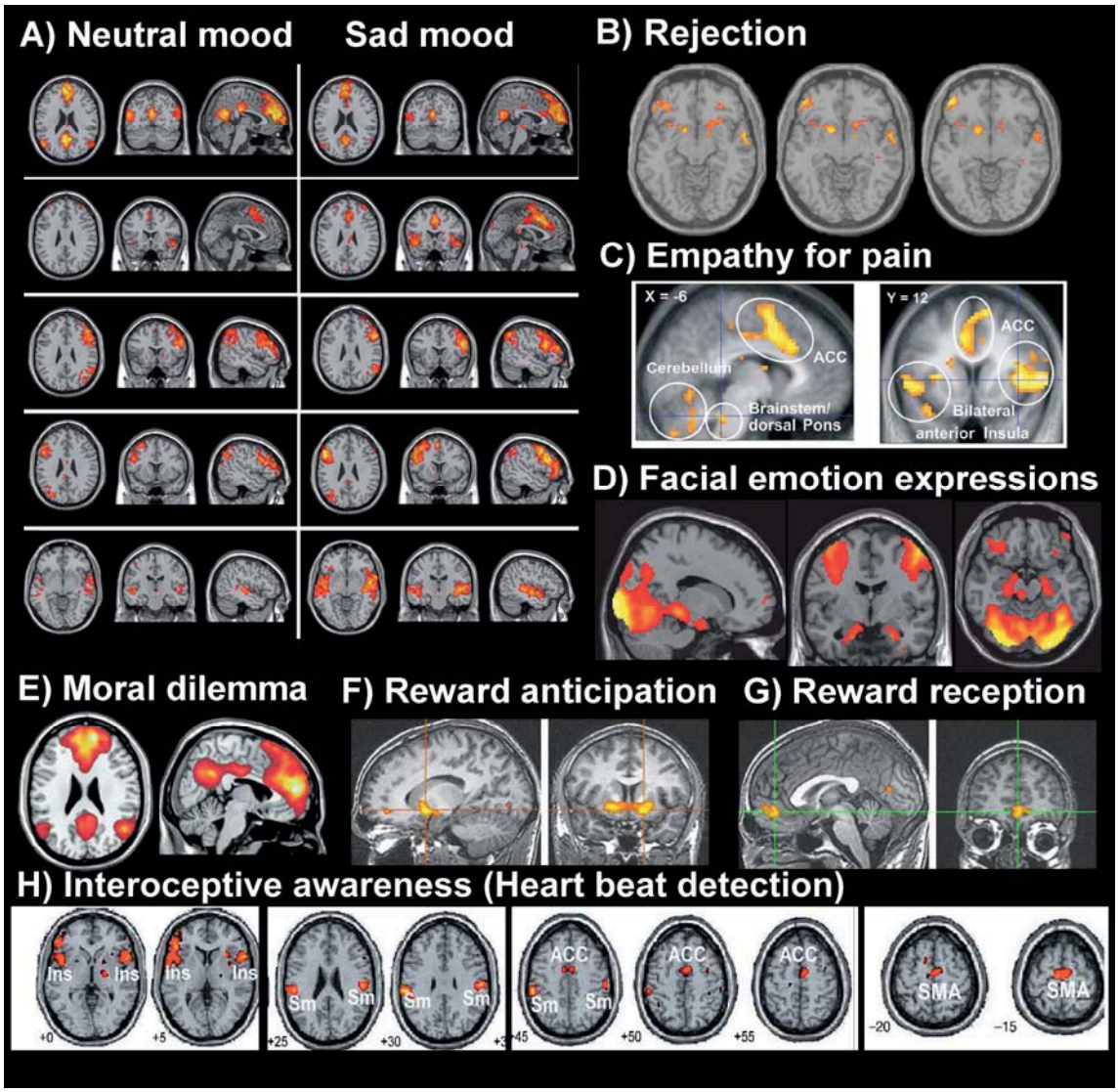


Figure 7. Examples of distinct brain regions and networks engaged in a variety of emotion contexts and affective states. A) Each row shows the changes in different functional brain networks (all relevant to the construction of a sustained negative affective state) from neutral to sad mood states. Major changes are observed in the default mode network (row 1), paralimbic network (row 2), bifrontal-biparietal network (rows 3 and 4) and paralimbic network (row 5). From Harrison et al, 2008a. **B)** Regions showing positive responses to rejection, mainly involving the ventral basal ganglia including the striatum and the inferior prefrontal cortex. The images correspond to a group of subjects who were in love and have been recently rejected compared with a group of in love subjects while viewing pictures of their couples or ex-couples. From Fisher et al, 2006. **C)** Activations occurring when painful stimulation was administered to a beloved person, i.e., regions encoding empathy for pain. This pattern clearly includes anterior insula, anterior cingulate cortex, cerebellum and brainstem, important regions for encoding the affective dimension of pain. From Singer et al, 2004. **D)** Brain activation map of responses when matching emotional faces compared to a control shape condition. The pattern includes the fusiform gyrus, amygdale extended to the hippocampus, and the bilateral dorsolateral prefrontal cortex. From Pujol et al, 2009. **E)** Brain activation map during a moral dilemma task, where the two possible choices would have negative emotional consequences for the person. From Harrison et al, 2008b. **F)** Nucleus accumbens (NACC) activation during anticipation of monetary, food, sexual, luxury, and other types of reward. From Peterson, 2005. **G)** The medial prefrontal cortex is activated when a preferred brand is seen or when a reward is received. From Peterson, 2005. **H)** Activity relating to interoceptive attention and awareness. Regional enhancement of brain activity during trials when attention was centered in detecting heart-beating and respiration compared to control trials when attention was centered in auditory perception. Ins: Insula. Sm: Somato-motor cortex, ACC, anterior cingulate cortex. SMA: Supplementary Motor Area. From Critchley et al, 2004.

Affective states are most often categorized into one of several "discrete emotions" such as "anger," "fear," "happiness," "sadness" and "disgust" or into broader affective dimensions, such as hedonic valence (positive/negative), arousal (high/low), or approach/withdrawal. Several observations can be made from figures 7, 8 and 9, which will be commented upon below.

Neuroimaging has markedly contributed to delineating the functional neuroanatomy involved in a large variety of forms of emotional processing. On the whole, results from this discipline, in keeping with relevant contributions from animal and human lesion studies, have provided data supporting the significance of most of the regions described by Papez and MacLean (limbic system) as relevant elements contributing to the brain's construction of emotion. Moreover, these studies and their integration have helped to elucidate the functional specialization of particular brain structures contributing to evoke specific emotional responses, such as the influence of the amygdala in mediating the processing of facial expressions of fear and fear conditioning, the involvement of the ventral striatum in the anticipation of reward and reward experiences as well as in feelings related to inter-personal attachment such as love, the participation of the orbitofrontal cortex in processing rewards and punishments, the insula in mediating disgust emotions and (as will be commented upon later) interoceptive awareness, and the region of the cingulate cortex in participating in a wide range of emotional experiences, with special relevance in self-related autobiographical recall and imagery or situations related to capturing the consequences of self behavior (see studies cited in the previous paragraph and figures 7, 8 and 9).

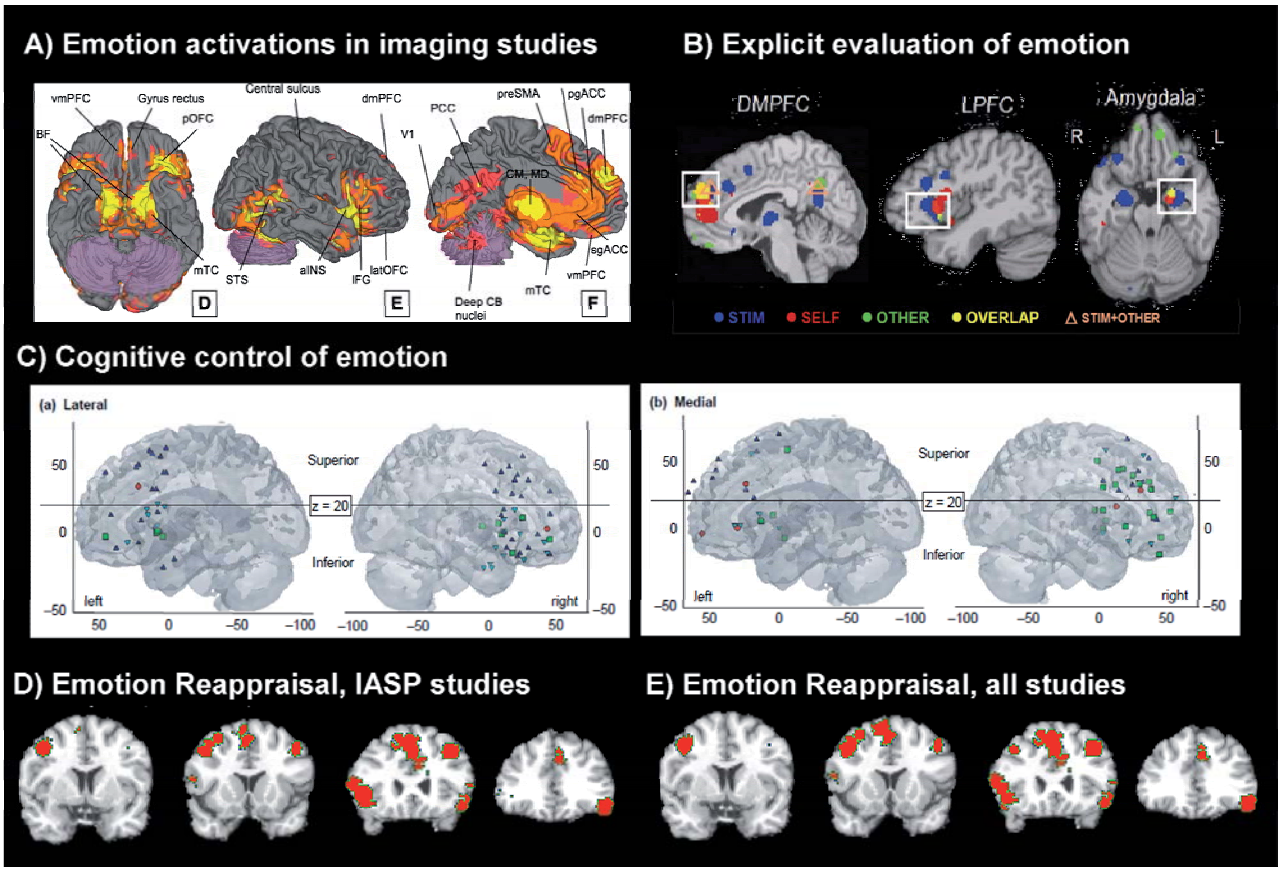


Figure 8. General meta-analyses of emotion processing. A) Regions that were consistently activated across neuroimaging studies of emotion, including medial and lateral regions of the frontal cortex, the anterior cingulate cortex, the insular cortex, the basal ganglia, amygdala and hippocampus, the hypothalamic region, the temporal pole, angular gyrus, posterior cingulate cortex and cerebellum. From Kober et al, 2008. **B)** Overlapping areas representing common brain networks underlying explicit evaluation of emotional content. The overlapping areas included the dorsomedial and dorsolateral prefrontal cortex and the amygdala. Different colours represent brain regions associated with different emotional evaluation tasks (Blue: brain regions activated by stimulus-focused evaluation (STIM), Red: brain regions associated with evaluation of one's own emotion (SELF), Green: brain areas associated with evaluation of others' emotions (OTHER), Yellow: Overlapping areas commonly associated with three tasks (OVERLAP). From Lee and Siegle, 2009. **C)** Activations in (a) lateral and (b) medial prefrontal cortex associated with different forms of cognitive control over emotional stimuli. Red dot: During attentionally distracting secondary task. Green square: emotion generation via anticipation. Purple triangle: Emotion regulation via reappraisal or placebo. Blue triangle: Emotion regulation via extinction or revearsal. From Ochsner and Gross, 2005. **D)** Consistent reappraisal-related activations across 9 studies that used IAPS (International Affective Picture System) pictures for negative emotion induction. From Kalisch, 2009. **E)** Consistent reappraisal-related activations across 13 studies using a variety of negative emotion elicitation methods. From Kalisch, 2009.

Neuroimaging studies have relevantly contributed to the study of the functional specialization within emotional processing in the anterior cingulate and extended medial prefrontal regions and the anterior-posterior and medial-lateral portions of the orbitofrontal cortex. It is interesting to observe (see figure 9 G, H, I) that a gradation seems to exist along the medial frontal wall in its contribution to emotion perception, so that more dorsal regions are more commonly activated during externally delivered potentially threatening stimulation (and usually co-activates with insular portions and SMA and pre-SMA regions) whereas more anterior and ventral parts appear to be of special relevance during emotional evaluation of self-related material. Specifically,

more dorsal cingulate regions would seem to be devoted to externally delivered aversive stimulation with a suffering component (such as acute painful stimulation, anxious-arousing situations, empathy for pain experienced by someone beloved or feeling the consequences of economic loss), whereas more self-related processing such as mentally evoking feelings of sadness or happiness, and expectations of reward or rewarding experiences, are generally likelier to engage more ventral parts (*pregenual* and *subgenual*) of the anterior cingulate cortex.

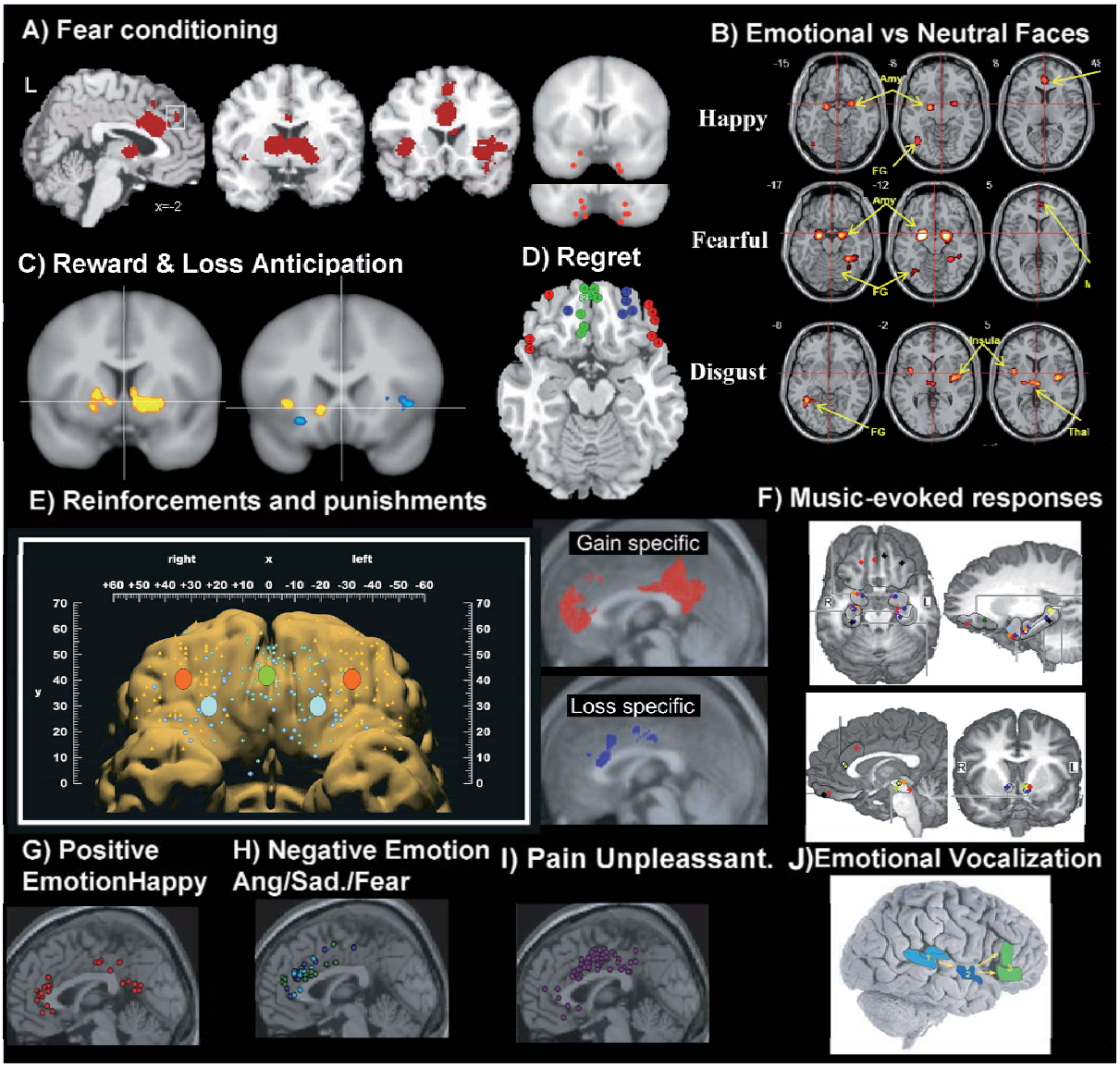


Figure 9. Specific meta-analyses of different emotions or emotional contexts. A) Meta-analyses of conditioned fear acquisition and instructed fear conditioning (anticipatory anxiety). Regions involved in such processes include the dorsal-rostral anterior cingulated cortex and extended medial prefrontal cortex, the thalamus extending to the basal ganglia, portions of the insular cortex and amygdala extended area. From LaBar and Cabeza, 2006 and Mechias et al, 2010. **B)** Brain maps of neural activation in response to happy, fearful and disgusted human faces compared with neutral faces. The amygdala is mostly involved in processing fearful and, to a lesser extent, happy faces, and the insular cortex is specially devoted to the processing of disgust expressions. Amy = amygdala; FG = fusiform gyrus; Thal=Thalamus. From Fusar-Poli et al, 2009. **C)** Meta-analysis results for gain anticipation (yellow, including mainly ventral basal ganglia) and loss anticipation (blue, mostly involving distinct parts of the insular cortex). From Knutson and Greer, 2008. **D)** The processing of regret in the orbitofrontal cortex. From Sommer et al, 2009. **E)** Orbitofrontal cortex representations of

reinforcements and punishments. Note two relevant distinctions: the mediolateral distinction, whereby medial orbitofrontal cortex activity is related to monitoring the reward value of many different reinforcers (blue and green ovals), whereas lateral orbitofrontal cortex activity (orange ovals) is related to the evaluation of punishers, which may lead to a change in ongoing behaviour. The second is a posterior–anterior distinction with more complex or abstract reinforcers/punishers (such as monetary gain and loss) represented more anteriorly in the orbitofrontal cortex (green ovals) than simpler reinforcers/punishers such as taste or pain. Orbitofrontal cortex meta-analysis by Kringelbach and Rolls, 2004. Medial wall figures show cingulate regions involved in the encoding of monetary reward and punishment. Data from Fujiwara et al, 2009. **F)** Illustration of some structures belonging to the limbic/paralimbic system. The diamonds represent music-evoked activity changes in these structures. Note the repeatedly reported activations of amygdala, nucleus accumbens and hippocampus, reflecting that music is capable of modulating activity in core structures of emotion. From Koelsch, 2010. **G) H) I)** Summary of the peak-activation sites in the cingulate cortex for positive emotion of happiness (G), for negative emotion of anger/sadness/fear (H) (blue: anger; blue: sadness; green: fear) and during noxious thermal stimulation of the skin (I) in previous studies. From Fujiwara et al, 2009. **J)** Schematic presentation of brain areas implicated in vocal emotional processing in a right sagittal view: primary, secondary and tertiary auditory cortex (light blue) extending to the anterior portion of the superior temporal sulcus (dark blue), from where projections reach inferior frontal gyrus and orbitofrontal gyrus (green). Arrows (yellow) indicate presumed processing directions (colors/numbers correspond to the processing stages of vocal emotional processing. From Schirmer and Kotz, 2006.

A relevant contribution from regions around the orbitofrontal cortex has been observed during a variety of emotion-relevant situations in neuroimaging studies (see figure 9, Kringelbach and Rolls, 2004), and has proved to be especially relevant in the processing of rewards and punishments in association with other brain regions. Specifically, medial orbitofrontal cortex activity is related to monitoring the reward value of many different reinforcers, whereas lateral orbitofrontal cortex activity is related to the evaluation of punishers which may lead to a change in ongoing behavior; also, more complex or abstract reinforcers (such as economic gain or loss) are represented more anteriorly in the orbitofrontal cortex than simpler reinforcers such as taste.

It is also noteworthy that emotion studies in humans have provided evidence suggesting a relevant role for structures that were not originally included as part of the emotion circuit in mediating different aspects of emotion perception and regulation. Such is the case for the bilateral insulae, and lateral frontal regions and the periaqueductal gray matter, among others. Frontal and insular regions are commonly associated with situations where emotion and cognitive processes take place in parallel, as normally occurs in most natural emotionally-relevant situations in humans. Regions within the anterior insulae and dorsal-rostral anterior cingulate cortex (ACC) have been consistently involved in mediating attention to interoceptive signals such as perception of heart-beat or respiratory awareness (Critchley et al, 2004; Craig et al., 2003; 2004). Additionally, although not comprehensively explored to date, frontal (prefrontal-premotor, mainly right-sided) and dorsal ACC-supplementary motor area (SMA) may have a relevant role in mediating motor aspects of emotional responses associated with

inhibition or initiation of behavior in a potentially threatening context (basic *fight-or-flight* stress responses).

The neuroimaging of emotion has also significantly complemented early conceptualizations of brain emotion processing by providing important data as to the role of the lateral aspects of the frontal cortex and specific portions of the frontal medial wall (rostral and dorsal ACC and extended medial prefrontal cortex) in the regulation of emotion and affective states (Kalisch et al, 2006; Kalisch, 2009; Phillips et al, 2003a; see figure 8C and 8D), significantly determining the "color" of our emotional experiences. It has been shown that voluntary and involuntary regulatory strategies to down-regulate aversive emotions such as those associated with pain (effortful down-regulation or placebo-mediated analgesia) or unpleasantness associated to the observation of aversive pictures, are substantially mediated by the lateral frontal cortex, with right-sided predominance (Wiech et al, 2006; 2008a;b). Modulating basic aspects of emotional responses is a key function for homeostatic regulation of behavior and it has been seen to be as severely affected in mood and anxiety disorders (and under chronic pain conditions) as other more primitive limbic or paralimbic regions (Apkarian et al, 2004; Baliki et al, 2006; Beauregard et al, 2006; Gundel et al, 2008).

As has already been pointed out in previous paragraphs, some studies have suggested an influential role of the right lateral aspect of the frontal cortex in modulating different components of emotion perception (i.e., specifically in bottom-up detection of salient biologically-relevant stimuli, withdrawal responses, preparation for action, inhibition of motor response, and emotion regulation [Corbetta and Shulman, 2002; Davidson, 2002a; Downar et al, 2000; Kalisch et al, 2006; Levesque et al, 2003; Ochsner et al, 2004; Paus, 2000; Paus and Barrett, 2004; Schutter et al, 2008; Wager et al, 2008; Wiech et al, 2008a]). These observations appear to be in agreement with more traditional ideas suggesting a relevant role for the right lateral frontal cortex in emotion perception (see introduction epigraph 1.3.2). Although important insight has been provided to date on the relevance of the right lateral frontal cortex role to affective processing, regional functional specialization within this part of the cortex is still elusive and requires further research to be understood. The specific study of the response dynamics of distinct regions within this part of the cortex may provide important feedback on the functional organization of this polyvalent structure with a marked contribution to the final emotional experience under healthy and

psychopathologic conditions.

It is important to note that one of the most relevant aspects of emotion processing deals with response-timing or the dynamic features associated to it. The characterization of the dynamics of brain emotional responses constitutes a major interest in affective neuroscience as it may importantly inform as to relevant aspects of the neurophysiology underlying different emotional experiences (Garrett and Maddock, 2001; 2006). Furthermore, the dynamic study of brain emotion responses may provide evidence as to the involvement of distinct circuits relevant in conferring distinct aspects of the unitary emotional experience. It is well known that specific emotional responses are characterized by being phasic in nature, such as the startle response or the experience of an unexpected threat or surprise, and are difficult to evoke several times during a short period, whereas others are characterized by being sustained and generating a more durable affective state such as sadness or unpleasantness associated with a variety of life situations. Therefore, although it is still mostly unexplored to date due to the limitation of previous neuroimaging techniques such as PET and to technical limitations associated with image analysis in fMRI, the dynamic study of emotions constitutes one of the most relevant aspects to be addressed in future studies in affective neuroscience.

As an integrated, though simplistic, summary of what has been exposed in figures 7, 8 and 9 and along the text, it could be stated that, when assessed from a neuroimaging perspective, the brain configuration of an emotion is complex in nature, involving a variety of regions and region networks that function in parallel and mutually influence each other, finally generating a unified experience of emotion, which is wholly dependent on the sound functioning of each and every element of the brain emotion circuit. On the whole, findings may be integrated as suggesting an important role of two neural systems in emotional perception: a ventral and a dorsal system. The ventral system, mostly including the amygdala, insula, ventral striatum, and ventral regions of the anterior cingulate gyrus, orbitofrontal and medial prefrontal cortex, is important in the identification of the emotional significance of environmental stimuli and the production of affective states and emotional memories. It is additionally important for the involuntary regulation and mediation of autonomic responses to emotive stimulation accompanying the production of affective states. A more dorsal system, including mostly dorsal regions of the medial wall, rostral-dorsal anterior cingulate and lateral aspects of the frontal lobe, provides relevant aspects associated with emotion-guided

behavior and emotion regulation that significantly determine the integrated and unitary perceived emotional experience. Finally, evidence from studies examining ventral and dorsal anterior cingulate gyral activity suggests a reciprocal functional relationship between both these neural systems. Taken together, these findings allow us to suggest that the extent to which a stimulus is identified as emotive and is associated with the production of an affective state and/or emotional behavior may be dependent upon levels of activity within a ventral neural system, important for the rapid appraisal of emotional material, the production of affective states, and autonomic response and a dorsal system, important for guiding appropriate behavioral responses to affective cues and regulation of resulting affective states. Specific abnormalities in the functioning of either or both of these two major neural systems (or subsystems within) may therefore be associated with abnormalities in emotional behavior and regulation typically observed in psychiatric mood disorders.

1.3.2.1. Specific contribution of neuroimaging findings in mood disorders

The study as to how the brain constructs emotions and affective states may be helpfully informed by exploring and further understanding the pathophysiology of core emotional disorders, such as major depressive disorder (MDD).

To date, numerous neuroimaging studies have been performed in MDD patients measuring resting-state alterations in metabolic activity across the whole brain mainly using PET and SPECT techniques (Drevets et al, 2008; Mayberg, 1997a; 2003; Phillips et al, 2003b; Price and Drevets, 2010; Savitz and Drevets, 2009). fMRI has incorporated the possibility of specifically challenging altered emotion systems in such patients using a variety of affective stimuli in a phasic or sustained manner (emotional faces or pictures, potentially-rewarding stimuli, paradigms of self-judgment and voluntary regulation of emotions, and incipiently, painful stimulation and its anticipation) (Price and Drevets, 2010; Rigucci et al, 2009). The overall impression obtained from such data is the existence of a global alteration involving several regions within the previously mentioned dorsal and ventral systems important in conferring distinct aspects of emotional experiences (Phillips, 2003b; Savitz and Drevets, 2009). As stated by Mayberg (2003), MDD may be summarized as involving a comprehensive dysregulation within limbic-cortical regions, which may hypothetically explain the combination of clinical symptoms observed in depressed patients (i.e. mood, motor,

cognitive, somatic and vegetative). On the whole, major depressive episodes are generally associated with resting-state metabolic decreases in dorsal neocortical regions, mainly affecting lateral frontal cortices and dorsal frontal medial wall, and relative metabolic increases in ventral limbic areas, mainly within pregenual and subgenual anterior cingulate cortex and extended ventro-medial prefrontal regions, orbitofrontal cortex and amygdala (Mayberg, 2003; Phillips et al, 2003b; Savitz and Drevets, 2009; Videbech, 2000).

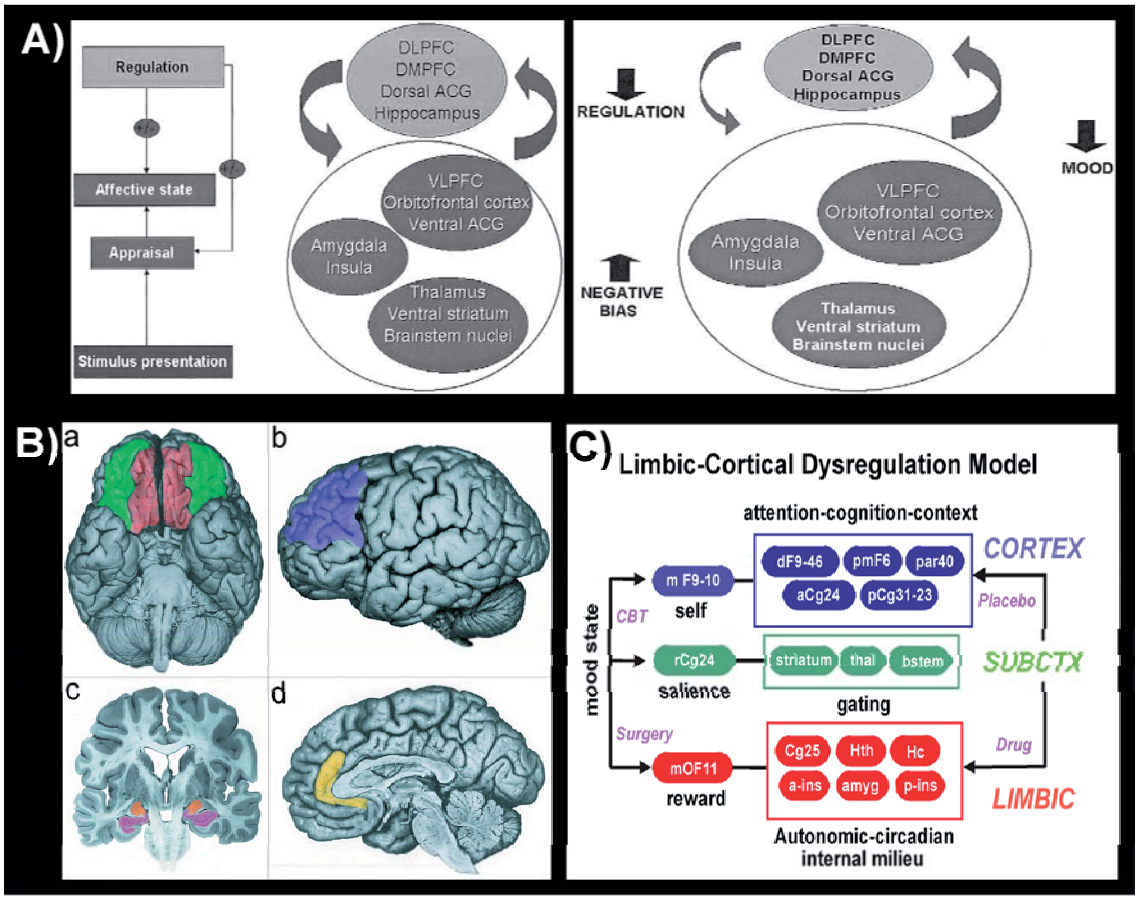


Figure 10. Classic models of brain emotion processing under healthy and pathological conditions of the affective sphere (such as MDD). A) Left panel: Phillips et al (2003a) view of the neural processes underlying emotion, a schematic representation. A predominantly ventral system is important for the identification of the emotional significance of a stimulus, the production of an affective state, which may be associated with autonomic response regulation (depicted in pale gray), whereas a predominantly dorsal system (depicted in dark gray) is important for the effortful regulation of the resulting affective states. A reciprocal functional relationship may exist between these two neural systems (depicted by the curved arrows). DLPFC, dorsolateral prefrontal cortex; DMPFC, dorsomedial prefrontal cortex; ACG, anterior cingulate gyrus; VLPFC, ventrolateral prefrontal cortex. **Right panel: Phillips et al (2003b) view** of the neural basis of the observed deficits in emotion perception and behavior in major depression, and the relationship between the neural alteration and the clinical symptom, in such patients. Volume reductions within the amygdala and other components of the ventral neural system, together with increased rather than decreased activity within these regions during illness, may result in a restricted emotional range, biased toward the predominant role of the amygdala in the perception of negative rather than positive emotions. Structural and functional impairments within regions of the dorsal system, associated with impairments in executive function and effortful regulation of emotional behavior (reduced size of the curved arrow representing the regulation of the ventral by the dorsal system), may perpetuate these phenomena, resulting in depressed mood and anhedonia. **B)** Key brain regions, as considered by **Davidson et al (2002b)** involved in affect and mood disorders. (a) Orbital prefrontal cortex (green) and

the ventromedial prefrontal cortex (red). (b) Dorsolateral prefrontal cortex (blue). (c) Hippocampus (purple) and amygdala (orange). (d) Anterior cingulate cortex (yellow). **C) Limbic-cortical dysregulation model by Mayberg, 1997a; 2003**. Regions with known anatomical interconnections. Failure of this regional network is hypothesized to explain the combination of clinical symptoms seen in depressed patients (i.e. mood, motor, cognitive, vegetative). Regions are grouped into 3 main compartments, cortical (blue), limbic (red) and subcortical (green). The frontal-limbic (dorsal-ventral) segregation additionally identifies those brain regions where an inverse relationship is seen across the different PET paradigms. Sadness and depressive illness are both associated with decreases in dorsal neocortical regions and relative increases in ventral limbic and paralimbic areas. The model, in turn, proposes that illness remission occurs when there is appropriate modulation of dysfunctional limbic-cortical interactions (solid black arrows) – an effect facilitated by various forms of treatment. Dorsal medial frontal (mF9), rostral anterior cingulate (rCg24) and medial orbital frontal cortex (oF11) are separated from their respective 'compartments' in the model to highlight their critical primary interactions both within and between 'levels' in the integration self-referential, emotionally salient, exogenous stimuli relevant to reward, punishment and learning in the healthy and depressed state. Abbreviations: mF, medial prefrontal; dF, prefrontal; pm, premotor; par, parietal; aCg, dorsal anterior cingulate; pCg, posterior cingulate; rCg, rostral cingulate; thal, thalamus; bstem, brainstem; mOF, medial orbital frontal; Cg25, subgenual cingulate; Hth, hypothamus; Hc, hippocampus; a-ins, anterior insula; amyg, amygdala; pins, posterior insula. Numbers are Brodmann designations.

fMRI has relevantly contributed to previous data by providing cumulative evidence with regard to abnormal MDD hyper-responsiveness to aversive stimuli in a variety of relevant regions such as the ACC, lateral frontal cortex, insula, amygdala, basal ganglia, fusiform and parahippocampal gyri (Anand et al, 2005a; Fu et al, 2004; Grimm et al, 2008; Ravindran et al, 2009; Sheline et al, 2001; Surguladze et al, 2005). MDD hyper-responses have also been widely reported during cognitive-executive fMRI paradigms, mainly within task-involved regions such as the frontal cortex and dorsal-rostral ACC, (Harvey et al, 2005; Matsuo et al, 2007; Mitterschiffthaler et al, 2008; Vasic et al, 2009; Wagner et al, 2006; 2008). Such brain response abnormalities were paralleled either by an absence of significant between-group differences in cognitive performance or by worse outcome for MDD patients, thus suggesting that greater activation magnitudes may be required to compensate inefficient cognitive brain processing in such patients. In contrast, some studies have found less activation of regions implicated in specific cognitive tasks (Okada et al., 2003; Wang et al., 2008), which have been more frequently accompanied by significantly reduced levels of performance in patients.

fMRI studies in MDD have additionally reported a significant failure to reduce activity in the subgenual-pregenual ACC and extended medial prefrontal cortex regions during tasks requiring external attention focusing, both during emotion processing (Grimm et al, 2009; Sheline et al, 2009) and cognitive-executive performance (Matthews et al, 2009; Mitterschiffthaler et al, 2008; Vasic et al, 2009; Wagner et al, 2006, 2008), which were partly associated with symptom severity (Grimm et al, 2009).

When looking at brain responses to appetitive rewarding stimuli in MDD patients,

Pizzagalli et al. (2009) found a reduction in fMRI responses to monetary gains in left accumbens and caudate bilaterally in agreement with results by Smoski et al. (2009), who found that the MDD group was characterized by reduced activation of striatal regions during reward selection, anticipation, and feedback. Congruently, Surguladze et al. (2005) found increasing levels of deactivations in the fusiform gyrus in MDD with increasing emotional intensity of happy expressions (compared to healthy controls showing quite the opposite pattern).

On the whole, previous data suggest a generally increased pattern of responses to aversive and cognitive stimulation in MDD patients, probably in line with augmented bias to negative stimulation and inefficient processing of cognitive demands, together with a specific and consistent reduction of brain resources destined to processing positive valence stimulation in such patients.

fMRI resting-state connectivity has just begun to show a very interesting pattern of functional disruption within cortico-cortical and cortico-subcortical regions in MDD patients (Anand et al, 2005a; 2009; Bluhm et al, 2009; Cullen et al, 2009), which may be in agreement with the hypothesized model of abnormal cortical regulation of limbic and subcortical regions traditionally suggested for MDD (Mayberg, 2003; Phillips et al, 2003b; Savitz and Drevets, 2009). Additionally, specific connectivity enhancements have also been identified involving regions of crucial importance to MDD pathophysiology, such as the subgenual ACC (Greicius et al, 2007).

Interestingly, MDD neuroimaging studies have provided evidence as to the effects of various forms of antidepressant treatment on altered brain responses in such patients. Treatment has proved to effectively revert pathologically enhanced responses in the amygdala (Chen et al, 2007; Fu et al, 2004; Sheline et al, 2001) and the orbitofrontal cortex and ventral striatum (Brody et al, 1999; Goldapple et al, 2004; Mayberg et al, 2005), in addition to increasing metabolic measurements in prefrontal and parietal neocortical regions (Mayberg, 2003). Previous research has shown the capacity of a wide variety of antidepressant treatment strategies (including antidepressant drugs, electroconvulsive therapy and deep brain stimulation) to modulate and normalize functional alterations in the subgenual-pregenual ACC and extended regions within the medial frontal cortex (for relevant examples see: Drevets et al, 2002; Mayberg et al, 2000; 2005; Nahas et al, 2007). Also, resting-state connectivity increases in cortico-

subcortical connectivity between regions showing baseline functional connectivity disruptions in MDD patients has recently been observed for the first time following pharmacological treatment (Anand et al, 2005b). Taken together, such findings suggest a state-dependent nature of important baseline brain functional abnormalities in MDD patients during active depressive episodes, which may be specifically targeted with various forms of antidepressant treatment.

Functional neuroimaging has also proved to be particularly useful in detecting possible baseline biomarkers predicting positive responders to specific forms of antidepressant treatment. A region involving the pregenual-subgenual ACC and their surroundings has emerged as having positive prognostic significance in depression, as depressed individuals who show higher metabolic activity in such regions prior to treatment seem to show a higher probability of positive responses to a variety of treatment modalities (Chen et al, 2007; Dougherty et al, 2003; Keedwell et al, 2010; Mayberg et al, 1997b; Saxena et al, 2003).

On the whole, a wide variety of data suggest the significant role of neuroimaging in progressively helping to describe and elucidate distinct aspects of brain pathophysiology in MDD patients. Specific state-dependent (versus trait-related) abnormalities have been suggested and await replication to further describe the functional neuroanatomy associated with the major depressive episode. Functional MRI has shown a relevant contribution to the characterization of abnormalities in MDD that were not possible to achieve by means of PET due to its technical constraints. Initial evidence has been provided in depression as to the potential relevance of studying functional connectivity abnormalities in specific states, suggesting that important alterations involving the dynamic functional coupling within relevant emotion regions and circuits may exist in such patients. Such a hypothesis would require further comprehensive assessment in future studies.

Another important challenge in MDD fMRI studies is to further contribute to developing specific paradigms to effectively capture the effects of treatment on relevant brain emotion processing circuits. To this end, the use of primary, urgent and salient stimulation of emotional content, such as acute noxious stimulation, may prove to be especially useful in mapping the effects of antidepressant treatment on such patients, and in obtaining basic functional biomarkers of potential responders to medical and

psychological therapies, which would serve to lay the foundations for a future science of individually-tailored antidepressant treatment.

Moreover, the requirement of studies using homogeneous and well-described samples of study in terms of age at onset, years of illness, subtype, medication status, and appropriate periods of medication wash-out are required to further disentangle the partial contradictions observed across studies, which suggest the existence of relevant confounding variables which may be controlled, or at least reported, in future studies.

1.4. Pain as an emotional experience

The experience of pain involves local sensations, cognitive-evaluative processes and basically relevant affective phenomena that depend on both stimulus features and the individual's receptive state (Price, 2000; Tracey and Mantyh, 2007). A painful experience, described in such terms on the basis of its emotional component, may even occur without a primary nociceptive input (using empathy paradigms, hypnotic methods etc. [Derbyshire et al, 2004; Eisenberger et al, 2003; Raij et al, 2005; 2009; Rainville et al, 1997; Singer et al, 2004]), further indicating that unpleasant emotional feelings are integral components of painful experiences, which often occur in a threatening and biologically-relevant context, such as during disease or physical trauma.

The affective domain of painful experiences usually involves quick defensive responses, urgent desires to terminate, reduce, or escape its presence (Melzack and Casey, 1968; Price, 1999) and later effortful regulatory mechanisms to cope with the evoked unpleasant feelings (Wiech et al, 2008a), suggesting that complex brain regional dynamics may parallel distinct aspects of such emotional processes. In the same way, it can be stated that part of the affective dimension of pain is the moment-by-moment unpleasantness caused by the noxious stimulus *per se*, consisting of emotional feelings that pertain to the present or short-term future, such as distress or fear. Another component of pain affect, "secondary pain affect," includes emotional feelings directed towards long-term implications of experiencing pain (e.g., "suffering") (Price, 2000).

Pain sensations are often more intense than other types of somatic sensations, as painful stimuli normally consist of greater quantities of mechanical, thermal, electrical or

chemical energy than other, similar in nature but non-painful, stimuli. In addition, pain presents characteristics of slow adaptation (i.e., persistence), temporal summation, spatial spread of sensation at suprathreshold levels, spatial summation, and unique sensory qualities, as implied by words such as stinging, burning, and aching (Melzack and Casey, 1968; Price, 1999). Sensory attributes dispose us to perceive pain as invasive and intrusive for both the body and consciousness (Price, 1999). Both neural and psychological processes associated with pain may be conceived as important causal links in the production of pain-related emotional disturbance. The persistence of pain enhances unpleasantness and aversive feelings over time, which may significantly alter all major functional aspects in life.

In agreement with the emotional experience profoundly associated with pain, painful stimuli not only target the somatosensory brain circuitry but also evoke comprehensive responses within the brain's emotional circuits, including limbic, paralimbic and neocortical prefrontal areas within the described ventral and dorsal brain emotion circuits (see figures 11 and 12 for a representative view of the circuitry involved in pain perception). Such systems would appear to be relevant in conferring the various aspects of the complete and complex emotional response to pain, from the early urgent withdrawal-related behavior to the later components of more sustained negative affect and with its active down-regulation or amplification (see figure 13A for a representation of the descending modulatory pathway involving medial and lateral frontal cortex regions associated to the effortful down-regulation of aversive emotions in general, from Weich et al, 2008a). See figure 13B for a representation of the main factors that influence nociceptive inputs and contribute to finally determining the subjective experience of pain.

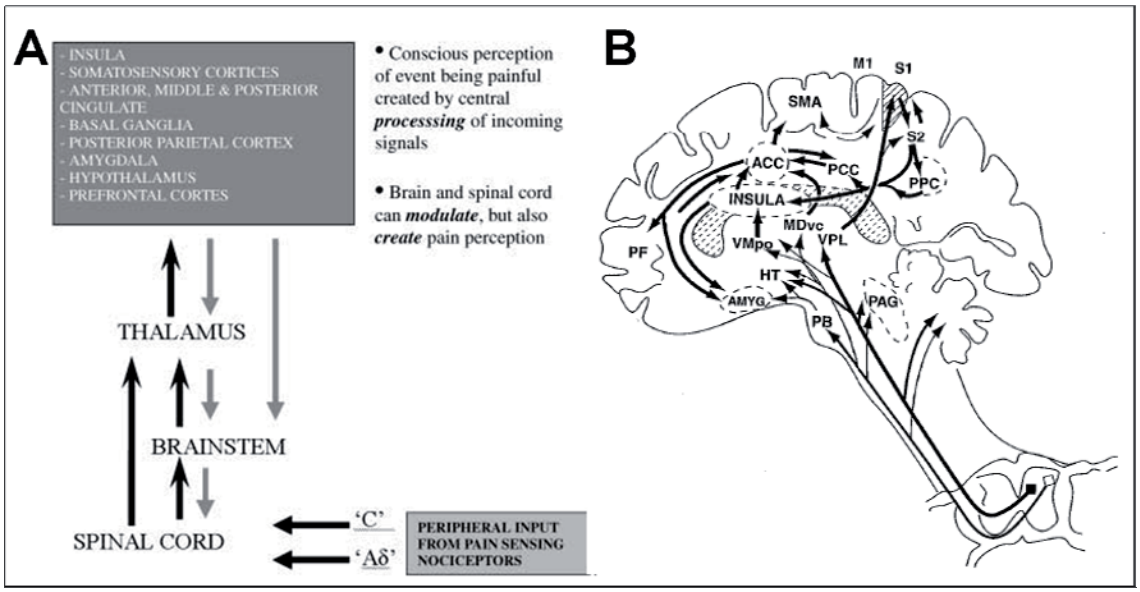


Figure 11. Simple schematic representations of nociceptive pathways from the periphery to supraspinal regions. A) Black arrows represent transmission of pain signals supraspinally, which is integrated at several levels along the neuroaxis, and at almost every level influenced by descending fibres (grey arrows). From Brooks and Tracey, 2005. B) Schematic medial wall representation of ascending pathways, subcortical structures, and cerebral cortical structures involved in processing pain (Price, 2000). PAG, periaqueductal gray; PB, parabrachial nucleus of the dorsolateral pons; VMpo, ventromedial part of the posterior nuclear complex; MDvc, ventrocaudal part of the medial dorsal nucleus; VPL, ventroposterior lateral nucleus; ACC, anterior cingulate cortex; PCC, posterior cingulated cortex; HT, hypothalamus; S-1 and S-2, First and second somatosensory cortical areas; PPC, posterior parietal complex; SMA, supplementary motor area; AMYG, amygdala; PF, prefrontal cortex.

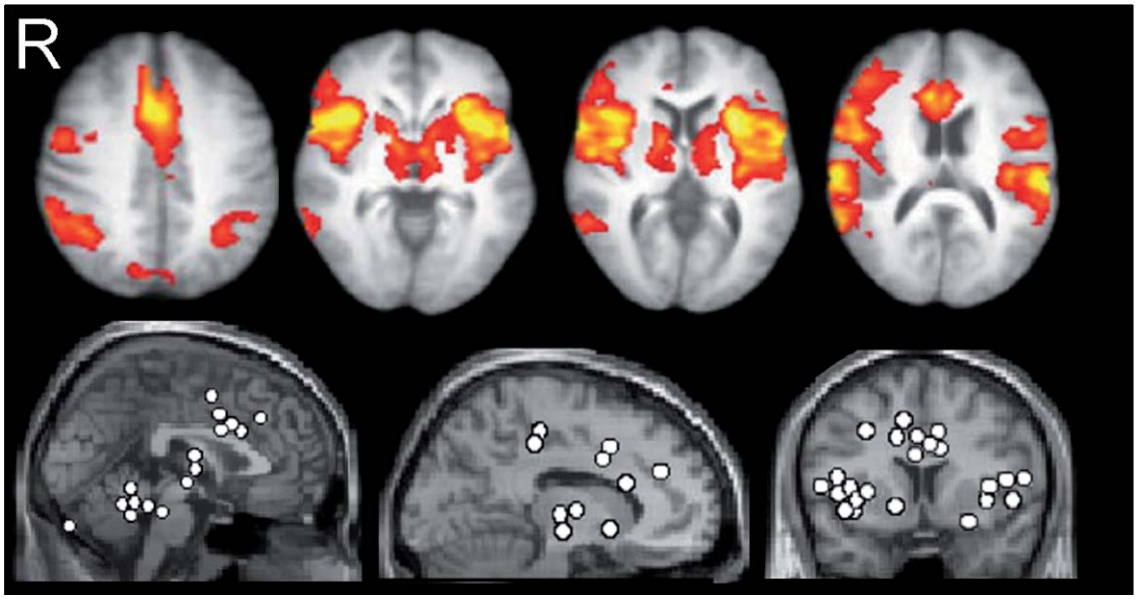


Figure 12. The cerebral signature of pain. The superior row illustrates increased BOLD activity in response to painful stimuli overlaid on a structural T1-weighted MRI. From Bingel and Tracey, 2008. The bottom row shows the results of a pain meta-analysis including a variety of PET studies of pain-related activation. From Peyron et al, 2000.

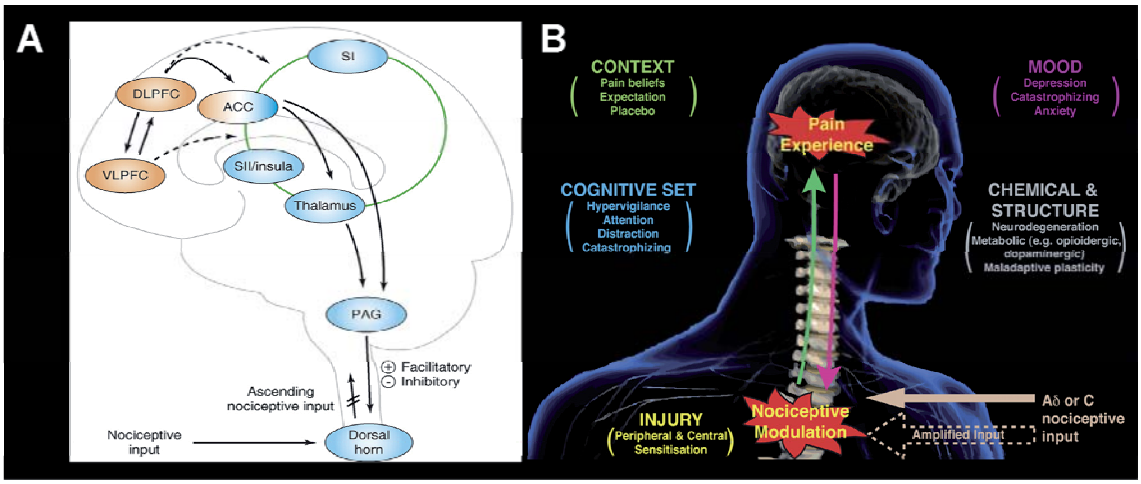


Figure 13. A) Possible neural pathways of cognitive pain modulation. From Wiech et al, 2008a. Cognitive modulations of pain are related to activation of prefrontal brain areas (DLPFC, VLPFC and ACC; shown in orange), which modulate activation in pain-associated regions in the cortex (ACC, SI, SII/insula and thalamus), brainstem and dorsal horn (e.g. the PAG and dorsal horn; shown in blue). Attention mechanisms have been shown to mainly engage the DLPFC and ACC, whereas reappraisal (voluntarily changing the interpretation and meaning of the painful stimuli) relates particularly to the VLPFC. Expectation has been associated with both densely interconnected prefrontal areas. The DLPFC is connected to the ACC, which, in turn, projects to thalamus and the PAG, a core component of the descending pain modulatory system. This system eventually facilitates and/or inhibits pain processing at the level of the spinal cord dorsal horn. Direct cortico–cortical modulations from VLPFC and DLPFC to pain-associated cortical areas are probable but have not been directly shown yet (broken lines). Areas most closely associated with pain (SI, ACC, SII/ insula and thalamus) are densely interconnected, as indicated by the green circle. For the sake of clarity, ascending projections are not fully shown. ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; PAG, periaqueductual gray; SI, primary somatosensory cortex; SII, secondary somatosensory cortex; VLPFC, ventrolateral prefrontal cortex. **B) Main factors that influence nociceptive inputs and contribute to build up the final pain perception**. From Tracey and Mantyh, 2007.

All in all, painful stimuli, given their basic emotional nature and particular ability to challenge the overall circuitry for primitive and more evolved forms of emotional regulatory processes, may be considered a valuable mean to study the brain's construction of emotional experiences from a dynamic perspective. The neuro-scientific study of pain as a non-static process involving the parallel work of several brain systems to generate an integrated and unitary perception may relevantly influence our knowledge not only as to how pain is generated but also in terms of how the brain serve the construction of emotional experiences in general.

The application of such a dynamic view may prove to be of special relevance in targeting abnormalities in pain disorders characterized by central sensitization manifested as augmented temporal summation of second pain and sustained distress associated with noxious input. The dynamic approach of study applied to clinical pain populations may be specifically tailored to capture the abnormal temporal processing within brain circuits associated with the augmented and sustained unpleasantness

typically observed in such patients. In this context, patients suffering from fibromyalgia may be a particularly relevant population to be studied, considering the misunderstood nature of their widespread and sustained pain of "non-physical" origin, which is normally accompanied by great distress and suffering, low quality of life and affective and anxious comorbidities (Wolfe et al, 1990; 1995).

1.5. Interest of our study and justification of the selected experimental contexts and study populations

The present work aims to assess a basic and relevant aspect of brain emotional responses and affective states that is still mostly unexplored in human affective neuroscience. Specifically, we aimed to comprehensively study the dynamic dimension of the emotional experience using functional magnetic resonance imaging. Two different approaches were employed. The first approach (i) was intended to dynamically characterize brain responses in emotion circuits when specifically targeted by aversive painful stimulation in healthy subjects and in selected clinical populations. The second approach (ii) specifically aimed to dynamically characterize the "baseline" functional organization of distinct emotion-processing circuits in healthy subjects and in a core affective disorder such as major depression.

1.5.1. Adequacy of the selected experimental contexts

(i) Painful stimulation was used to evoke dynamic responses in the major emotion-related brain networks. Specifically, painful stimulation was optimal to challenge brain structures relevant to affective processes including the cingulate and medial prefrontal cortex, dorsal and lateral frontal areas, insula, basal ganglia, thalamus, hypothalamus, periaqueductal gray and, to some extent, the amygdala-hippocampus region. In this context, the dynamic assessment of brain response to basically aversive and biologically salient noxious stimulation may be useful to further characterize core features of the complex emotional experience in healthy subjects, and may also contribute to better understanding pain processing in patient populations specifically showing aberrant central mechanisms contributing to the chronification of a pain-related suffering state. Such an approach may also prove to be useful in satisfactorily detecting functional

abnormalities in disease-relevant emotion circuits in core affective disorders such as major depression. Finally, the dynamic assessment of brain response to painful stimulation as a primary emotion elicitor may prove to be sensitive in capturing functional brain changes over time associated with clinical recovery in such patients following effective antidepressant treatment.

(ii) The second approach involved the study of the baseline dynamic organization (i.e., functional connectivity or activity synchrony between distinct brain regions) of major emotional networks in resting-state conditions when no active task was being performed. This new strategy may provide relevant information as to the dynamic equilibrium of brain emotional networks in healthy conditions during different sustained states of the brain (and mind). Such strategy may also be relevant in revealing the existence of a baseline pattern of abnormal organization of emotion-related networks in populations suffering from severe affective disorders, thus providing valuable new information as to the pathophysiology of such disorders from a mostly unexplored dynamic perspective.

1.5.2. Adequacy of the selected populations

Our study was based on three populations of interest:

- A sample of healthy subjects to dynamically assess brain activity in relevant emotion circuits during painful stimulation and in the resting-state.

- A homogeneous sample of female fibromyalgia (FM) patients showing severe and durable pain symptoms was studied to test whether the dynamic analysis of fMRI data of the patient group was useful both (i) to further characterize the abnormal processing of painful stimulation in this clinical population and (ii) to increase sensitivity of fMRI as a tool for the clinical use in such a context.

Fibromyalgia syndrome is characterized by the presence of widespread pain for more than three months and hyperalgesia in at least 11 out of 18 tender points when a 4kg/cm^2 mechanical stimulus is administered (Wolfe et al, 1990; 1995). The chronicity of this process favors a high prevalence of emotional disturbances that may further

modify the experience of pain (Buskila and Cohen, 2007). Psycho-physiological studies suggest that relevant alterations within the temporal domain of cerebral pain processing occur in FM associated with central sensitization mechanisms and temporal summation effects. Such aberrant central nervous system processes further contribute to aggravating the suffering component of the pain experience in FM patients. In particular, fibromyalgia patients have been shown to require longer periods of time to recover from the aversive pain sensation after painful stimulation is removed (Staud, 2002; Staud et al, 2001; 2007). This information suggests that potentially relevant pathophysiology mechanisms may be detected in such patients by using a dynamic approach when assessing brain responses to pain.

- A sample of severe MDD patients was selected to identify possible alterations in the dynamic dimension of brain activity. Major (mood) depression is a paradigmatic disorder in which functional intrinsic imbalance within emotion-related brain networks may be expected. Emotion processing was firstly assessed under resting-state conditions and then using aversive painful stimulation to challenge brain regions relevant to emotion experience and to further investigate MDD pathophysiology in this context.

1.6. Study strategy

In order to characterize the dynamic dimension of the emotional experience in a multidimensional way, we developed the following studies:

1) Firstly, we comprehensively studied brain response dynamics to mechanical painful stimulation and its cued anticipation in a group of healthy subjects. Our study showed the existence of a complex pattern of certainly different dynamics of response underlying different functional aspects of the brain's construction of pain perception. Specifically, we observed distinct onsets, durations and shapes of response among separate brain regions, including those relevant in conferring the emotional quality of pain. We specifically assessed regional specialization on the basis of the response dynamics within distinct regions pertaining to the right lateral frontal cortex. The idea emerged from previous observations showing that the right lateral frontal cortex plays an important and multifaceted role in modulating emotional experiences, as it had been

involved in promoting withdrawal responses from aversive stimulation and in actively down-regulating and reducing emotional experiences of an aversive nature.

2) Considering the results from the previous study showing the existence of distinct response dynamics in different brain regions relevant in conferring the emotional quality of pain perception, a second study was performed to test whether such temporal information may be useful to better characterize specific abnormalities in the brain response to pain in a chronic disorder involving widespread somatic complaints with a relevant contribution from affective symptoms, such as FM. The application of the dynamic approach to the study of brain responses to painful stimulation in FM patients was particularly relevant when considering the specific difficulty of such patients to recover from the painful experience, once it had been fully elicited. On this basis, we expected that the temporal coupling between the duration of the painful stimulus and the corresponding brain response constructing the subjective experience of pain would be distorted in FM in comparison with healthy subjects, specifically in the regions processing the affective component of pain.

3) A large overlap exists between emotional brain systems actively responding during externally provoked pain, and brain networks that appear to be affected in the most paradigmatic clinical population suffering from an affective disorder, i.e., major depression. Specifically, in this study we aimed to assess the particularities in the dynamic equilibrium of the emotion-related functional brain networks in a sustained pathologically negative affective state, such as that observed in MDD patients during unconstrained resting-state conditions. To guide the functional analysis of the dynamic alteration of brain emotion circuits in depression we used the sites showing maximal anatomical alterations in the MDD group as regions of interest.

4) Emotional experiences in general and the subjective experience of pain in particular significantly vary in intensity and quality as a function of the individual's receptive state. It is thus also to be expected that both the experience of painful stimulation and the underlying brain responses relevant in conferring such experience may significantly change following the improvement of the individuals' affective state by means of effective medical treatment. Our final study was intended to capture the dynamic changes in brain responses to painful stimulation within relevant emotion-related

circuits underlying the improvement in the overall affective state in MDD patients following pharmacological treatment.

1.7. Objectives

The general objective of this study was to assess the dynamic features of the emotional brain response in healthy and clinical conditions in which emotional symptoms have special relevance. We used painful stimulation as the primary elicitor of emotional responses and assessed functional connectivity during resting-state in emotion-related networks. fMRI served to carry out this functional assessment of brain activity, taking advantage of recent technical advances in the analysis of fMRI data.

Specific study objectives were as follows:

1) To study regional specialization within the right lateral aspect of the frontal cortex important for the affective modulation of pain perception on the basis of their response dynamics during mechanical painful stimulation and its cued anticipation in a group of healthy subjects.

2) To test whether the use of information concerning the actual brain response dynamics (time-courses of brain responses) to painful stimulation in a group of fibromyalgia patients (and in healthy subjects) may help to better characterize their overall subjective pain experience and the specific contribution of brain emotional processing abnormalities to such a clinical disorder.

3) To assess possible alterations in the dynamic equilibrium of the emotion-related functional brain networks during resting-state conditions in a group of MDD patients, characterized by a continuous and severe negative affective state.

4) To study the temporal changes in the abnormal responses to aversive painful stimulation following one and eight weeks of medical treatment observed within relevant emotion brain circuits in MDD, and the specific brain correlates of affect-related symptomatic improvement in such patients.

1.8. Hypotheses

Previous research has convincingly demonstrated that fMRI is an optimal technique to identify different elements of the emotional brain system using specific stimulation and to assess their organized functional activity during a variety of states. Nevertheless, data as to the ability of fMRI to characterize different dynamics within distinct elements of the emotion circuitry are scarce and have only provided information relating to partial aspects of the phenomenon.

The hypothesis of this study was that fMRI would be sensitive enough to dynamically identify relevant aspects of brain emotion processing by actively targeting affective networks using biologically-salient and essentially aversive painful stimulation and when assessing their basic functional disposition during unconstrained resting-state conditions.

Specifically, we predicted that:

1. Different response dynamics to painful mechanical stimulation would occur within specific regions of the right lateral aspect of the frontal cortex, which would be distinctly associated with subjective pain perception in healthy subjects.

2. The use of a dynamic approach to assess brain response to painful stimulation in fibromyalgia patients would increase fMRI sensitivity to detect relevant alterations to FM pathophysiology, specifically within regions conferring the affective dimension of pain perception. Such functional alterations would be significantly correlated with the abnormally enhanced pain perception commonly reported by patients.

3. The dynamic study of resting-state functional connectivity within emotion-related brain systems in major depressive disorder would comprehensively detect relevant alterations in the basic organization of brain emotion circuits when no active task was being performed. Such abnormalities would be associated with the severity of the depressive state in MDD.

4. Functional MRI would prove effective in isolating pain-related activation changes within altered emotion brain circuits in MDD parallel to the improvement in the affective state of such patients following one and eight weeks of antidepressant treatment. Specifically, normalization of altered baseline activation in regions of particular relevance to MDD pathophysiology, such as the subgenual anterior cingulate cortex, would occur following successful treatment of affective symptoms.

2. Methodology

The introduction section (see epigraph *1.3.1. Functional magnetic resonance imaging*), provides insight on the justification of the methodological approach employed in the present work as particularly suitable to fully characterize the dynamic dimension of brain emotion processing by means of fMRI under healthy and pathological conditions. The specific characteristics of the methodology employed in this PhD thesis have been described in detail in each particular manuscript (see Results section). We will briefly summarize the methodological particularities of each study below.

Study 1.

In this study we assessed brain responses to a mechanical painful stimulus and a preceding anticipatory cue using a dynamic fMRI analysis approach. The study sample included twenty-five right-handed subjects, including 9 males and 16 females ranging from 28 to 62 years. A complete medical interview was carried out to exclude subjects with relevant medical or neurological disorders, history of substance abuse, psychiatric illness or chronic pain complaints. No subject was undergoing medical treatment or suffering pain symptoms before the fMRI assessment.

All subjects received mechanical (pressure) stimulation delivered by means of a specially designed hydraulic device capable of transmitting controlled pressure to a 1 cm^2 surface placed on the subject's right thumbnail. This system consisted of a hard rubber probe attached to a hydraulic piston that was displaced by mechanical pressure. The painful stimulus involved a pressure of 6 kg/cm^2 applied for 10 seconds.

Specifically, the fMRI paradigm consisted of a block design comprising three periods per stimulation cycle repeated 12 times during a 7-minute run: a rest period with pseudorandom variable duration, a 6-second anticipatory period starting with a brief auditory stimulus that cued the subsequent pain period, and the actual painful period involving the application of 6 kg/cm2 of pressure for 10 seconds. Immediately after image acquisition, each subject rated the overall pain intensity and unpleasantness experienced during the 12 painful stimulation cycles. MRI acquisition parameters are fully described in the method section of the manuscript.

Image preprocessing involved motion correction, spatial normalization and smoothing using a Gaussian filter (full-width half-maximum [FWHM], 8 mm). Data were normalized to the standard SPM-EPI template and re-sliced into 2 mm isotropic voxels in Montreal Neurological Institute (MNI) space.

Our fMRI analysis aimed to characterize the temporal evolution of brain activations across a representative activation cycle (anticipation, pain and post stimulation) using data from the 12 trials included in the fMRI run. To do so, we employed the finite impulse response (FIR) analysis approach (Dale and Buckner, 1997) as implemented in SPM5 to obtain 15 activation maps covering the activation cycle with a temporal resolution of 2 seconds (1 scan). Further details on this method can be seen in the method section of the manuscript and on the introduction section, epigraph 1.3.1.
The 15 whole-brain activation maps from the group analyses were used to create a movie sequence that dynamically illustrated the temporal evolution of brain anticipatory and pain responses within four representative brain views.

To graphically represent the temporal dynamics (activation cycle time course) for each significant brain area, we plotted the activation measurements (both the mean percent signal change with its associated standard error and also t values) against the 15 time points (scans). The activation measurements were obtained from the region coordinate showing peak activation across the cycle.

To test statistically for differences in the temporal dynamics of the right frontal cortex regions, individual BOLD signal changes were expressed as a percentage of the group mean peak activation for each region during the cycle. Paired Student *t*-tests were used to specifically compare regional activation measurements at time points when each of the three frontal subregions were mostly activated (above 90% of cycle maximum).

Pearson's correlation was used to assess, across subjects, the linear relationships between the activation strength in right lateral frontal regions and in three representative areas of the pain-processing network. In addition, we correlated the strength of right frontal cortex activations with individuals' subjective ratings of pain intensity and unpleasantness. Finally, a backward regression analysis was performed to determine the

combination of right frontal cortex measurements explaining the greatest variance of unpleasantness ratings.

Study 2.

In this study, we aimed to further characterize brain response to pain in patients with severe fibromyalgia and healthy subjects using an fMRI data driven dynamic approach (Hu et al, 2005; McKeown, 2000). We assessed the temporal dynamics of the actual brain response to local painful pressure in pain-related regions with Independent Component Analysis (ICA). The results were then used to generate fMRI maps adjusted for the duration of brain responses that showed more complete activation patterns in patients and in control subjects and stronger correlation with reported subjective pain.

Twenty-seven subjects participated in the study, including nine patients with fibromyalgia showing severe and durable pain symptoms and two groups of nine healthy subjects (control group 1 and 2) matched to patients for gender and age, and recruited from the same sociodemographic environment. Control group 1 served to compare brain response to a fixed mechanical stimulus pressure able to provoke severe pain in fibromyalgia patients. Control group 2 was matched to fibromyalgia patients for levels of perceived pain by increasing stimulus intensity.

Pressure stimuli were delivered using a specially designed hydraulic device capable of transmitting controlled pressure to 1-cm^2 surface placed on the subject's thumbnail, as in study number 1. In a preliminary session, each subject was acclimatized to the mechanical stimuli and trained to rate perceived pain intensity using a numerical rating scale (NRS) ranging from 0 (no pain) to 100 (the worst pain possible). Pain thresholds were also assessed during the session and the intensity of pressure producing severe pain in both patients and control subjects was estimated. To determine individual thresholds, different stimulus intensities were applied lasting 5 seconds each, with an inter-stimuli interval of 20 seconds. The selected pressure stimuli, ranging from 2–9 kg/cm2, were administered pseudo-randomly.

Fybromialgia patients and the control group 1 received an identical pressure stimulus (4 kg/cm^2) delivered to their right thumbnail. The control group 2 received 6.8 kg/cm^2,

which produced a pain severity level similar to that experienced by fibromyalgia patients using 4 kg/cm^2 (numerical rating scale 0-100 for pain intensity above 70). An fMRI block-design paradigm was used consisting of 21-second resting-state periods interleaved with pressure stimulation blocks of nine seconds. MRI acquisition parameters are fully described in the method section of the manuscript.

Imaging data were processed using MATLAB version 7 and Statistical Parametric Mapping software. Preprocessing involved motion correction, spatial normalization and smoothing using a Gaussian filter (full-width half-maximum, 6 mm). Data were normalized to the standard SPM-EPI template and resliced to 3 mm isotropic resolution in Montreal Neurological Institute (MNI) space.

fMRI data are commonly analyzed using 'model-based' statistical methods that require a specific assumption about the time courses of activation. Typically, model-based analyses estimate the contrast between signal intensity of images obtained during stimulus application and signal intensity of images obtained without stimulation or during a control condition. In experiments where response durations cannot be completely anticipated, as in pain assessment and in the assessment of emotions in general, the standard model-based approach may underestimate the evoked brain response. In contrast, ''data-driven'' statistical methods are used to identify actual brain activation without a priori hypothesis on the expected activation time course. These methods estimate the best fitting of the data, but do not directly test the statistical significance of the activations. In the current study, we used a data-driven approach based on Independent Component Analysis (ICA) to generate a study-specific time course model, which was used as a regressor in conventional SPM analyses to statistically test between-group differences for the activation pattern. The specific characteristic of every analysis approach is fully described in the methods section of Study 2.

Finally, we mapped voxel-wise correlations between subjective pain scores and brain activation. Separate correlation maps were obtained for both the data-driven and model-driven approaches including 18 study subjects (patients and control group 1). Correlations were considered significant at a P value less than 0.05 False Discovery Rate (FDR) corrected for the volume of activated regions (pain network). In addition,

we assessed the extent to which brain activation in the region showing the highest correlation with subjective pain (the anterior cingulate cortex) was able to account for group differences in perceived pain. This was carried out by comparing group differences in subjective reported pain both before and after controlling for (regressing out) the effect of cingulate activation using analysis of covariance (ANCOVA).

Study 3.

By means of this third study we aimed to assess possible alterations in the dynamic equilibrium of the emotion-related functional brain networks during resting-state conditions in a group of MDD patients, characterized by a continuous and severe negative affective state.

Twenty-seven MDD patients were recruited from the Mood Disorders Unit of the University Hospital of Bellvitge. All patients met DSM-IV criteria for MDD with no psychotic features. Exclusion criteria included the presence or past history of other Axis I diagnoses, relevant medical or neurological disorders and abnormal clinical MRI upon radiological inspection. A group of 27 healthy volunteers comparable in gender, age, handedness and years of education also participated in the study. The study included an antidepressant medication wash-out of 15 days before the MRI assessment. The HAMD-17 was used to assess mood and general state of the patient group (and also the healthy group).

The MRI examination included: (i) A high-resolution anatomical 3 dimensional-T1 sequence to obtain a sample-specific pattern of MDD structural abnormalities; and (ii) a four-minute resting-state assessment with closed eyes where the subjects were asked to refrain from moving and relax avoiding following asleep. The details of the sequence acquisition parameters are contained in the methods section of the manuscript.

Imaging data were processed using MATLAB version 7 (The MathWorks Inc, Natick, Mass) and Statistical Parametric Mapping software (SPM5; The Welcome Department of Imaging Neuroscience, London). Standard preprocessing steps were applied to the original anatomical scans following the unified segmentation-normalization approach provided in SPM5 (Ashburner and Friston, 2005), which are fully explained in the

manuscript. Voxel-wise regional volume between-group differences (absolute and relative) were assessed by means of second-level random-effect group analyses in SPM5.

Functional image preprocessing involved motion correction, spatial normalization and smoothing using a Gaussian filter (full-width at half-maximum, 8 mm). Functional data were normalized to the standard SPM-EPI template and re-sliced into 2 mm isotropic voxels in Montreal Neurological Institute (MNI) space. We performed a detailed set of seed-based functional connectivity analysis of subjects' resting-state imaging sequences using the identified anatomical alterations (i.e. regional volumetric reductions) to guide the functional analysis. The time course of each selected volume of interest (seed) was used as a regressor to be correlated with the time course of all the voxels throughout the brain. Additional nuisance signals (white matter, cerebro-spinal fluid, and global signal) were calculated and included in the model as covariates of no interest. These nuisance signals are typically adjusted for in resting-state functional connectivity studies because they reflect global signal fluctuations of nonneuronal origin (eg, physiological artifacts associated with variables such as cardiac and respiratory cycles, CSF motion, and scanner drift; Fox and Raichle, 2007). The analysis was based on the method fully described in the manuscript and in a recent study by our group (Harrison et al, 2009). The placement of the seeds of interest corresponded to the coordinates showing the maximal between-group anatomical difference obtained in the previously mentioned structural analysis. Group patterns of functional connectivity for each seed region and between-group differences were assessed by means of second-level random-effects group analyses, using a two-sample t-test model.

We performed voxel-wise correlation analyses in SPM5 to test for the linear relationship between patients' overall symptom severity (assessed using HAM-17 total score) and the strength of resting-state functional connectivity within each network of interest.

Study 4.

This last piece of work aimed at studying the temporal changes in the abnormal responses to aversive painful stimulation following one and eight weeks of medical treatment observed within relevant emotion brain circuits in MDD, and the specific brain correlates of affect-related symptomatic improvement in such patients.

Fifteen patients were consecutively recruited from the Mood Disorders Unit of the University Hospital of Bellvitge. All patients met DSM-IV criteria for MDD with no psychotic features. Exclusion criteria included the presence or past history of other Axis I diagnoses and relevant medical or neurological disorders including chronic pain syndromes, and abnormal clinical MRI upon radiological inspection. From the original 15-subject sample, 13 patients made up our final study sample. A comparison group of 21 healthy volunteers comparable in age, gender, handedness and years of education also participated in the study, from which 20 made up our final study sample. All the sample details are fully explained in the method section of the manuscript.

For all patients, the study included an antidepressant medication wash-out of 15 days before treatment onset. Patients received antidepressant treatment with oral duloxetine, a serotonin-norepinephrine reuptake inhibitor, starting at 60 mg/day in a single dosage for 4 full weeks. The following clinical scales were used to assess mood, somatic and general treatment-related response: HAMD-17, Brief Pain Inventory, Symptom Questionnaire- Somatic Subscale and the Clinical Global Impression of Severity.

The study consisted of three fMRI assessments, which were carried out at week 0 (before treatment), and following 1 and 8 weeks of treatment. Control subjects also underwent fMRI assessments at baseline, week 1 and week 8, which served to control for task repetition effects on brain responses to painful stimulation.

The Contact Heat-Evoked Potential Stimulator (CHEPS) system was used, which has been designed to provide controlled thermal stimuli (CHEPS, Medoc Ltd., Advanced Medical Systems, Israel). This system is able to provoke pain by direct stimulation of A delta and C nociceptive fibers on a relatively large skin area (via the 27 mm-diameter thermode) through very rapid local heating (70ºC/s rate). In our experiment, painful heat

stimulation was applied to the right volar forearm in 10-second blocks each including eleven 50ºC spikes (full-width at half-maximum duration of each spike: 125 ms), starting from a baseline temperature of 32ºC.

An fMRI block design was used consisting of three conditions per stimulation cycle repeated 12 times during a 7-minute image acquisition run: a rest condition with pseudorandom variable duration (duration range: 12 to 26 s), a 6-second anticipatory condition that began with a brief auditory stimulus (600-ms tone) cuing the subsequent pain condition, and the actual 10-second painful condition (involving the application of the 50ºC spike stimuli). Immediately after the entire fMRI sequence was completed, each subject rated the overall pain intensity and unpleasantness experienced during the 12 painful stimulation cycles. MRI acquisition parameters are fully described in the method section of the manuscript.

Imaging data were processed using MATLAB version 7 and Statistical Parametric Mapping software. Image preprocessing involved motion correction, spatial normalization and smoothing using a Gaussian filter (full-width at half-maximum, 8 mm). Data were normalized to the standard SPM-EPI template and resliced into 2 mm isotropic voxels in Montreal Neurological Institute (MNI) space.

Our analyses aimed to identify (i) between-group differences in brain activation at baseline (pretreatment); (ii) treatment effects at week 1, (iii) treatment effects at week 8, and (iv) the pattern of correlations between clinical measurements (and experimental pain ratings) and fMRI treatment effects. Single-subject 1st level analyses were implemented in SPM5 to model fMRI time-series using four box-car regressors; two representing anticipation and painful stimulation periods respectively, and two representing the rest period divided into two parts: a 6-second post-stimulation period and a variable (6-20 s) remaining rest period, considering a hemodynamic delay of 4 seconds. For further details please refer to the method section of the manuscript. A contrast image showing fMRI signal differences between the painful stimulation condition and the second part of the rest period (modeled by the corresponding box-car regressors in SPM5) was calculated for each subject. Second-level random-effects (group) analyses were performed. One-sample t-statistic maps were calculated to obtain baseline (pre-treatment) activation (and deactivation) patterns for each group, and a

two-sample t-test was performed to map between-group baseline brain activation (and deactivation) differences. In order to assess treatment effects on brain response to pain, we performed two separate (week 1 and week 8) 2nd-level mixed ANOVA analyses including the within-subject factor 'time moment' (baseline versus reassessment), and the inter-subject factor 'group' (patient versus control) as independent variables. Group-by-time interaction t-statistic maps were then calculated to identify activation changes that were greater in MDD patients than in healthy control subjects. Specific correlation analyses were performed in SPM5 to test for linear relationships between clinical improvement in the two symptomatic dimensions of interest, i.e. core emotional and somatic symptoms and fMRI brain activation changes at week 1 and week 8 in relation to baseline. An exploratory two-sample t-test analysis was additionally performed to assess treatment-related fMRI activation changes associated with remission. To specifically test whether baseline (pretreatment) regional activations were able to predict positive clinical responders to duloxetine, an exploratory two-sample t-test analysis was performed to compare the patterns of baseline activation of clinical responders and non-responders. Additional correlation analyses were conducted in SPM5 to investigate the relationship between experimental pain perception and brain activation in regions showing significant treatment effects in MDD.

3. Results (Accepted and Submitted Manuscripts)

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Dynamic assessment of the right lateral frontal cortex response to painful stimulation

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ABSTRACT

The lateral surface of the right frontal lobe has a relevant role in modulating behavioral responses to aversive stimuli and may significantly influence pain experience. Imaging studies suggest that this modulatory role is multifaceted, but no studies have assessed the regional specialization of this cortex on the basis of its response dynamics during pain processing. We aimed to investigate functional specialization within the right lateral frontal cortex using a dynamic fMRI approach. Brain responses to a mechanical painful stimulus and a preceding anticipatory cue (auditory tone) were assessed in 25 healthy subjects. Functional data were decomposed into 15 sequential activation maps covering the full anticipation-painful stimulation cycle using a finite impulse response (FIR) analysis approach. Movie sequences showing the temporal evolution of brain activation illustrate the findings. A region involving premotor–prefrontal cortices was activated soon after the anticipatory cue and showed a significant correlation with both anterior cingulate cortex activation and subjective pain ratings. The frontal operculum also showed a significant anticipatory response, but the most robust activation followed painful stimulation onset and was strongly correlated with insula activation. The anterior prefrontal cortex showed full activation during late painful stimulation and was negatively correlated with pain unpleasantness. In conclusion, different elements within the right lateral frontal cortex showed distinct activation dynamics in response to painful stimulation, which would suggest relevant regional specialization during pain processing. These findings are congruent with the broad functional role of the right frontal cortex and its influence on crucial aspects of human behavior.

Introduction

The experience of pain involves local sensations, cognitive-evaluative processes and general affective phenomena that depend on both stimulus features and the individual's receptive state (Price, 2000; Tracey and Mantyh, 2007). Nociceptive stimuli promptly engage brain networks able to trigger quick defensive responses (Price, 2000) and later modulatory mechanisms (Wiech et al., 2008a) to cope with the evoked unpleasantness.

Functional imaging has notably contributed to characterizing the functional anatomy of the brain network mediating pain responses (Peyron et al., 2000). Consistent neural activations occur in the somatosensory and adjacent parietal cortex, the operculo-insular region, the anterior cingulate cortex (ACC), the prefrontal cortex and the thalamus (Apkarian et al., 2005). Although the brain response to pain is largely bilateral, there is evidence for a right-hemisphere dominance mostly involving the right lateral frontal cortex (Peyron et al., 1999; Symonds et al., 2006; Wiech et al., 2006, 2008a). Imaging literature on the human pain-processing system has been concerned primarily with the spatial anatomy of pain-related activity, although a growing number of studies suggest that rich information also exists in the temporal domain (Apkarian et al., 1999; Chen et al., 2002; Lui et al., 2008; Moulton et al., 2005; Niddam et al., 2002; Porro et al., 1998; Pujol et al., 2009; Qiu et al., 2006; Ringler et al., 2003; Staud et al., 2008).

The overall role of the right lateral frontal cortex during pain processing appears to be multifaceted. On one hand, the right lateral prefrontal cortex is involved in the cognitive reappraisal of aversive stimulation (Kalisch et al., 2006; Levesque et al., 2003; Ochsner et al., 2004; Wager et al., 2008) and is specifically relevant in mediating attenuation of pain perception via cognitive control mechanisms (Lieberman et al., 2004; Salomons et al., 2007; Wiech et al., 2006, 2008a,b). On the other hand, this region is involved in directing

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attention to nociceptive stimuli, which ultimately enhances pain perception (Peyron et al., 1999), and in mediating suggestion-induced pain (Raij et al., 2009). Furthermore, the lateral surface of the frontal cortex is necessary for the pain experience to be completed, as damage of this cortex (Daum et al., 1995) and its experimental inhibition (Graff-Guerrero et al., 2005) diminish the degree of perceived pain. The right lateral frontal cortex is also an important mediator of early responses to alerting signals (Corbetta and Shulman, 2002), which in the context of pain expectancy may promote anticipatory protective behavior.

All in all, converging data support the relevant role of the right lateral frontal cortex in modulating pain responses. Nevertheless, no studies have specifically investigated the regional specialization of this part of the cortex on the basis of its response dynamics during pain processing. In the present study, we assessed brain responses to a mechanical painful stimulus and a preceding anticipatory cue using a dynamic fMRI analysis approach. We aimed to identify dynamically distinct regions within the right lateral frontal cortex and to assess their functional significance using objective and subjective measurements of the individuals' pain response. Movie sequences of brain activation illustrate the findings.

Materials and methods

Subjects

Twenty-five right-handed subjects, including 9 males and 16 females ranging from 28 to 62 years (mean: 46.8 years; SD: 9.1 years) with a mean ± SD education level of 14.2 ± 2.5 years, made up the study sample. A complete medical interview was carried out to exclude subjects with relevant medical or neurological disorders, history of substance abuse, psychiatric illness or chronic pain complaints. No subject was undergoing medical treatment or suffering pain symptoms before the fMRI assessment (mean ± SD resting pain in a 101-point numerical rating scale [NRS] prior to fMRI assessment was 2.0 ± 6.0 points). From an original sample of 29 participants, we excluded one subject as he experienced no pain during fMRI assessment and 3 subjects due to excessive head movement (*z*-axis translation >2 mm). All subjects gave written informed consent to participate in the study, which was approved by the research and ethics committee of the University Hospital of Bellvitge.

Stimulus

Mechanical (pressure) stimulation was delivered using a specially designed hydraulic device capable of transmitting controlled pressure to a 1 cm^2 surface placed on the subject's right thumbnail. As in other studies (Gracely et al., 2002, 2004), this system consisted of a hard rubber probe attached to a hydraulic piston that was displaced by mechanical pressure. In a preliminary session, each subject received the stimulus that was later used during the fMRI assessment and was trained to rate perceived pain intensity using an NRS ranging from 0 (no pain) to 100 (the worst pain possible), and perceived unpleasantness using a 9-point verbal descriptor scale ranging from “not at all unpleasant” to “extremely unpleasant.” The painful stimulus involved a pressure of 6 kg/cm^2 applied for 10 s, which was briefly (1 s) removed halfway through the period to avoid tissue damage. The pressure stimulus was capable of producing moderate-to-severe pain during the pre-scan assessment (mean ± SD in the NRS, 62 ± 19 points).

Real-time assessment of subjective pain intensity

A separate behavioral experiment was conducted to assess the temporal evolution in subjective pain intensity during the painful stimulation cycle. A group of 10 different subjects, comparable in age (mean 46.5 years, SD 9.4, range 27 to 59 years) and gender distribution (4 males and 6 females) to the study sample, received the full painful stimulation paradigm while rating their subjective pain intensity on a real-time basis. Painful stimulation was applied to the right thumb and subjects used their left hand to squeeze a pneumatic device connected to a pressure register system. We used a highly sensitive (0–7.5 kPa) pressure transducer connected to a computer interface and software environment (National Instruments USB acquisition card and Labview 8.0, Austin, TX) that was able to display and register changes in pressure sampled 4000 times per second. Using this system, a vertical color bar display provided direct feedback to the subjects about the evolution of the registered pressure by the sensor on a 0 to 100-point visual scale, where 0 was no pain and 100 was the maximum pain produced during the stimulation block. Subjects were trained to match the pressure register system with the subjective pain experience and the experiment was carried out when they were confident with the accuracy of their responses. At the end of the assessment, each participant rated the amount of pain intensity that was experienced globally during the 12 stimulation blocks. The data were analyzed by scaling each individual time course to the global subjective rating (range from 0 to 100) and results were expressed as group mean with 95% CI (Fig. 1). The obtained time course therefore represents the average of 120 trials (10 subjects by 12 cycles each).

fMRI pain paradigm

A block design was used consisting of three periods per stimulation cycle repeated 12 times during a 7-min run: a rest period with pseudorandom variable duration (duration range: 12 to 26 s), a 6-s anticipatory period starting with a brief auditory stimulus (600-ms tone) that cued the subsequent pain period, and the actual painful period involving the application of 6 kg/cm^2 of pressure for 10 s. Immediately after image acquisition, each subject rated the overall pain intensity and unpleasantness experienced during the 12 painful stimulation cycles.

MRI acquisition

A 1.5 Tesla Signa system (General Electric, Milwaukee, WI) equipped with an eight-channel phased-array head coil and single-shot echoplanar imaging (EPI) software was used. Functional sequences consisted of gradient recalled acquisitions in the steady-state (time of repetition [TR], 2000 ms; time of echo [TE], 50 ms; pulse angle, 90°) within a field of view of 24 cm, a 64 × 64-pixel matrix, and slice thickness of 4 mm (inter-slice gap, 1.5 mm). Twenty-two slices

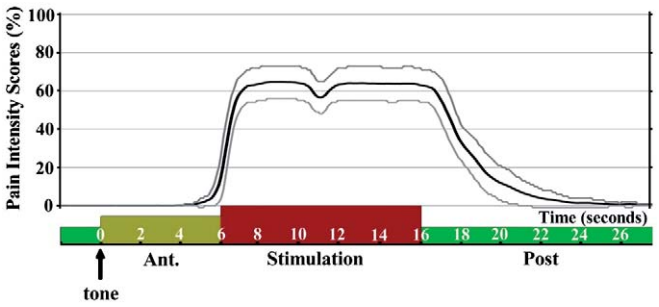


Fig. 1. Real-time assessment of subjective pain intensity. The time course represents the continuous evolution in subjective pain intensity (0 = no pain; 100 = the worst pain) in response to the mechanical stimulus applied in our paradigm. The black line represents the mean curve obtained from 120 trials (10 subjects, 12 trials per subject) and gray lines indicate the 95% CI. Note the existence of a small anticipatory effect (Ant., anticipation period); stable ratings during stimulation (with a short decrease coinciding with the brief stimulus removal halfway through the stimulation period); and a progressive intensity decrease after stimulation, which notably varied across subjects.

parallel to the anterior–posterior commissure line covered the whole-brain. The sequence included 4 additional dummy volumes to allow the magnetization to reach equilibrium.

Image preprocessing

Imaging data were processed using MATLAB version 7 (The MathWorks Inc, Natick, Mass) and Statistical Parametric Mapping software (SPM5; The Welcome Department of Imaging Neuroscience, London). Image preprocessing involved motion correction, spatial normalization and smoothing using a Gaussian filter (full-width half-maximum [FWHM], 8 mm). Data were normalized to the standard SPM-EPI template and re-sliced into 2 mm isotropic voxels in Montreal Neurological Institute (MNI) space.

Temporal analysis of brain response to pain

Our fMRI analysis aimed to characterize the temporal evolution of brain activations across a representative activation cycle (anticipation, pain and post stimulation) using data from the 12 trials included in the fMRI run. To do so, we employed the finite impulse response (FIR) analysis approach (Dale and Buckner, 1997) as implemented in SPM5 to obtain 15 activation maps covering the activation cycle with a temporal resolution of 2 s (1 scan). Specifically, fMRI time-series were modeled using 14 box-car regressors corresponding to 14 consecutive scans (28 s) covering the activation cycle and commencing from the second scan after the auditory tone. The model included an implicit baseline of 8 s (4 scans) from each trial (on average). A separate FIR analysis was performed to obtain one between-trial transition map that corresponded to the first scan after the auditory tone with a shorter implicit baseline of 6 s. For each subject, 'contrast images' were calculated for the 15 regressors that expressed the relative blood oxygenation level-dependent (BOLD) signal change from baseline throughout the activation cycle.

The contrast images were then entered in 15 group random-effects analyses (one-sample *t*-tests) to generate whole-brain activation *t*-statistic maps for each scan (TR). Activation maps were thresholded at $p<0.05$, whole-brain false discovery rate (FDR) corrected.

The 15 whole-brain activation maps from the group analyses were used to create a movie sequence that dynamically illustrated the temporal evolution of brain anticipatory and pain responses within four representative brain views (an axial slice, $z=3$ in MNI space; a sagittal slice, $x=6$ in MNI space; and right and left lateral surface views generated using MRIcron (v.8) considering an overlay depth representation of 16 mm). The transition between consecutive images was displayed using a previously described method (Pujol et al., 2006) that involved composing a 24-step morphing sequence (using Fantamorph software, v. 2.0, Abrosoft, Devon, UK). This procedure was relevant in conferring the notion of smooth activation progression in a pain-related context as a function of time (Supplemental Movies 1 and 2). In a recent report, Windischberger et al. (2008) also combined fMRI and FIR analyses to generate time-resolved brain activation movie displays.

To graphically represent the temporal dynamics (activation cycle time course) for each significant brain area, we plotted the activation measurements (both the mean percent signal change with its associated standard error and also *t* values) against the 15 time points (scans). The activation measurements were obtained from the region coordinate showing peak activation across the cycle (Table 1).

To test statistically for differences in the temporal dynamics of the right frontal cortex regions, individual BOLD signal changes were expressed as a percentage of the group mean peak activation for each region during the cycle. Paired Student *t*-tests were used to specifically compare regional activation measurements at time points when the premotor–prefrontal (scan 3), the opercular (scan 5) and the anterior prefrontal (scan 7) regions were mostly activated (above 90% of cycle maximum). To further assess whether the identified frontal clusters corresponded to anatomically and histologically separated regions, the WFU_PickAtlas toolbox implemented in SPM5 (Maldjian et al., 2003) was used. In addition, to discard smoothing-related dependency between clusters, we calculated the distance for each pair of peak-activation coordinates and verified that all distances were greater than 2*FWHM (16 mm.), where the influence of smoothing effects is almost zero (Mechelli et al., 2005).

Table 1
Significant peak activations observed during the stimulation cycle.

Activated regions	Peak *t*-value	*X* : *Y* : *Z*	*K*	Scan of peak activation
Right auditory	11.13	59 : −32 : 11	2442	Scan 3
Left auditory	7.82	−59 : −38 : 15	1661	
Right premotor-PFC	7.34	42 : 6 : 42	3710	
Left PFC	4.52	−38 : −5 : 50	299	
Anterior midbrain	5.52	−10 : −12 : −8	181	Scan 5
PAG	5.21	4 : −27 : −5	306	
Right insula/operculum	9.65	38 : 23 : −1	4316	Scan 6
Left insula/operculum	9.57	−44 : 0 : 4	4030	
Right pre-SMA	6.41	8 : 16 : 49	1748	
Left basal ganglia	5.93	−18 : 8 : −4	1677	
Right basal ganglia	5.92	12 : 10 : −2	181	
Anterior cingulate cortex	5.70	0 : 6 : 35	2366	
Right thalamus	4.54	18 : −6 : 6	411	
Left thalamus	4.17	−16 : −13 : 6	602	
Right SII	10.41	57 : −24 : 21	1199	Scan 7
Left SII	9.09	−59 : −22 : 25	1348	
Left cerebellum	5.12	−20 : −73 : −28	914	
Left SI	9.40	−56 : −25 : 47	2071	Scan 8
Right anterior PFC	8.07	44 : 41 : 0	1618	Scan 9

Coordinates (mm) are in standard Talairach space. *K*, cluster size (number of significantly activated voxels, $p<0.05$ False Discovery Rate (FDR) whole-brain corrected). PFC, prefrontal cortex; PAG, periaqueductal gray matter; SMA, supplementary motor area; SII, second somatosensory cortex; SI, primary somatosensory cortex. Scan number 1 corresponds to the first scan after the auditory tone (see Fig. 2).

Correlation analysis of right lateral frontal cortex temporal dynamics

A specific analysis was carried out to assess the correlations between the temporal dynamics of the three right frontal regions and those of all activated voxels in the brain. This analysis allowed us to map the regions gathered by the distinct right frontal cortex dynamics. We used a "seed-based" approach in which the time course of each frontal region was used as a regressor to be correlated with the time course of all the activated voxels throughout the brain. The analysis was based on the method fully described in a recent study (Harrison et al., 2009). The placement of the seeds of interest corresponded to activation maxima within the three right frontal clusters (peak coordinates in MNI space: $x=42$, $y=4$, $z=46$ for the premotor–prefrontal, $x=48$, $y=22$, $z=0$ for the opercular and $x=44$, $y=42$, $z=2$ for the anterior prefrontal region). For each frontal location, seeds were defined as 3.5-mm radial spheres (sampling approximately 25 voxels with 2 mm of isotropic resolution) using MarsBaR region-of-interest toolbox in Montreal Neurological Institute stereotaxic space (Brett et al., 2002). Signal values for the seeds were calculated as the average signal of all the included voxels at each data point.

Contrast images were generated for each subject (first-level) by estimating the regression coefficient between the seed time series and each brain voxel signal using SPM5. These images were then included in three distinct one-sample *t*-test group (second-level) random-effects analyses. The resulting *t*-maps were thresholded using a false discovery rate correction of $P_{FDR}<0.05$ for the whole pain-matrix volume with a minimum cluster extent of 20 contiguous voxels. Also, paired *t*-test group random-effects analyses were performed to test

for significant differences across the three frontal seed-based correlation maps. The resulting maps of the difference were also thresholded at $P_{FDR}<0.05$ for the whole pain-matrix volume with a minimum cluster extent of 20 contiguous voxels.

Correlation analysis of right lateral frontal cortex activation magnitudes

Pearson's correlation was used to assess, across subjects, the linear relationships between the activation strength in right lateral frontal regions and in three representative areas of the pain-processing network. We selected the cortical areas most frequently activated in pain experiments (Apkarian et al., 2005), including the second somatosensory area, as part of the sensory-discriminative component of brain response to pain, the ACC as part of the affective component and the insular complex participating in both aspects of the pain experience (Brooks and Tracey, 2005). We estimated the activation strength in these regions for each subject by averaging percent signal change from the scans where the region showed significant activation at the group level ($P_{FDR}<0.05$ whole-brain corrected). Percent signal change measurements for each region were extracted at the coordinates showing peak activation across the cycle (reported in Table 1). For the insula and the second somatosensory area (typically showing bilateral activation in most pain experiments Peyron et al., 2000), data from left and right hemispheres were averaged to provide single measurements of pain response at these functional stages. In addition, we correlated the strength of right frontal cortex activations with individuals' subjective ratings of pain intensity and unpleasantness. Finally, a backward regression analysis was performed to determine the combination of right frontal cortex measurements explaining the greatest variance of unpleasantness ratings.

Age effects

As the age range of our sample was relatively large, we tested for a possible age effect on subjective pain ratings (pain intensity and unpleasantness) by comparing younger (28 to 46 years, $n=12$) and older subjects (47 to 62 years, $n=13$) using a Student t-test. In addition, to test for possible age effects on brain activation magnitude and dynamics, both groups were compared using a mixed repeated-measures ANOVA for each significantly activated brain area ($n=19$) that included the 15 time-resolved measurements.

Results

Subjective pain ratings

The mean ± SD subjective ratings of the overall pain experienced during fMRI were 65.0 ± 16.9 points for pain intensity (101-point NRS) and 3.8 ± 1.7 points for unpleasantness (9-point verbal descriptor scale). The younger ($n=12$) and older subjects ($n=13$) did not differ in either of these ratings ($t=-0.3$ and $p=0.775$ for pain intensity, and $t=1.5$ and $p=0.146$ for unpleasantness).

A separate experiment was performed to determine the temporal evolution of subjective pain intensity during the painful stimulation cycle in our paradigm. The overall pain experienced during this assessment (101-point NRS, 64.8 ± 14.1) did not differ from the ratings obtained during fMRI ($t=0.1$; $p=0.962$). Fig. 1 summarizes the group results of this continuous pain intensity rating and shows a period of sustained pain that progressively decreased after stimulation ended. Pain intensity decreased to half maximum (32 points in the 101-point NRS) 12 s after stimulus onset and was significant up to 14 s after stimulus onset (11 points in the 101-point NRS, one sample t-test; $t=2.5$ and $p=0.039$).

Dynamics of the overall brain response to painful stimulation

Figs. 2 and 3, Table 1, Supplemental Figs. 1–5 and the Supplemental Movies show the temporal evolution of brain responses to painful mechanical stimulation. All data are based on signal changes measured with a temporal resolution of 2 s (1 scan) with no correction for the hemodynamic delay of the BOLD response.

Strong anticipatory activations were observed in the auditory areas and the right premotor–prefrontal cortex. Auditory areas, particularly in the right hemisphere, showed a rapid signal increase during the second and third scans of the cycle (i.e., the initial changes were delayed 2 to 4 s with regards to the auditory tone and the peak activation was at 4 to 6 s). The right premotor–prefrontal activation showed similar initial dynamics, but remained significant for a longer period (see below). Most of the relevant pain-processing regions (somatosensory cortices, anterior insulae, frontal opercula, pre-supplementary motor area [preSMA] and ACC) showed significant anticipatory activation, although their maximum response occurred thereafter. For these regions, a BOLD signal increase was already prominent in the second scan (2 to 4 s) after the onset of mechanical stimulation, showing further general enhancement during the next scan. The left cerebellum and the right anterior prefrontal cortex were the last implicated regions, showing an activation peak in the fourth scan (6 to 8 s) after painful stimulation onset. The decline of region activations also showed varying dynamics. Response to actual painful stimulation in the thalamus, basal ganglia and ACC did not exceed the 10-s stimulus duration, whereas in the right and left insulae, the frontal opercula and pre-SMA, a robust response to the painful stimulus (i.e., t values above 3) was observed during 14 s. Activation of these core regions was weaker for the rest of the cycle with a mild activation increase at its final stage (Fig. 3). The most durable activation response was observed for the somatosensory cortices and supramarginal gyri, which showed a robust signal increase (t values above 3) up until the end of the cycle, indicating that the response to actual painful stimulation lasted up to 20 s (Fig. 3 and Supplemental Movies).

The complete activation pattern described above appeared to be bilateral, with the exception of frontal and auditory cortex activations that were more prominent in the right hemisphere (Fig. 4 and Table 1). It is relevant to mention that the cerebellar activation was lateralized to the left side (Fig. 3 and Table 1), which is congruent with the observed right-sided dominance within the cerebral hemispheres (considering that cerebral-cerebellar connections are mostly crossed Schmahmann, 1991).

Right lateral frontal cortex dynamics

The right lateral frontal cortex activation involved separate regions showing distinct response dynamics. As can be appreciated in Fig. 4 and Supplemental Movie 1, three main activation clusters were gradually and sequentially engaged across the activation cycle. The first activated region mainly involved the premotor cortex, extending to the adjacent prefrontal cortex (BA 6, 8 and 9). Significant activation in this "premotor–prefrontal" region was observed soon (2 to 4 s) after the anticipatory cue and showed its maximum response during the next scan. An "opercular" region (BA 44 and 45) also showed significant anticipatory changes, but reached most of its activation magnitude 2 to 4 s after actual painful stimulation onset. Finally, an "anterior prefrontal" region (BA 46 and 10) was activated later showing the first activation peak 6 to 8 s after painful stimulation onset.

To test statistically for differences in the temporal dynamics of the right frontal regions, we compared the activation magnitude between regions at the time points when the premotor–prefrontal, opercular and anterior prefrontal regions were mostly engaged (scans 3, 5 and 7)

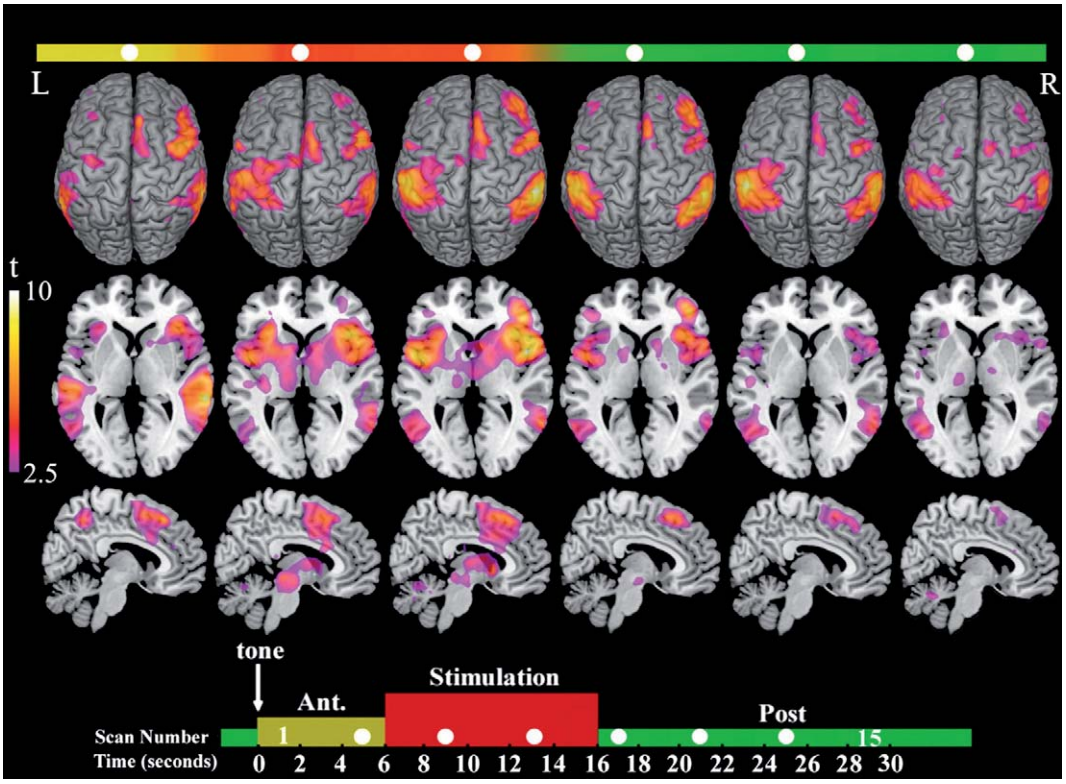


Fig. 2. A selection of sequential activation maps depicting the temporal evolution of brain responses throughout the fMRI activation cycle, considering no hemodynamic delay. Each column of brain images corresponds to the scan indicated below within the stimulation cycle representation (white circles). Activations corresponding to $P_{FDR}<0.05$ whole-brain corrected are displayed.

using relative activation measurements (percentage of region peak activation). The right premotor–prefrontal region showed a mean ± standard error of 100% ± 14% of its maximum activation during scan 3, whereas, at this time point, the opercular region showed 55% ± 11%, and the anterior prefrontal region 10% ± 13%. The difference in this measurement between premotor–prefrontal and opercular regions was significant showing $t=3.1$ and $p=0.005$; and between premotor–prefrontal and anterior prefrontal regions was significant showing $t=5.3$ and $p=0.00002$. Activation of the opercular region reached 96% ± 11% of its maximum during scan 5, whereas the anterior prefrontal region showed a significantly lower value at this time point (34% ± 19%; $t=5.6$, $p=0.000008$). Finally, the late-activated anterior prefrontal region reached 100% ± 13% of its maximum activation during scan 7.

The three right frontal regions showed full activation (see Supplemental Movies) up to 18 to 20 s after the auditory tone. These clusters showed a gradual activation reduction during the two following scans, although scattered activation foci were observed until the end of the cycle.

Correlation analysis of right lateral frontal cortex temporal dynamics

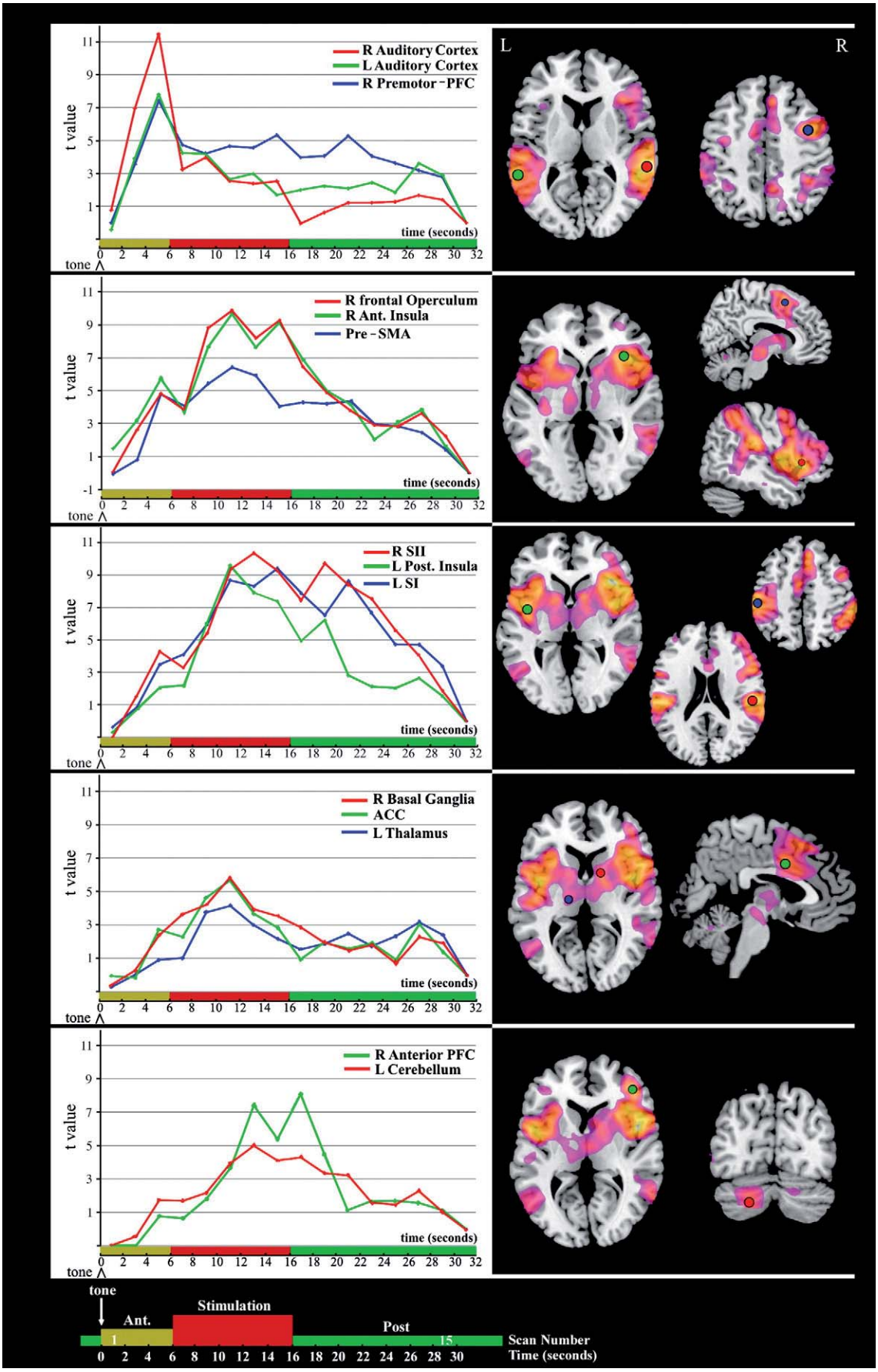
Fig. 5 maps the correlation of the distinct frontal cortex time courses with time courses of all activated voxels in the brain. The three frontal regions significantly correlated with an important number of pain-related structures. Some areas were common to the three maps, including bilateral somatosensory cortices, insulae, basal ganglia and portions of the frontal cortex. Nevertheless, relevant differences were also observed between the three correlation patterns (reported in Supplemental Fig. 6). Relevantly, the premotor–prefrontal region showed the strongest correlation with the ACC and adjacent SMA and pre-SMA and the opercular region showed the most robust and extensive correlation with the operculo-insula-basal ganglia complex bilaterally.

Correlation patterns for right lateral frontal cortex activation magnitudes

To assess the functional significance of the dynamically distinct right frontal activations, a correlation analysis was carried out using different measurements of subjects' pain response. We used the amount of second somatosensory area, ACC and insula activation for each individual as representative measurements of the objective brain response to painful stimulation, and individual ratings of pain intensity and unpleasantness as measurements of the subjective pain experience.

Second somatosensory area activation was significantly correlated across subjects with activation of the right opercular region ($r=0.60$; $p=0.002$). ACC activation was significantly correlated with activation of the right premotor–prefrontal region ($r=0.48$; $p=0.015$) and showed a trend toward significance with right opercular activation ($r=0.37$; $p=0.072$). Activation of the insular cortex showed a significant correlation only with the right opercular region ($r=0.83$; $p<0.0001$). The right anterior prefrontal region did not show any significant correlation with the selected areas.

In the analysis of subjective pain ratings, pain intensity was significantly correlated with activation in the right premotor–prefrontal region ($r=0.54$; $p=0.005$), but not with the other frontal regions. Similarly, unpleasantness ratings were significantly correlated with the right premotor–prefrontal region ($r=0.54$; $p=0.005$) and showed a tendency to be negatively correlated with the right anterior prefrontal region ($r=-0.36$; $p=0.078$). In a post hoc evaluation, we found that unpleasantness ratings showed a significant negative correlation specifically with the late activation peak of the right anterior prefrontal region (BOLD signal increase at scan 9) showing $r=0.47$ and $p=0.017$. Notably, a backward regression analysis including this late activation peak and peak activation of the right premotor–prefrontal region (scan 3, corresponding to anticipatory changes) indicated that both


R Auditory Cortex
L Auditory Cortex
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Pre–SMA
R SII
L Post. Insula
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R Basal Ganglia
ACC
L Thalamus
R Anterior PFC
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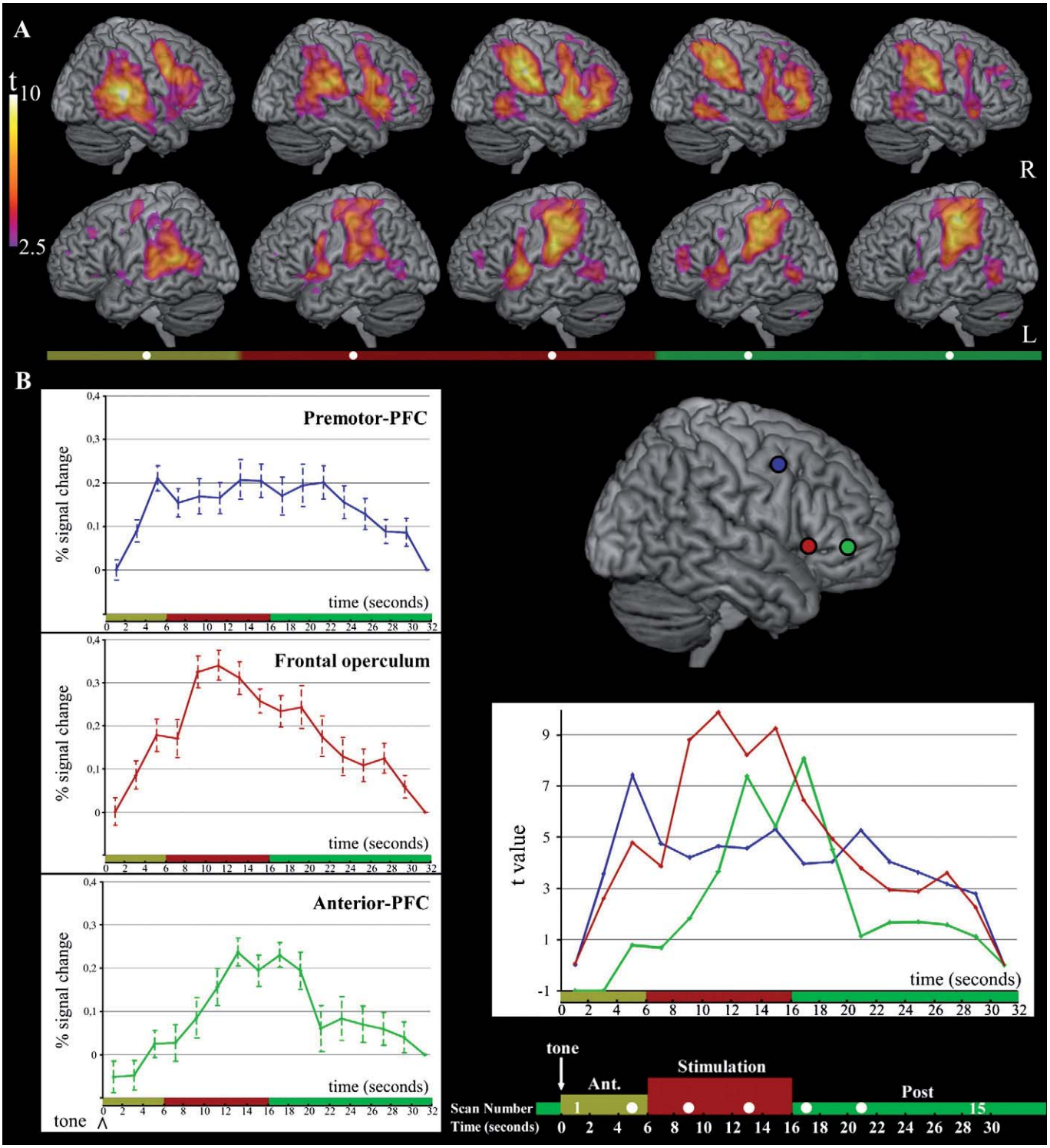

Fig. 4. Lateral frontal cortex response to painful stimulation (with no adjustment for hemodynamic delay). (A) A selection of sequential activation maps illustrating the temporal evolution of brain activations on the right (top row) and left (bottom row) surface views. Activations corresponding to $P_{FDR}<0.05$ whole-brain corrected are displayed. (B) Circles on the right surface view indicate the peak coordinates of the three frontal regions subsequently engaged along the activation cycle, as illustrated in the plot (PFC, prefrontal cortex).

measurements were significant and independent (tolerance = 0.99) predictors of subjective unpleasantness with opposite correlation signs. Specifically, higher unpleasantness was associated with higher early premotor–prefrontal activation ($t = 2.98$; $p = 0.007$) and lower late anterior prefrontal activation ($p = -3.09$; $p = 0.005$), with a total explained variance of 40%.

Fig. 3. Time courses for relevant activated areas (with no adjustment for hemodynamic delay). The five plots combine regions showing similar activation dynamics (see Supplemental figures for additional data showing group mean percent signal change and its standard error). PFC, prefrontal cortex; ACC, anterior cingulate cortex; SMA, supplementary motor area; SII, second somatosensory area; SI, primary somatosensory area. Color circles in the brain images are at the coordinates where the plotted data were extracted for each region.

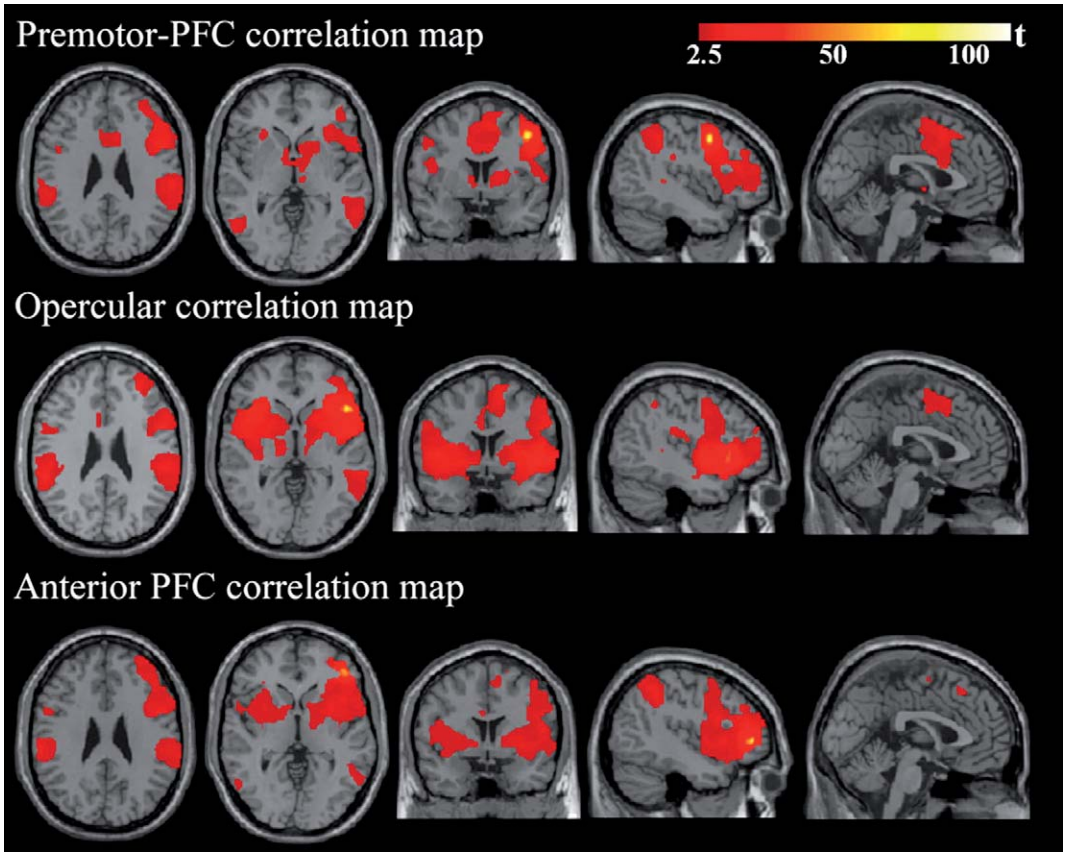

Fig. 5. Maps showing significant correlations between the temporal dynamics of the three right frontal regions and those of all activated voxels in the brain. All voxels show $P_{FDR}<0.05$ corrected. PFC, prefrontal cortex. Images are displayed in neurological convention (Right = Right).

Age effects

We found no age-group by time interaction (brain activation dynamics) and no main effect for age-group (activation magnitude) in the mixed repeated measurements ANOVA performed for each significantly activated brain area across the 15 time-resolved measurements.

Discussion

The current study presents a dynamic analysis of the brain response to mechanical painful stimulation and to its cued anticipation. We describe the time courses for significantly activated regions and illustrate the results using a movie display. We used this approach to specifically investigate functional specialization within the right lateral frontal cortex and found distinct temporal dynamics in three separate regions. The identified frontal activations showed different correlation patterns with brain responses in other pain-related areas and with subjective pain ratings.

Our whole-brain temporal analysis showed remarkable differences between the involved regions in terms of the moment at which significant changes emerged and the regions reached their peak activation. We identified relatively brief and strong auditory cortex activation close to the presented tone, followed by the progressive engagement of most regions of the pain-processing network during the anticipatory period. Previous studies have also consistently reported anticipatory activation in such regions (Burgmer et al., 2009; Koyama et al., 2005; Ploghaus et al., 1999; Porro et al., 2002, 2003; Salomons et al., 2007; Wise et al., 2007; Yaguez et al., 2005), which suggests that to some extent, subjects anticipate the actual pain experience in the context of associative learning related to anticipatory cues. In agreement with these studies, the extent and magnitude of anticipatory activations increased during painful stimulation in most regions (with the exception of the premotor–prefrontal region). Subsequently, activation in different regions persisted for variable durations and several areas showed significant responses beyond the end of the stimulation period, as reported in previous studies using other nociceptive stimuli (Apkarian et al., 1999; Chen et al., 2002; Lui et al., 2008; Moulton et al., 2005; Niddam et al., 2002).

The time course of subjective pain intensity in our behavioral experiment (Fig. 1) showed a small anticipatory effect followed by sustained pain during stimulation, progressively decreasing after mechanical pressure was removed. The total duration of experienced pain was approximately 14 s. This experiment provides a temporal reference for the fMRI results, where we observed (i) regions with activation mainly restricted to the period of sustained pain (ACC, thalamus and basal ganglia), (ii) regions with robust activation during all 14 s (insulae, frontal opercula and pre-SMA), and (iii) regions strongly activated beyond this period (somatosensory cortices).

The whole-brain temporal assessment provided us with a reference framework to characterize frontal cortex activation dynamics in response to painful stimulation. The frontal cortex, specifically its right lateral surface, has become a growing focus of interest in neuroimaging studies of pain due to its relevant role in modulating pain experience via resources related to individuals' cognitive and affective state (for comprehensive reviews see Bingel and Tracey, 2008; Wiech et al., 2008a; see also Lorenz et al., 2003; Salomons et al., 2007; Wiech et al., 2006, 2008b). Nevertheless, despite its potential relevance, regional specialization of the right lateral frontal cortex during pain processing has not been comprehensively investigated (Tracey, 2008; Wiech et al., 2008a). Our findings further characterize such regional specialization by identifying different response dynamics within the right frontal cortex anatomy that were distinctly

associated with subjective pain perception and showed different correlation patterns with the rest of pain-processing regions.

The "premotor–prefrontal" region was promptly activated after the alerting anticipatory cue and showed a right-sided sustained response throughout the activation cycle. The temporal dynamics of this region was notably coupled with the dynamics of frontal medial wall structures (ACC-SMA complex). This region was also positively correlated with the overall ACC activation across individuals as well as with their subjective pain ratings. The premotor and adjacent prefrontal cortices are primarily involved in movement preparation and execution (Picard and Strick, 2001; Wise, 1985). This area shows early activation after the presentation of conditioned cues and is related to anticipatory aspects of motor and cognitive responses (Chouinard and Paus, 2006; Picard and Strick, 2001). The most rostral part of the premotor cortex mediates responses specifically related to auditory conditioned stimuli (Kurata et al., 2000). In the context of our paradigm, the premotor–prefrontal region may play a role in the selection and planning of adaptive (fight or flight) behaviors, but may also be related to "active" motor control of such prompt responses as subjects were required to refrain from moving inside the scanner. Previous EEG and transcranial magnetic stimulation (TMS) studies have suggested a relevant role for the right lateral frontal cortex in mediating both "withdrawal" behavior from aversive stimulation and negative emotions in the context of normal and abnormal mood states (Coan and Allen, 2004; Davidson, 2002; Pascual-Leone et al., 1996; Paus and Barrett, 2004; Schutter et al., 2008). Additionally, on the basis of its anatomical and temporal features, the premotor–prefrontal region may also be an element of the right-lateralized frontal and parieto-temporal network involved in re-orienting attention to salient sensory stimuli (Corbetta and Shulman, 2002; Downar et al., 2000; Paus, 2000).

The frontal "opercular" region was significantly engaged during anticipation and showed maximum activation after painful stimulation onset with temporal dynamics markedly parallel to the bilateral insula-basal ganglia complex. Opercular activation is usually considered part of the core network mediating pain responses (Peyron et al., 2000; Apkarian et al., 2005). In accordance with previous studies, activation in this region was mainly bilateral, although in our case, there was a tendency to higher response magnitude and extent in the right hemisphere. Individual activation in the opercular region was strongly correlated with the second somatosensory area and the insular cortex. Interestingly, no significant correlations emerged between individual activation in the opercular region and subjective ratings of pain intensity or unpleasantness. This finding agrees with a previous study (Lorenz et al., 2003) showing a strong reduction in the correlation between activation in the anterior insular cortex adjacent to our opercular region and subjective pain ratings when the right prefrontal cortex was recruited, as was also the case in our study (see below).

The right "anterior prefrontal" region was the last to engage during the activation cycle and showed maximum activation during the second half of the stimulation course. Global activation in the anterior prefrontal cortex showed a tendency to be negatively correlated with individual ratings of perceived unpleasantness. Specifically, low unpleasantness ratings were significantly predicted by the combination of reduced "premotor–prefrontal" and increased "anterior prefrontal" activation. The right prefrontal cortex has been previously associated with down-regulation of aversive emotions and painful experiences. Indeed, different mechanisms may mediate the attenuation of aversive emotions, including diverting attention from the unpleasant stimulus (e.g., diverting attention from pain, see Bantick et al., 2002; Peyron et al., 1999; Valet et al., 2004) and the cognitive reappraisal of stimulus meaning while self-detaching from its emotional qualities, which commonly involves right dorsolateral prefrontal activation (Kalisch et al., 2006; Levesque et al., 2003; Ochsner et al., 2004; Wager et al., 2008). In the context of pain perception, these frontally mediated "strategies" involve the reduction of stimulus intensity expectancies or the enhancement of perceived control, ultimately making pain less threatening (for a review see Wiech et al., 2008a; see also Lieberman et al., 2004; Salomons et al., 2004, 2007; Wager et al., 2004; Wiech et al., 2006, 2008b). Interestingly, the cognitive modulation of pain perception via attention resources and expectation changes seems to involve elements of the descending pain modulatory system ultimately inhibiting—or facilitating- noxious input from dorsal horn neurons (Bingel and Tracey, 2008). This phenomenon appears to be primarily orchestrated by the lateral prefrontal cortex, including our "anterior prefrontal" region (Bingel and Tracey, 2008; Wiech et al., 2008a).

In a different context, it is relevant to mention that the prefrontal cortex has shown substantial and selective tissue degeneration in patients with chronic pain (Apkarian et al., 2004). A variety of frontal lobe alterations in this clinical situation have also been reported in other structural (Geha et al., 2008; Schmidt-Wilcke et al., 2005), functional (Baliki et al., 2006; Berman et al., 2008; Gundel et al., 2008) and biochemical (Grachev et al., 2000; Sorensen et al., 2008) studies, which would suggest a significant implication of the frontal lobe in the pathophysiology of abnormal chronic pain states.

It is important to recognize that the temporal resolution of fMRI does not permit the assessment of fast synaptic processes that occur in the order of milliseconds. As discussed by Windischberger et al. (2008), the temporal sequence of the neuronal response cannot be fully characterized from the presented approach, given the variability of hemodynamic response latencies across brain regions (Handwerker et al., 2004). Other imaging analysis approaches (e.g., dynamic causal modeling Friston, 2009), combined with validating electrophysiological studies may be able to characterize regional temporal order effects with more accuracy. Additionally, we suggest that further experimental and correlational fMRI studies using a more comprehensive assessment of affect and cognition are necessary to better delimit the behavioral significance of the described frontal cortex dynamics during pain perception.

In conclusion, the temporal assessment of brain activations allowed us to identify different dynamics within the right lateral frontal surface with distinct functional correlates suggesting the specialization of this cortex during pain processing. Overall, our findings are consistent with the broad functional role of the right lateral frontal cortex and its influence on crucial aspects of human behavior that can relevantly modify the final experience of pain.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2010.01.031.

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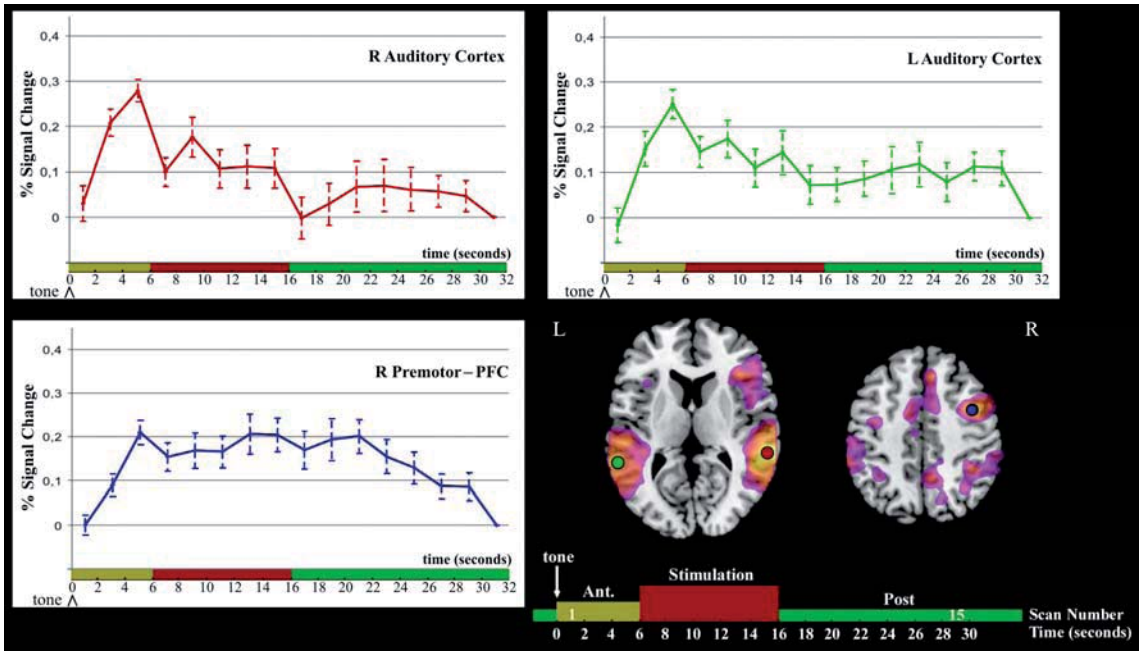
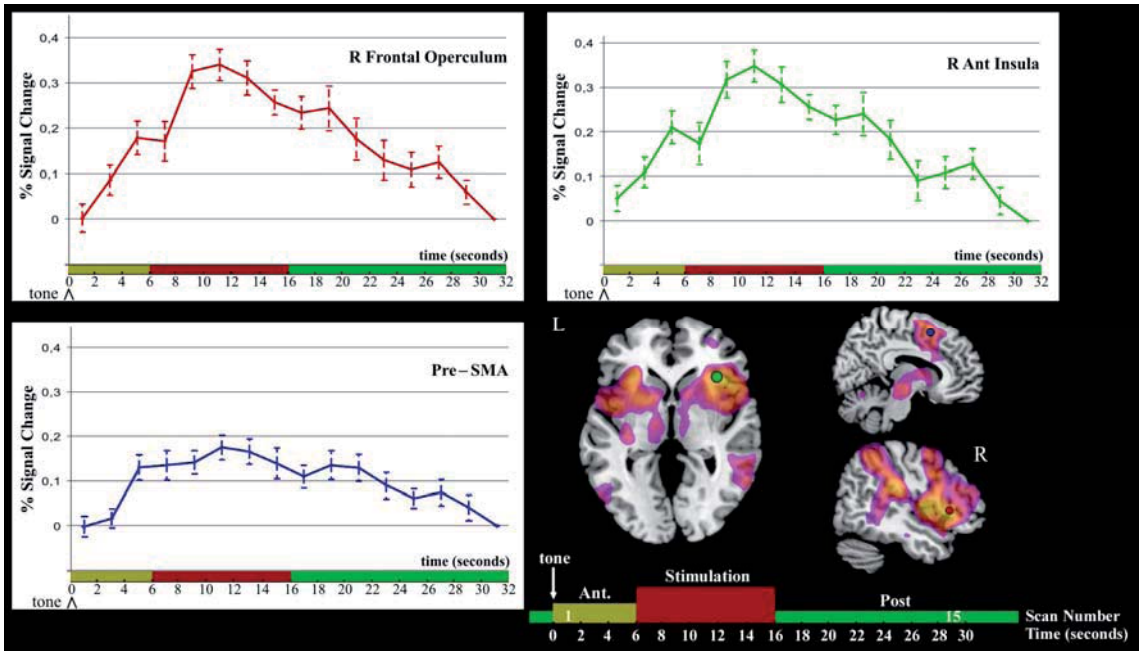
Supplemental Material Figures

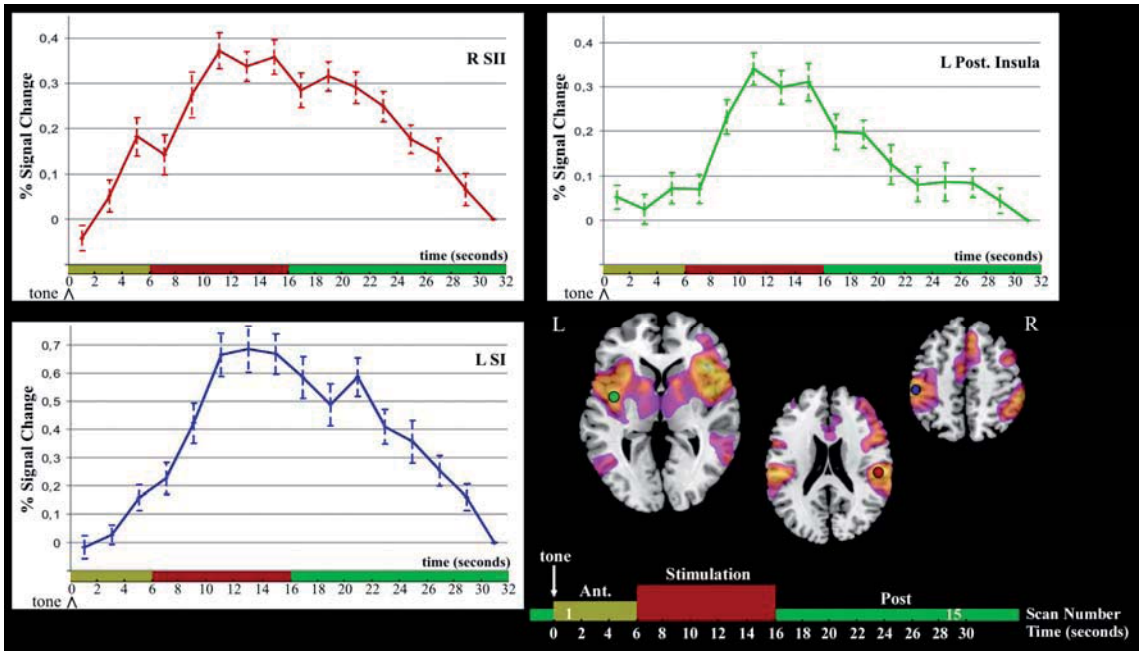


Supplemental Figure 1. Auditory and right premotor-PFC (prefrontal cortex) activation time courses expressed as mean percent signal change (± SEM), with no adjustment for hemodynamic delay. This figure complements the first plot in main text Figure 3.

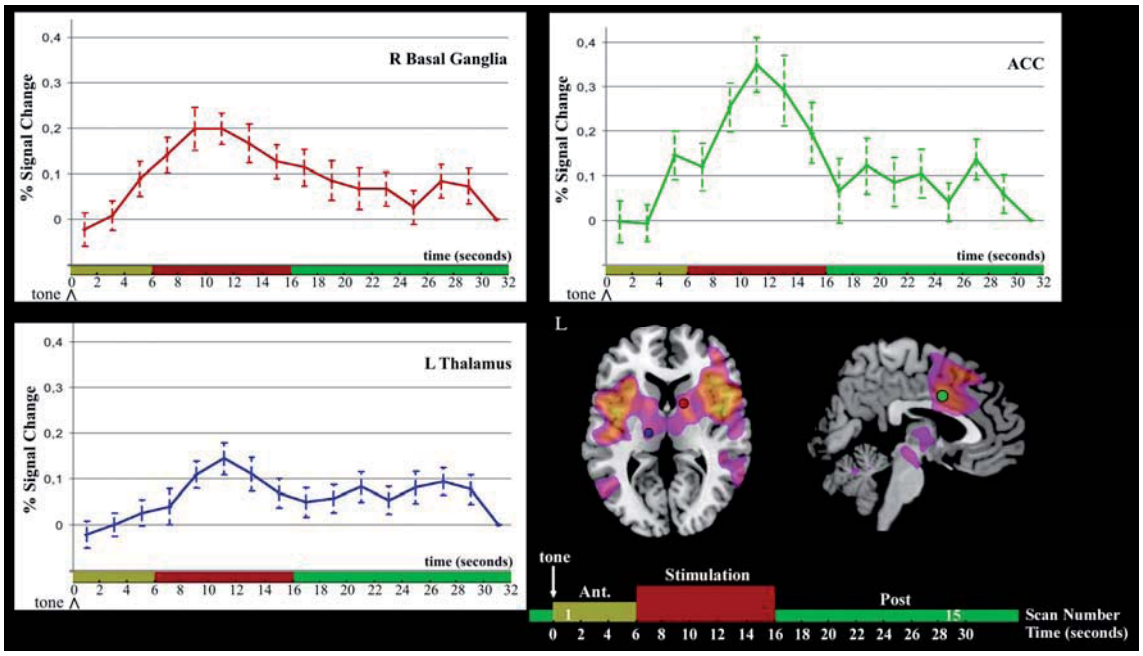


Supplemental Figure 2. Right frontal operculum, anterior insula and pre-SMA (supplementary motor area) activation time courses expressed as mean percent signal

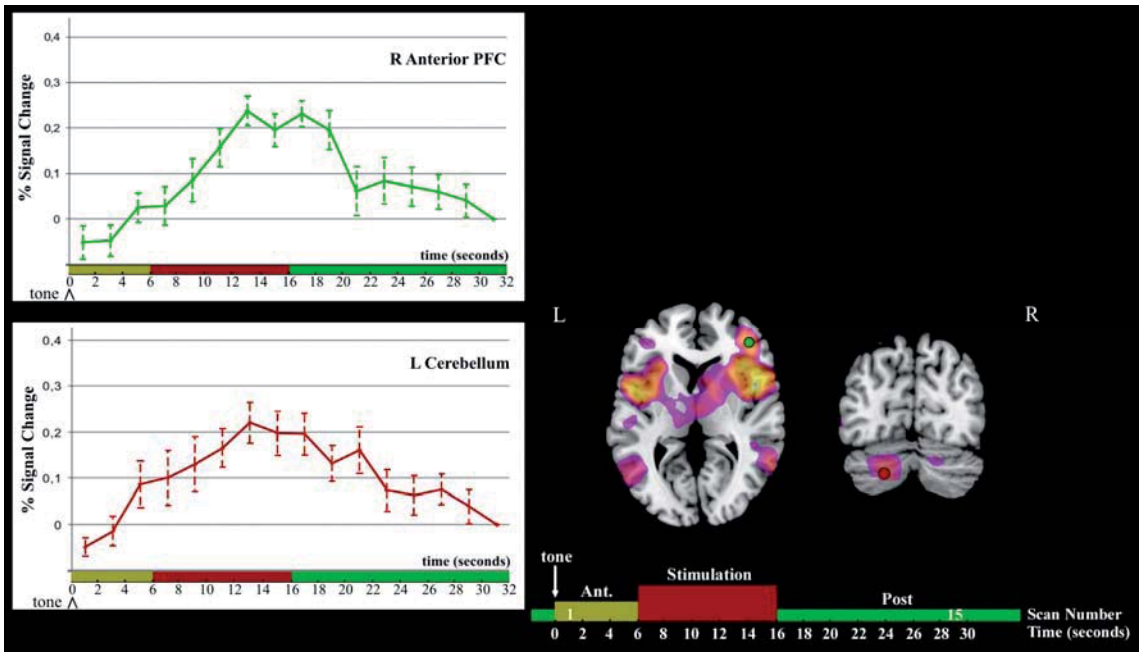
change (± SEM), with no adjustment for hemodynamic delay. This figure complements the second plot in main text Figure 3.



Supplemental Figure 3. Right SII (second somatosensory), left posterior insula and SI (primary somatosensory) activation time courses expressed as mean percent signal change (± SEM), with no adjustment for hemodynamic delay. This figure complements the third plot in main text Figure 3.

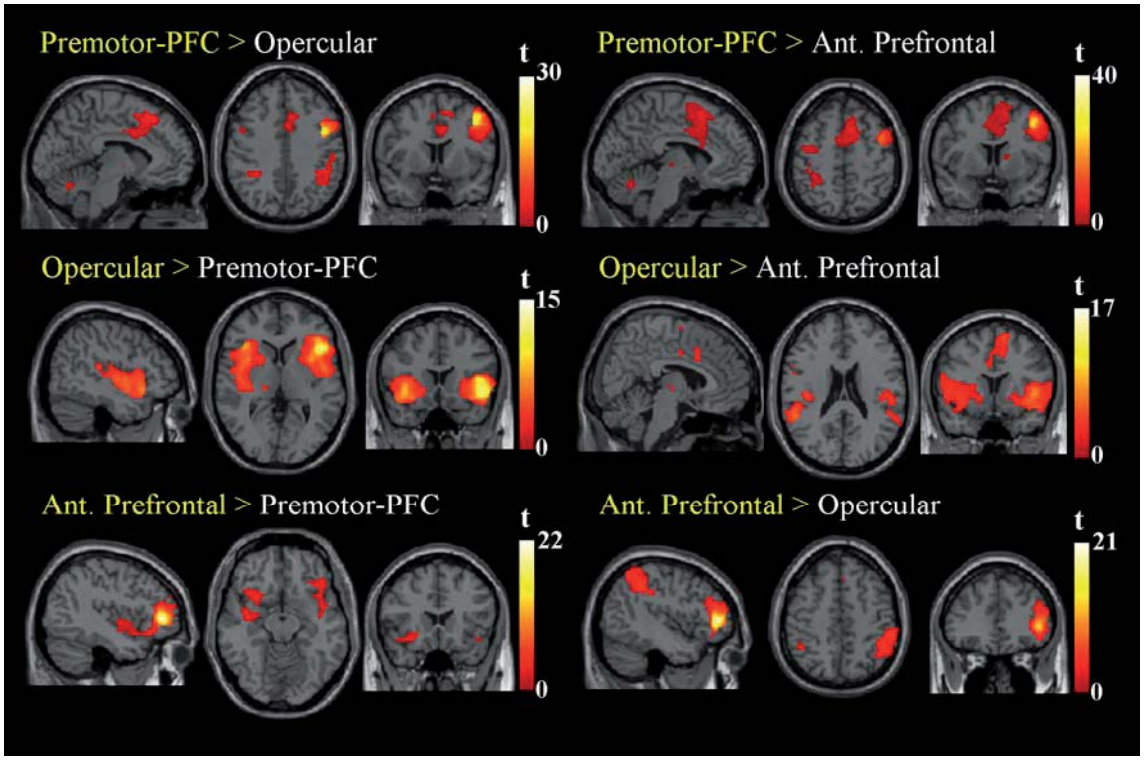


Supplemental Figure 4. Right basal ganglia, ACC (anterior cingulate cortex) and left thalamus activation time courses expressed as mean percent signal change (± SEM), with no adjustment for hemodynamic delay. This figure complements the fourth plot in main text Figure 3.



Supplemental Figure 5. Right anterior PFC (prefrontal cortex) and left cerebellum activation time courses expressed as mean percent signal change (± SEM), with no

adjustment for hemodynamic delay. This figure complements the fifth plot in main text Figure 3.



Supplemental Figure 6. Statistical t-maps displaying significant differences across the three frontal correlation maps reported in main text Figure 5 ($P_{FDR}<0.05$). PFC, prefrontal cortex. Ant., anterior. Images are displayed in neurological convention (Right=Right).

Supplemental Movie 1. Movie sequence dynamically illustrating the temporal evolution of brain responses to painful stimulation on right and left lateral surface views. The activation cycle is represented in the color bar including a cued (tone) anticipation period (Ant.) and the actual painful stimulation. Activations are thresholded at $P< 0.05$, whole-brain false discovery rate (FDR) corrected (activation range, t values from 2.5 to 10).

Supplemental Movie 2. Movie sequence dynamically illustrating the temporal evolution of brain responses to painful stimulation on representative brain views (axial slice, z= 3 and sagittal slice, x= 6 in Montreal Neurological Institute [MNI] space). The activation cycle is represented in the color bar including a cued (tone) anticipation period (Ant.) and the actual painful stimulation. Activations are thresholded at $P< 0.05$, whole-brain false discovery rate (FDR) corrected (activation range, t values from 2.5 to 10).

3.2. Study 2. Published in the international journal *PLoS One*

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Mapping Brain Response to Pain in Fibromyalgia Patients Using Temporal Analysis of fMRI

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Abstract

Background: Nociceptive stimuli may evoke brain responses longer than the stimulus duration often partially detected by conventional neuroimaging. Fibromyalgia patients typically complain of severe pain from gentle stimuli. We aimed to characterize brain response to painful pressure in fibromyalgia patients by generating activation maps adjusted for the duration of brain responses.

Methodology/Principal Findings: Twenty-seven women (mean age: 47.8 years) were assessed with fMRI. The sample included nine fibromyalgia patients and nine healthy subjects who received 4 kg/cm^2 of pressure on the thumb. Nine additional control subjects received 6.8 kg/cm^2 to match the patients for the severity of perceived pain. Independent Component Analysis characterized the temporal dynamics of the actual brain response to pressure. Statistical parametric maps were estimated using the obtained time courses. Brain response to pressure (18 seconds) consistently exceeded the stimulus application (9 seconds) in somatosensory regions in all groups. fMRI maps following such temporal dynamics showed a complete pain network response (sensory-motor cortices, operculo-insula, cingulate cortex, and basal ganglia) to 4 kg/cm^2 of pressure in fibromyalgia patients. In healthy subjects, response to this low intensity pressure involved mainly somatosensory cortices. When matched for perceived pain (6.8 kg/cm^2), control subjects showed also comprehensive activation of pain-related regions, but fibromyalgia patients showed significantly larger activation in the anterior insula-basal ganglia complex and the cingulate cortex.

Conclusions/Significance: The results suggest that data-driven fMRI assessments may complement conventional neuroimaging for characterizing pain responses and that enhancement of brain activation in fibromyalgia patients may be particularly relevant in emotion-related regions.

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Introduction

Nociceptive stimulation can trigger complex behavioral responses involving both local pain sensations and general affective phenomena [1]. Responses to painful mechanical stimuli typically persist after their application for a time that largely depends on stimulus features and the individual's receptive state [2,3].

Functional imaging has notably contributed to delineating the functional anatomy of the brain network mediating pain responses [4]. The most consistent activations in this "pain matrix" involve somatosensory and adjacent parietal cortex, the operculo-insular region and the anterior cingulate cortex [see specific reviews 1,4,5]. Interestingly, only a few imaging studies have explored nociception temporal dynamics, suggesting that pain-related activity may persist well beyond the specified stimulation periods [3,6–10].

Fibromyalgia is a syndrome expressed mainly as chronic complaints involving augmented subjective pain of mechanical origin [11]. Previous functional magnetic resonance imaging (fMRI) studies assessing the anatomy of brain activations have suggested that brain responses to mechanical stimuli are abnormally increased in fibromyalgia patients [12]. In this study, we aimed to further characterize brain response to pain in patients with severe fibromyalgia and healthy subjects using an fMRI data-driven approach [13,14]. We assessed the temporal dynamics of the actual brain response to local painful pressure in pain-related regions with Independent Component Analysis (ICA). The results

were then used to generate fMRI maps adjusted for the duration of brain responses that showed more complete activation patterns in patients and in control subjects and stronger correlation with reported subjective pain.

Methods

Ethics statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Ethics and Institutional Review Board of the Autonomous University of Barcelona (reference number SAF2007-62376). All patients and healthy subjects provided written informed consent for clinical and fMRI assessment and subsequent analyses.

Subjects

Twenty-seven subjects participated in the study, including nine patients with fibromyalgia and two groups of nine healthy subjects (control group 1 and 2) matched to patients for gender and age, and recruited from the same sociodemographic environment. Control group 1 served to compare brain response to a fixed mechanical stimulus pressure able to provoke severe pain in fibromyalgia patients. Control group 2 was matched to fibromyalgia patients for levels of perceived pain by increasing stimulus intensity.

The patients were consecutively selected during clinical follow-up to make up a homogeneous sample showing severe and durable symptoms. The series included nine right-handed females with a mean±SD age of 47.9±9.4 years and education level of 11.0±2.1 years. All patients met the American College of Rheumatology criteria for fibromyalgia [11]. Mean illness duration was 8.2±5.6 years. The number of tender points upon study assessment was 16.7±2.3. General Perception of Health according to the 36-Item Short-Form Health Survey [15] scored 11.1±13.2 (maximum score, 100). The Fibromyalgia Impact Questionnaire (FIQ) [16] total score was 73.2±13.8 (maximum score, 100). Hospital Anxiety and Depression Scale (HADS) ratings [17,18] were 13.4±4.0 and 10.3±4.7. One patient had a co-morbid clinical diagnosis of major depression, 2 patients a dysthymic disorder and 3 patients an adjustment disorder with mixed anxiety and depressed mood.

Patients were allowed to continue with their stable medical treatment, but were required to refrain from taking analgesic drugs 72 hours prior to fMRI. Six patients were on anti-inflammatory drugs in a stable regime (2 were also taking benzodiazepines, 1 antidepressants and 1 carbamazepine). The remaining 3 patients were taking: antidepressants, benzodiazepines and carbamazepine (1 patient), antidepressants and benzodiazepines (1 patient), and no medication (1 patient).

The control group 1 included nine right-handed females with a mean age of 47.2±8.9 years and education level 12.4±4.3 years, and the control group 2 nine right-handed females with a mean age of 48.2±5.5 years and education level 13.0±3.0 years. Subjects with relevant medical or neurological disorder, substance abuse, or psychiatric disease were not considered for inclusion. None of the healthy subjects was undergoing medical treatment.

Stimuli

Pressure stimuli were delivered using a specially designed hydraulic device capable of transmitting controlled pressure to 1-cm^2 surface placed on the subject's thumbnail. As in other studies [19,20], this system involved a hard rubber probe attached to a hydraulic piston that was displaced by mechanical pressure. In a preliminary session, each subject was acclimatized to the mechanical stimuli and trained to rate perceived pain intensity using a numerical rating scale (NRS) ranging from 0 (no pain) to 100 (the worst pain possible).

Pain thresholds were also assessed during the session and the intensity of pressure producing severe pain in both patients and control subjects was estimated. To determine individual thresholds, different stimulus intensities were applied lasting 5 seconds each, with an inter-stimuli interval of 20 seconds. The selected pressure stimuli, ranging from 2–9 kg/cm^2, were administered pseudo-randomly. Conventional pain thresholds corresponded to the least pressure intensity at which subjects perceived pain in two trials. In this session, pain threshold was 1.6±0.5 kg/cm^2 in the 9 patients and 4.0±1.0 kg/cm^2 in the 18 healthy subjects ($P<0.0005$). The minimum pressure intensity to provoke severe pain (NRS above 70) in patients was 3.6±0.9 kg/cm^2 and 6.8±1.4 kg/cm^2 in healthy subjects ($P<0.0005$).

fMRI pain paradigm

During the primary study assessment, identical stimulation was applied to both patients and healthy subjects (control group 1). A block-design paradigm was used consisting of 21-second resting-state periods interleaved with pressure stimulation blocks of nine seconds. During pressure blocks, sustained 4 kg/cm^2 pressure was delivered to the subjects' right thumbnail. Pressure was partially removed for 1 second in the middle of each pain block to reduce the probability of tissue damage in the thumb. The entire imaging sequence involved 12 rest-pressure cycles lasting 6 minutes in total. Immediately after image acquisition, each subject provided a single score to globally rate pain intensity perceived during the 12 pressure blocks.

The control group 2 was assessed using identical procedures, but applying 6.8 kg/cm^2, which produced a pain severity level similar to that experienced by fibromyalgia patients using 4 kg/cm^2 (NRS above 70).

MRI acquisition

A 1.5 T Signa system (General Electric, Milwaukee, WI) equipped with an eight-channel phased-array head coil and single-shot echoplanar imaging (EPI) software was used. Functional sequences consisted of gradient recalled acquisition in the steady-state (time of repetition [TR], 3,000 ms; time of echo [TE], 50 ms; pulse angle, 90°) within a field of view of 24 cm, a 96×64-pixel matrix, and slice thickness of 5 mm (inter-slice gap, 1 mm). Seventeen slices parallel to the anterior-posterior commissure line covered the whole-brain. The first two images in each run were discarded to allow the magnetization to reach equilibrium.

Image preprocessing

Imaging data were processed using MATLAB version 7 (The MathWorks Inc, Natick, Mass) and Statistical Parametric Mapping software (SPM5; The Wellcome Department of Imaging Neuroscience, London). Preprocessing involved motion correction, spatial normalization and smoothing using a Gaussian filter (full-width half-maximum, 6 mm). Data were normalized to the standard SPM-EPI template and resliced to 3 mm isotropic resolution in Montreal Neurological Institute (MNI) space. We excluded data from two subjects from an original sample of 29 subjects due to excessive head movement (z-axis translation>2 mm).

Image analysis

fMRI data are commonly analyzed using 'model-based' statistical methods that require a specific assumption about the time courses of activation. Typically, model-based analyses estimate the contrast between signal intensity of images obtained during stimulus

application and signal intensity of images obtained without stimulation or during a control condition. In experiments where response durations cannot be completely anticipated, as in pain assessment and in the assessment of emotions in general, the standard model-based approach may underestimate the evoked brain response. In contrast, "data-driven" statistical methods are used to identify actual brain activation without a priori hypothesis on the expected activation time course. These methods estimate the best fitting of the data, but do not directly test the statistical significance of the activations [13,14]. In the current study, we used a data-driven approach based on Independent Component Analysis (ICA) to generate a study-specific time course model, which was used as a regressor in conventional SPM analyses to statistically test between-group differences for the activation pattern.

Independent Component Analysis

Spatial Independent Component Analysis is a data-driven statistical analysis method that is able to decompose whole brain fMRI data into independent networks of brain regions (spatial components) involving voxels following similar temporal dynamics. Results are presented as a set of spatial maps with their associated time courses.

Group ICA for fMRI Toolbox was used (GIFT v1.3c; http://icatb.sourceforge.net), with previously described algorithms [21,22]. After subject-wise data concatenations, a separate spatial ICA was performed for each study group in three stages: *Stage 1:* The dimensionality of the fMRI data and the optimal number of components for each group were estimated using the minimum description length (MDL) criterion in GIFT [23]. Principal component analysis (2 reduction steps) was then used to reduce individual subject data in dimensionality (for computational feasibility) to the number of components estimated by the MDL criterion. *Stage 2:* Group estimation of spatially independent sources was then performed using the Infomax algorithm. *Stage 3:* During the final stage of back-reconstruction to the original dimensionality, individual subject image maps and time courses were estimated using the group solution [21,22]. This step was followed by the process of grouping components across subjects to produce group component maps and group-average time courses.

Temporal analysis of brain response to pain

Group ICA results were used to identify actual response functions (i.e., normalized time courses) of the brain regions activated by nociceptive stimulation. In selecting these time courses for further analysis, we considered those components involving regions known to mediate brain response to pain [4] and showing a consistent signal increase (activation) coinciding with each pain stimulation block, irrespectively of the duration of the activation.

Mapping brain response to pain: analyses of main task effects

1st-level (single-subject) SPM contrast images were estimated to characterize the functional anatomy of pain-related brain activations. For this analysis, the BOLD response at each voxel was modeled using (i) data-driven response function generated from the Group ICA; and (ii) conventional (SPM5) model-driven canonical hemodynamic response function. Resulting 1st-level contrast images for each subject were then carried forward to 2nd-level random-effects (group) analyses using one-sample t-tests. A two-sample t-test analysis was performed to compare activation maps between study groups. Spatial coordinates from the obtained maps were then converted to standard Talairach coordinates [24] using a non-linear transform of SPM standard space to Talairach space [25].

Mapping brain response to pain: correlation maps

We mapped voxel-wise correlations between subjective pain scores and brain activation. Separate correlation maps were obtained for both the data-driven and model-driven approaches including 18 study subjects (patients and control group 1). Correlations were considered significant at a P value less than 0.05 False Discovery Rate (FDR) corrected for the volume of activated regions (pain network).

In addition, we assessed the extent to which brain activation in the region showing the highest correlation with subjective pain (the anterior cingulate cortex) was able to account for group differences in perceived pain. This was carried out by comparing group differences in subjective reported pain both before and after controlling for (regressing out) the effect of cingulate activation using analysis of covariance (ANCOVA).

Results

Pain rating during fMRI assessment

The range of subjectively reported pain varied from 20 to 100 points across the 18 subjects (9 patients and 9 healthy subjects from the control group 1) assessed using 4 kg/cm^2 of pressure. Healthy subjects reported mild-to-moderate pain and fibromyalgia patients the most severe scores during this stimulation (mean$\pm$SD for healthy subjects = 41.1$\pm$20.1 and for patients 88.8$\pm$11.6; $t = 6.2$ and $P<0.0001$). The group of healthy subjects (n = 9) receiving 6.8 kg/cm^2 (control group 2) reported severe pain at rating levels comparable to the fibromyalgia group (80.2$\pm$10.7; $t = 1.6$ and $P = 0.123$).

Temporal analysis of brain activation at 4 kg/cm^2 of pressure

ICA estimated 34 spatially independent components in patients and 31 in healthy subjects (control group 1). The time course of nine components in patients and three components in healthy subjects showed a signal increase (i.e., activation) coinciding with each pain stimulation block. Two such components involved pain-related brain regions in each study group. That is, in both patients and healthy subjects, a "somatosensory" and an "insular" component met the double criterion of showing signal increase in each pain block and involving regions known to mediate brain response to pain.

The somatosensory component included bilateral parietal cortex in both groups and a small portion of the dorsal anterior cingulate cortex in fibromyalgia (Figure 1). The associated time course was very similar in patients and healthy subjects showing evoked signal changes that persisted after stimulus removal in each stimulation block. Block-average time courses (Figure 1) revealed an early fMRI signal increase that returned to the baseline level only after 18 seconds in both groups (twice the duration of the applied stimulus). Time to peak activation since stimulus onset was 6.9$\pm$5.1 s in patients and 6.3$\pm$4.8 s in control subjects (control group 1), showing $t = 0.28$ and $P = 0.782$. Activation duration was 18.6$\pm$3.6 s in patients and 18.9$\pm$3.6 s in control subjects, showing $t = -0.19$ and $P = 0.848$.

The "insular" component involved bilateral insulo-opercular cortex in both groups. In fibromyalgia patients, the time course of this component followed the dynamics of the somatosensory component, showing a fast initial signal increase and duration of 18 seconds. By contrast, healthy subjects, showed much less consistent signal changes in the insular region, as not all the stimulation blocks showed a definite signal increase (see Figure 1B).

Mapping brain response to 4 kg/cm^2 of pressure

The time course of the somatosensory component was averaged across groups (patients and control group 1) and was used as the

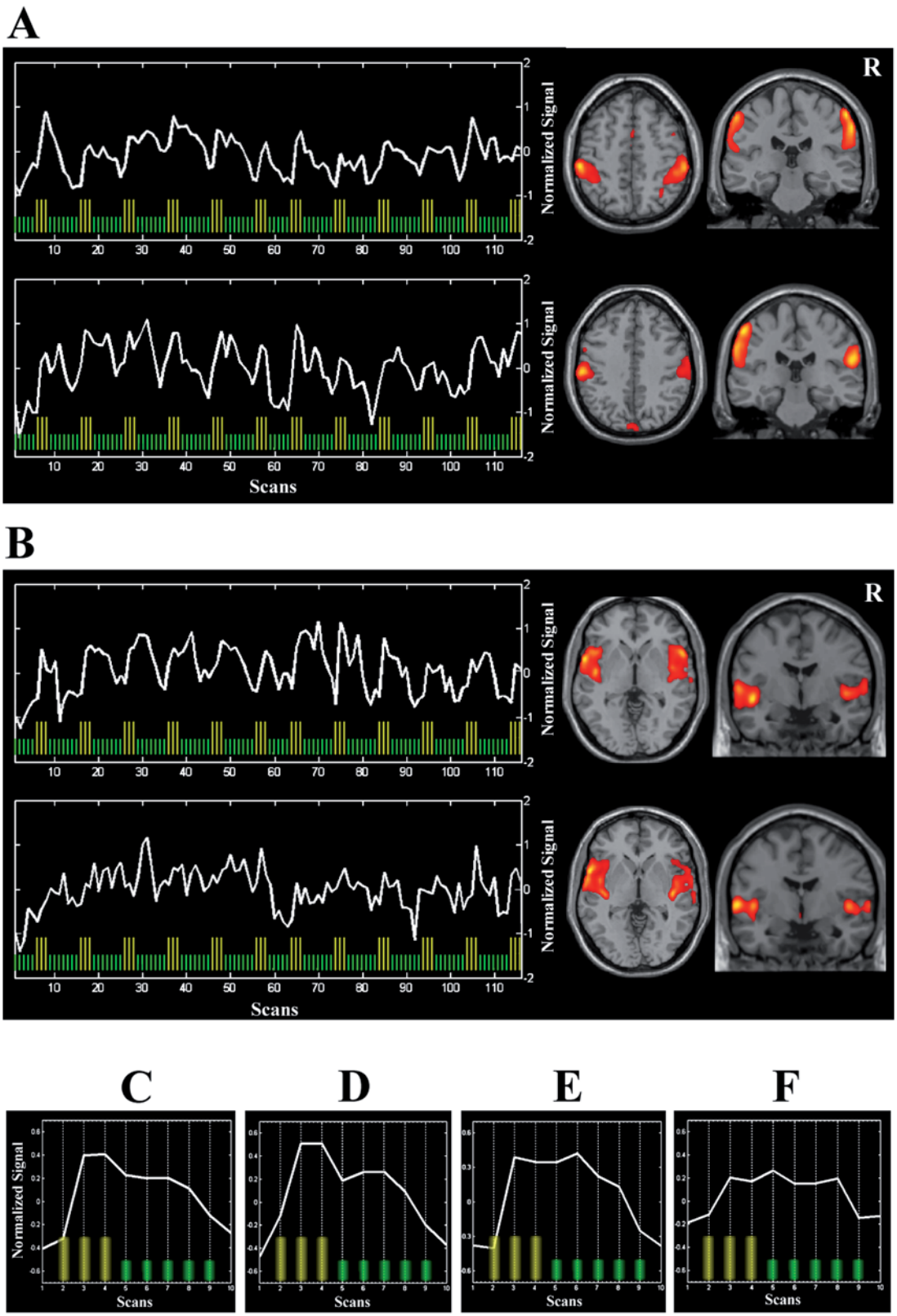

Figure 1. Temporal dynamics of the brain response to painful stimulation. (A) shows time courses and representative brain slices for the somatosensory component in fibromyalgia patients (top) and healthy subjects (bottom) derived from activation temporal analysis. (B) shows the corresponding data for the insular component in patients (top) and healthy subjects (bottom). (C–F) show block-average time courses for the somatosensory component in patients (C) and healthy subjects (D), and the insular component in patients (E) and healthy subjects (F). Yellow bars identify stimulation scans. R indicates right hemisphere.
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reference function in a conventional fMRI analysis. Figure 2 and Table 1 report brain activations obtained using this data-driven model. In fibromyalgia patients, activations involved all relevant regions of the pain network, including contralateral somatosensory and motor cortices, bilateral inferior parietal areas, the opercula, the insula, the basal ganglia, the supplementary motor area, the

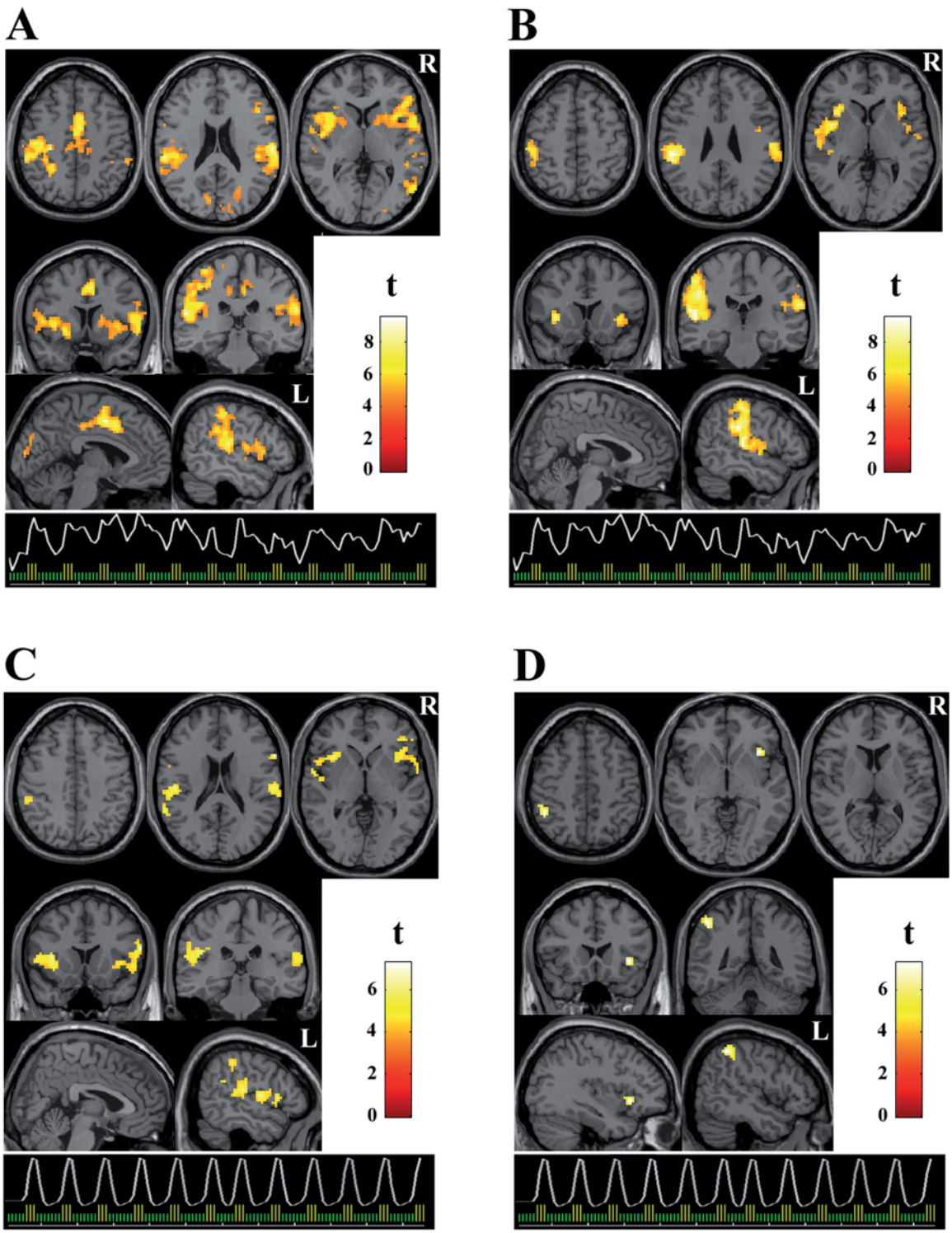

Figure 2. Brain activation maps. Brain response to 4 kg/cm^2 of pressure applied on the right thumb. Statistical parametric maps (SPM) are shown adjusted for response duration in fibromyalgia patients (A) and healthy subjects (B), and for stimulus duration in patients (C) and healthy subjects (D). Graphs illustrate the reference function models used in the SPM analysis (i. e., the time course from the somatosensory component averaged across groups in both A an B, and conventional canonical hemodynamic response function in C and D). Display threshold, $P<0.0005$, 20 voxels for all the data. R and L indicate right and left hemispheres. The names of the regions are shown in Table 1 and 2.
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Table 1. Brain Activations Adjusted for Response Duration (Data-Driven Analysis).

	Fibromyalgia		Healthy Controls		Patients>Controls	
	z	*X:Y:Z*	*z*	*X:Y:Z*	*z*	*X:Y:Z*
Sensory-Motor Cortex	4.4	−36:−17:59	4.9	−51:−27:48	3.9	−45:−15:42
Inferior Parietal - SII	4.8	−48:−28:26	5.1	−54:−28:26	3.0	−45:−36:30
	4.0	59:−37:21	5.0	56:−17:17		
	5.4	−59:−28:18	5.0	−59:−23:15	3.7	−59:−28:18
	5.1	56:−17:17				
Insula	4.9	−39:15:5	4.8	−36:15:2	3.5	−45:18:7
	4.9	36:15:−1	4.3	36:15:−1		
Anterior Cingulate - SMA	5.1	0:14:38			3.9	0:14:38
	5.3	3:2:44			4.1	3:2:44
	4.4	0:0:53				
Basal Ganglia	4.5	−27:6:−3			2.8	−27:6:−3
	4.4	15:14:−6			3.5	15:14:−6
Other Regions:						
Angular Gyrus	4.5	54:−64:6				
Visual Cortex	4.0	21:−78:18				
Frontal Operculum					4.0	48:29:4

Group activations show $P<0.05$ False Discovery Rate (FDR) whole brain corrected. The contrast patients>controls shows $P<0.05$ FDR corrected for the volume of activated regions (pain network). Coordinates (mm) are in the standard Talairach space. SII, second somatosensory cortex, SMA, supplementary motor area.
doi:10.1371/journal.pone.0005224.t001

anterior cingulate cortex and the cerebellum. In healthy controls, activation was mainly observed in the inferior parietal cortex involving the supramarginal gyrus, and in the insula. Statistical differences between both groups are reported in Table 1.

The assessment of brain activations from the conventional block-design analysis adjusted to stimulus duration (i.e., model-based) resulted in notably smaller pain-related activation in patients and control group 1 (Figure 2, Table 2).

Correlation maps

We mapped the correlation of subjective pain scores with brain activations during stimulation at 4 kg/cm^2 of pressure (i.e., voxel-wise regression of the activation patterns with subjects' pain scores). Pain scores were widely correlated with brain activation in the data-driven approach involving the contralateral sensory-motor cortex, supplementary motor area, anterior cingulate cortex, anterior insula and basal ganglia (Figure 3, Table 3). By contrast, subjective pain showed no significant correlation with the activation pattern identified using the conventional model-driven approach.

The plot in Figure 3 shows a relatively graded correlation between subjective pain and anterior cingulate cortex activation when including all subjects stimulated at 4 kg/cm^2 of pressure. Nevertheless, it is evident that patients and healthy subjects are at opposite extremes of the pain score range. Using ANCOVA, cingulate cortex activation was found to account largely for the differences between both groups in perceived pain. In this analysis, group differences in subjective pain scores were highly significant before controlling for the effect of anterior cingulate cortex activation ($F=38.0$, $P<0.0001$); a finding that was reversed ($F=1.8$, $P=0.195$) when removing (regressing out) this effect.

Table 2. Brain Activations Adjusted for Stimulus Duration (Model-Based Analysis).

	Fibromyalgia		Healthy Controls		Patients>Controls	
	z	*X:Y:Z*	*z*	*X:Y:Z*	*z*	*X:Y:Z*
Sensory-Motor Cortex	4.2	−54:−33:43	4.4	−51:−44:49	3.7	−27:−39:46
Inferior Parietal - SII	4.3	−45:−25:23			3.4	−56:−46:22
	4.3	59:−17:20				
Insula	4.5	−39:18:5			3.8	−45:18:7
	3.9	36:15:0	4.5	36:20:−4		
Frontal Operculum					3.4	51:29:4

Group activations show $P<0.05$ False Discovery Rate (FDR) whole brain corrected. The contrast patients>controls shows $P<0.05$ FDR corrected for the volume of activated regions (pain network). Coordinates (mm) are in the standard Talairach space. SII, second somatosensory cortex.
doi:10.1371/journal.pone.0005224.t002

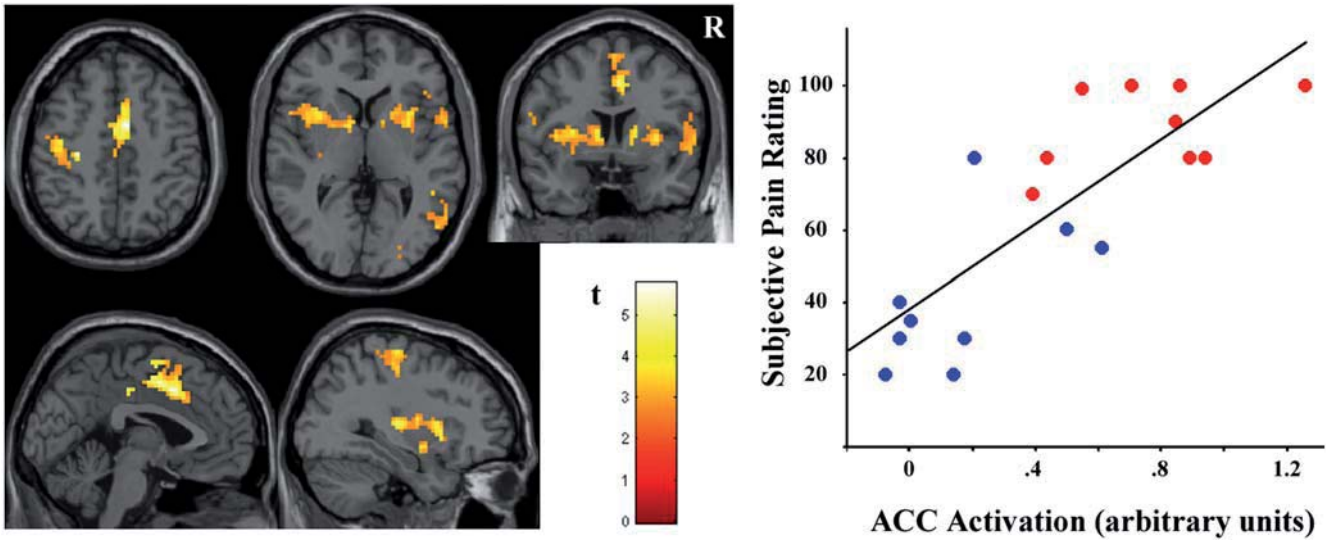

Figure 3. Correlation map between subjective pain scores and brain activations. (Adjusted for response duration -data-driven analysis- including all individuals). Display threshold, $P<0.01$, 10 voxels. R indicates right hemisphere. The plot illustrates the correlation at peak activation in anterior cingulate cortex (ACC) ($r=0.82$, $P<0.0001$ and adjusted $r^2=0.66$). A.u., arbitrary units. Red and blue dots correspond to patients and control subjects, respectively. The names of the regions are shown in Table 3.
doi:10.1371/journal.pone.0005224.g003

Comparing patients and controls subjects matched for pain levels

An ICA was carried out for the control group stimulated at pressure 6.8 kg/cm^2 and reporting severe pain (control group 2). This procedure estimated 37 spatially independent components. As in the above analysis, the time course of the obtained somatosensory component was averaged with the somatosensory time course of fibromyalgia patients and was used as the reference function in a new conventional fMRI analysis to compare patients with this control group. Table 4 shows the activation pattern obtained in both groups and the significant between-group differences. Brain response was comprehensive both in patients and control subjects involving most of the pain-related regions. Response in regions involved in the sensory aspects of nociception was similar, showing a tendency for higher activation in the somatosensory cortex in control subjects. Patients, however, showed significantly greater activation in the anterior insula and basal ganglia bilaterally, and in the SMA (Table 4, Figure 4).

Table 3. Correlation of Subjective Pain Scores with Brain Activations Adjusted for Response Duration (Data-Driven Analysis) (n = 18).

	Pearson	*z* score	Talairach Coor.
	r		*X:Y:Z*
Sensory-Motor Cortex	.73	3.4	−45:−15:42
Inferior Parietal - SII	.74	3.5	−59:−28:18
Insula	.69	3.2	−39:15:5
	.73	3.4	33:12:−1
Anterior Cingulate – SMA	.81	4.1	3:11:38
	.82	4.2	6:2:44
	.74	3.5	3:0:55
Basal Ganglia	.68	3.1	−33:9:5
	.63	2.8	18:12:−1
Other Regions:			
Angular Gyrus	.62	2.7	54:−61:3
Frontal Operculum	.64	2.9	56:6:3

All correlations show $P<0.05$ False Discovery Rate (FDR) corrected for the volume of activated regions (pain network).
doi:10.1371/journal.pone.0005224.t003

Discussion

This study aimed to characterize brain response to local pressure stimulation in fibromyalgia patients using an fMRI approach based on the temporal analysis of brain activation. Somatosensory areas showed consistent activation to each block of pressure stimulation that characteristically persisted beyond stimulus application. The fMRI maps adjusted for response duration showed robust activations in regions known to mediate brain responses to pain. Importantly, a strong correlation was observed between the rating of subjective pain during the fMRI assessment and the magnitude of the activation. Fibromyalgia patients showed significantly greater activation than comparative control subjects. Response enhancement was observed in fibromyalgia patients for most pain-related regions compared to the control subjects receiving identical stimulation, and for specific regions when the groups were matched for subjective pain levels.

This data-driven imaging analysis allowed us to compare specific temporal and anatomical features of nociceptive processing between fibromyalgia patients, who reported severe subjective pain to the relatively mild local pressure stimulus, and healthy subjects reporting only mild-to-moderate pain from this stimulation. We observed a similar activation time course in somatosensory cortices in both groups, which suggested relevant and durable responses to mechanical stimulation at the "sensory" stage of nociceptive processing, irrespectively of subjective pain severity. For the insula component, consistent long-lasting responses were observed only in fibromyalgia patients.

The anatomy of the activations in response to 4 kg/cm^2 of pressure differed between patients and control subjects (control

Table 4. Comparison analysis matching groups for subjective pain levels.

	Fibromyalgia (4 kg/cm^2)		Healthy Controls (6.8 kg/cm^2)		Patients>Controls	
	z	*X:Y:Z*	*z*	*X:Y:Z*	*z*	*X:Y:Z*
Sensory-Motor Cortex	4.6	−51:−27:43	5.8	−54:−15:48		
	4.0	54:−15:48	5.0	−33:−29:62		
			4.4	54:−21:48		
Inferior Parietal - SII	5.0	−48:−20:18	4.8	−60:−22:26		
	4.7	56:−16:23	4.9	56:−16:23		
Insula	5.0	−33:−3:8	4.8	−48:−20:18	3.6	−42:12:5
	4.6	−45:−8:6	4.4	−33:−2:11	3.6	38:18:−1
	4.7	39:17:−1	4.2	39:−3:−2		
Anterior Cingulate - SMA	4.8	0:−1:44	4.9	−6:−1:36	3.0	0:−4:44
	4.5	0:0:55	4.0	0:0:55		
Basal Ganglia	4.3	−27:3:−3			2.7	−27:3:8
	4.3	15:12:−1			3.6	30:12:−3
Other Regions:						
Frontal Lobe	3.7	56:10:16	4.0	54:13:35		
Left Cerebellum			4.8	−30:−62:−17		

Group activations show $P<0.05$ False Discovery Rate (FDR) whole brain corrected. The contrast patients>controls show $P<0.05$ FDR corrected for the volume of activated regions (pain network). Coordinates (mm) are in the standard Talairach space. SII, second somatosensory cortex, SMA, supplementary motor area. No significant findings were obtained in the contrast controls>patients.

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group 1). Healthy subjects showed mainly a sensory response with relevant activation in contralateral somatosensory cortices and moderate activation in the insular cortex. By contrast, fibromyalgia patients showed a full response to pain with robust sensory, limbic and motor activations. Functional MRI changes in these regions showed a significant correlation with the severity of experienced pain and largely accounted for group differences in subjective pain scores at low pressure stimulation. That is, increased activation in pain-related regions explained the increased subjective pain ratings in fibromyalgia patients.

It is noteworthy that all the "efferent" elements of the pain response (brain regions directly related to motor or visceral output) are represented in the voxel-wise map of the correlation between pain severity and brain activations, including contralateral sensory-motor cortex, supplementary motor area, anterior cingulate cortex, anterior insula and basal ganglia. Several fMRI studies have reported a close relationship between anterior cingulate cortex activation and the subjective experience of pain or its "suffering" component [2,4]. This has been an especially robust finding in fMRI pain studies [2,4,26–28] and our results further support such an association. In addition, the reported map suggests that the other elements of the efferent pain response may also participate in the subjective experience of pain. Staud et al. [10] have reported a near identical pattern by mapping the

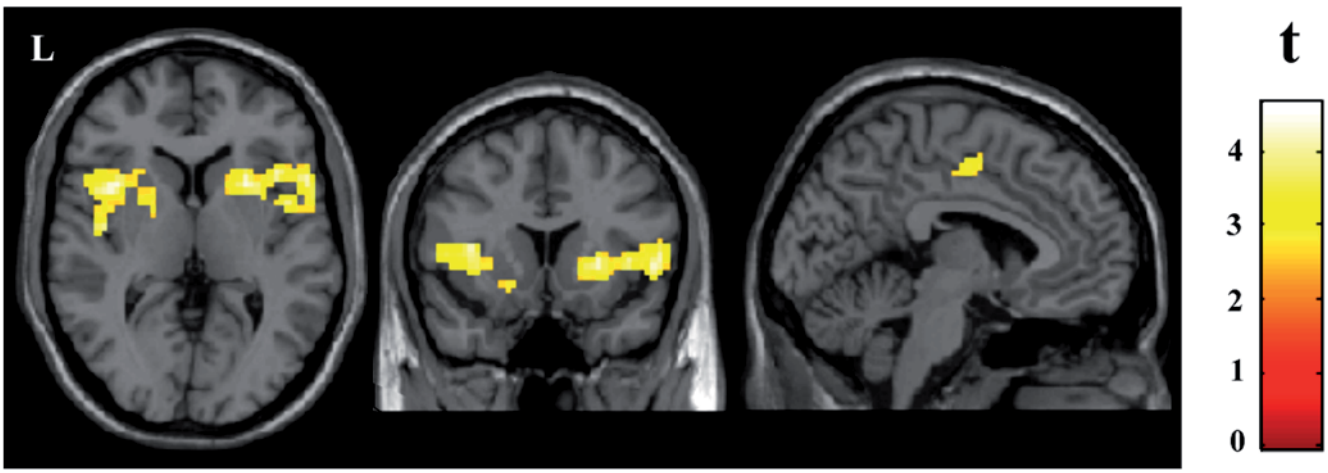

Figure 4. Comparison of fibromyalgia patients with healthy subjects matched for subjective pain levels. The statistical parametric map (SPM) adjusted for response duration shows the regions where patients receiving 4 kg/cm^2 of pressure showed greater activation than control subjects receiving 6.8 kg/cm^2. Display threshold, $P<0.01$, 10 voxels. L indicates left hemisphere. The names of the regions are shown in Table 4.

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correlation of perceived pain and brain activation related to temporal summation of "second pain" (late c-fiber evoked responses) during painful heat stimulation. Nevertheless, we did not obtain specific measurement of affect or unpleasantness during fMRI (only pain intensity ratings were recorded), which is a major limitation of our study. It would be of interest in future studies to map the correlation of brain activation during painful stimulation and individual affect ratings in addition to the reported correlation with pain intensity.

This closer correlation of subjective pain with the efferent brain response seems to further support proposed mechanisms for enhancement of emotions, including pain. Such models suggest that efferent somatic and visceral bodily responses to emotive stimuli originate backward afferent stimulation of the body representation in the brain, in turn amplifying emotional states [29–31]. Interestingly, the map showing the correlation of perceived pain with brain activations in our study largely coincides with the neural network related to interoceptive awareness in recent fMRI studies, which is proposed to mediate subjective feeling states arising from brain representations of bodily responses [32,33]. Our data indeed suggest that fibromyalgia patients show enhanced responses in regions related to the individuals' emotion expression that may be part of the subjective pain experience. Nevertheless, these activations are not necessarily the result of augmented responses in the basic levels of nociceptive processing. A very recent study by Burgmer et al. [34] showed that abnormal brain responses in emotion-related regions in patients with fibromyalgia may be delayed with respect to peripheral painful stimulation, suggesting that their painful experience enhancement is likely to originate from central factors related to the patients' affect and cognition. Our study is limited in that the influence of these factors (e.g., patients' anxiety and depression) was not controlled in the analysis.

Our results are consistent with most of previous fMRI studies on fibromyalgia, but expand the reported data by assessing the temporal dynamics of brain activity, which led to a more comprehensive activation mapping. All the reports coincide in showing abnormal brain responses to painful stimuli in fibromyalgia patients [20,35,36] when comparing patients to control subjects receiving identical stimulus intensity. In general, the data are consistent with a model of enhanced normal pain response and argues against the occurrence of "aberrant" nociception [20,37]. However, when matching both groups for perceived pain we observed larger activations in patients for specific regions. In this matching comparison, Gracely et al. [20] did not report significant differences between patients and control subjects with stimulation producing moderate pain. More recently, Staud et al. [9] specifically assessed the temporal summation of second pain using heat stimulation and also found no brain activation differences when stimulus strength was adjusted to induce moderate pain in both groups. In contrast with these two studies, more intense stimulation was used in our assessment and both patients and this control group reported severe pain. Fibromyalgia patients showed greater activation in the insula, basal ganglia and the anterior cingulate cortex, which are part of the brain network mediating efferent aspects of the pain response, and not in somatosensory cortices, where control subjects even had a tendency to show larger activation. Overall, our findings may be consistent with the notion of augmented brain response to pain in fibromyalgia, but the functional alterations may be particularly relevant in emotion-related (paralimbic) regions.

Functional MRI research is now focused on assessing the different dimensions of nociceptive processing. The presence of mood depression in fibromyalgia patients was associated with increased activation in regions processing affective components of pain [38]. Pain "catastrophizing," or characterizations of pain as awful, horrible and unbearable, was related to increased activation in the attentional, affective and motor domains, independently of the influence of depression [19]. Another study suggested that patients' beliefs about pain-control (locus of control for pain) may influence nociceptive processing at the sensory-discriminative stage [39]. In this context, mapping brain activations adjusted to the temporal dynamics of each nociception dimension in different clinical and experimental situations may be of interest to further characterize the complex phenomenology of pain responses. Interestingly, Burgmer et al. [34] suggested that patients with fibromyalgia may show different temporal dynamics in different elements of the brain pain network.

Conventional block-design fMRI is based on detecting brain activations following a specified paradigm of stimulus duration. These methods provide reliable and accurate activation patterns when stimulus duration corresponds well to brain activation (typical in most sensory and motor tasks). Nevertheless, for painful or emotional stimuli that may evoke responses of variable duration, the temporal analysis of brain activity may provide more informative activation maps and correlate better with subjective pain scores. Data-driven methods, however, are inherently biased to the actual response in a given population or experiment, which may hinder the generalization of conclusions [13,14]. For example, between-group comparisons may be difficult when the data-driven analyses identify different time courses for each group. In our study, it was feasible to compare groups using a common temporal model, as both patients and controls showed similar time courses for the somatosensory component.

Despite the small number of subjects included in this study, we observed robust activation maps reflecting the consistency of brain activation across all 12 pressure stimulation blocks. This may have particular relevance in the clinical fMRI setting as discussed in recent studies [40] where obtaining consistent findings at the individual case level is most desirable. Nonetheless, further studies will be needed to extrapolate our findings to the general population of fibromyalgia patients. In this context, it is also of interest to better establish the possible confounding effects of relevant clinical variables as, for example, the medication history of patients. In our study, no analgesic drugs were permitted 72 hours prior to fMRI, but patients were allowed to continue with their stable medical treatment, involving drugs with potential ability to modify the central nociceptive processing. In our patients, however, it is unlikely that the observed response enhancement to painful stimuli was a consequence of ongoing medical treatments, as the available data suggest the opposite effect [41–44]. Indeed, psychotropic medication showed no significant changes or ameliorative effects on abnormal functional neuroimaging measurements [43], while antidepressants reduced limbic activation during emotional processing [41], benzodiazepines reduced brain activity associated with anticipation to pain [44], and non-steroidal anti-inflammatory drugs suppressed pain-induced activation in most regions involved in pain processing [42].

Fibromyalgia has often been a controversial medical syndrome since patient identification is based largely on subjective symptoms [45]. In this and other studies [12], fMRI has demonstrated increased brain responses in patients labeled with this clinical diagnosis. Future research will establish the clinical usefulness of imaging tools for the objective assessment of subjective symptoms in both this and related disorders.

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Author Contributions

Conceived and designed the experiments: JP JCV CSM NC JD. Performed the experiments: JP MLS HO JCV NC JD. Analyzed the data: JP MLS HO BJH MY CSM. Contributed reagents/materials/analysis tools: JP HO JCV MY NC. Wrote the paper: JP MLS BJH.

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3.3. Study 3. Under review in the international journal Biological Psychiatry

Functional connectivity alterations in the mood depression state identified from changes in brain anatomy

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Abstract

Background: Major depressive disorder (MDD) is characterized by a dominant alteration of mood state that substantially interferes with all major aspects of brain functioning. Neuroimaging studies have provided evidence for both functional and anatomical abnormalities in MDD patients within the brain systems relevant to mood regulation. Although fMRI has recently emerged as a useful technique to assess the brain's functional organization during sustained states, state-dependent functional abnormalities in depressed patients have been only partially characterized using fMRI dynamic approaches. *Methods:* We used regions showing MRI gray matter volume alterations to guide a comprehensive analysis of resting-state functional connectivity in MDD. A total of 27 patients and 27 healthy subjects were assessed. Anatomical alterations were identified using conventional voxel-based morphometry tools that served to identify the regions of interest for "seed-based" functional connectivity analyses. *Results:* We found significant functional connectivity reduction in most networks related to MDD pathophysiology, congruent with a dominant hypo-functional brain state. Areas affected included the amygdala-hippocampal region, basal ganglia, insula-operculum region, dorsal-medial frontal cortex, *default mode network* and orbitofrontal areas. The identified functional connectivity alterations partly correlated with the overall severity of the depressive episode. Relevantly, significant increase in functional connectivity was also observed within (and between) structures critical to MDD, involving the subgenual part of the anterior cingulate cortex, the hypothalamus and the right amygdala. *Conclusions:* As a novel approach, the anatomically-guided assessment of functional connectivity in depressed patients was useful to further characterize relevant alterations compromising the intrinsic brain dynamic organization during the depressive state.

Introduction

Major depressive disorder (MDD) is characterized by a low mood state that pervades all aspects of life, the inability to experience pleasure, low self-esteem with negative thoughts and common discomfort from bodily sensations (1). Functional abnormalities in inter-connected brain regions relevant to affective processing (2) have been commonly reported in MDD, generally showing reductions in baseline brain activity in neocortical regions, particularly within dorsal medial and lateral frontal regions, together with activity enhancements in specific ventral limbic and subcortical structures (3-6).

fMRI has recently emerged as a successful technique for the non-invasive study of the brain's intrinsic functional organization in a variety of sustained states (7-10). State-dependent fMRI signal oscillations mostly reflect spontaneously fluctuating neural activity that synchronizes between regions (11, 12) showing direct or indirect anatomical connections (13-18). Although resting-state functional brain networks have shown a highly reproducible anatomical pattern across species and across different situations in humans (18-21), the strength of dynamic coupling between brain regions has a functionally-significant range for variation across different brain conditions such as those characterizing psychiatric disorders (14, 22). MDD resting-state functional connectivity studies have provided partial but compelling evidence for functional disruptions within specific brain networks (23-26), and particular connectivity enhancements within regions of crucial importance to MDD pathophysiology (27) such as the subgenual anterior cingulate cortex (ACC). Nevertheless, there are no studies

aimed at identifying a complete pattern of functional connectivity alterations involving most of the brain systems relevant to mood modulation.

Considering the potentially reversible nature of major depressive episodes, pathophysiologically relevant state-dependent brain alterations in MDD patients may arguably be more probable in the functional domain. Nevertheless, significant changes affecting brain anatomy have been frequently reported in patients, which hypothetically may either predispose to mood depression or follow enduring functional disturbances in the context of continuous fine remodelling of brain anatomy (6,28). Gray matter reductions frequently observed in MDD highly overlap brain circuits showing functional abnormalities in such patients (28), including the medial prefrontal cortex, amygdala, hippocampus, temporal cortex, ventral striatum and, to some extent, the insular cortex (3,6,28-33). Previous findings in a variety of brain pathologies have suggested a parallel occurrence of resting-state functional connectivity abnormalities and structural alterations in overlapping brain regions (13,14,16,22,34,35). Despite its potential relevance to further understanding MDD pathophysiology from a holistic perspective, to date no study has investigated possible resting-state functional abnormalities in relevant brain networks defined by existing anatomical alterations in such patients. In the present study, we used the regions showing the largest gray matter volume differences between MDD and comparable healthy subjects to guide a comprehensive functional connectivity resting-state “seed” analysis.

We specifically hypothesized that (i) the anatomically-guided resting-state functional connectivity analysis would successfully map functional brain networks relevant to MDD pathophysiology and provide a comprehensive picture of the altered dynamic

brain organization underlying the depressive state, and that (ii) greater clinical severity of the depressive episode would be significantly associated with greater magnitudes of resting-state functional connectivity abnormalities in functionally relevant networks.

Materials and Methods

Subjects

Twenty-seven MDD patients were recruited from the Mood Disorders Unit of the University Hospital of Bellvitge. All patients met DSM-IV criteria for MDD with no psychotic features. MDD patients were assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders-Clinician Version (SCID, [36]) that was conducted by two senior psychiatrists who reached a consensus for all items. At inclusion, all patients had a Hamilton Depression Scale (HAM-D 17, [37,38]) score equal to or greater than 18. Exclusion criteria included the presence or past history of other Axis I diagnoses, relevant medical or neurological disorders and abnormal clinical MRI upon radiological inspection. A group of 27 healthy volunteers comparable in gender, age, handedness and years of education also participated in the study. A complete medical interview was carried out to exclude subjects with relevant medical or neurological disorders, history of substance abuse and psychiatric illness. The characteristics of study groups are reported in Table 1. All patients and control subjects gave written informed consent to participate in the study, which was approved by the Research and Ethics Committee of the University Hospital of Bellvitge.

Study design and MRI acquisition parameters

The MRI examination included: (i) A high-resolution anatomical 3 dimensional-T1 sequence to obtain a sample-specific pattern of MDD structural abnormalities; and (ii) a four-minute resting-state assessment with closed eyes. For the patient group, the MRI examination was carried out on the 15th day of an antidepressant wash-out period.

A 1.5 Tesla Signa system (General Electric, Milwaukee, WI) equipped with an eight-channel phased-array head coil and single-shot echoplanar imaging (EPI) software was used. The high-resolution axial T1-weighted anatomical image was acquired for each subject using a 3-dimensional fast spoiled gradient inversion-recovery prepared sequence with 130 contiguous slices (repetition time, 11.8 milliseconds; echo time, 4.2 milliseconds; flip angle, 15°; field of view, 30 cm; 256 x 256 pixel matrix; slice thickness, 1.2 mm). The functional EPI sequence consisted of gradient recalled acquisitions in the steady-state (repetition time [TR], 2,000 ms (120 volumes); echo time [TE], 50 ms; pulse angle, 90º) within a field of view of 24 cm, a 64 x 64 pixel matrix, and a slice thickness of 4 mm (inter-slice gap, 1.5 mm). Twenty-two slices parallel to the anterior-posterior commissure line covered the whole-brain. The sequence included 4 additional dummy volumes to allow the magnetization to reach equilibrium.

Preprocessing and analysis of MRI data

Imaging data were processed using MATLAB version 7 (The MathWorks Inc, Natick, Mass) and Statistical Parametric Mapping software (SPM5; The Welcome Department of Imaging Neuroscience, London).

Anatomical imaging sequences. All images were checked for artifacts and the anatomical scans were co-registered to the SPM-T1 template. Standard preprocessing steps were applied to the original anatomical scans following the unified segmentation-normalization approach provided in SPM5 (39). Briefly, image preprocessing involved the following steps: (1) optimally normalizing and segmenting gray matter, white

matter, and cerebrospinal fluid (CSF) using tissue-specific probability maps provided in SPM5; (2) modulating voxel values from spatial normalization data to preserve volumetric information; and (3) spatial smoothing using a 12-mm full-width at half-maximum isotropic Gaussian kernel.

Voxel-wise regional volume differences (absolute and relative) were tested by means of second-level random-effect group analyses in SPM5. Two distinct models were employed: (i) two-sample t-test without covariates and (ii) two-sample t-test including the *total intracranial volume* as a covariate (the total volume resulting from the addition of gray matter, white matter and CSF volumes). As an exploratory evaluation of anatomical alterations and to guide the functional connectivity analysis (selecting the "seeds"), regions showing group differences with a minimum cluster extension of 200 voxels at $p<0.01$ (uncorrected) were considered in both the absolute and relative analyses.

Functional imaging. Functional image preprocessing involved motion correction, spatial normalization and smoothing using a Gaussian filter (full-width at half-maximum, 8 mm). Functional data were normalized to the standard SPM-EPI template and re-sliced into 2 mm isotropic voxels in Montreal Neurological Institute (MNI) space. All image sequences were routinely inspected for potential artifacts.

We performed a detailed set of seed-based functional connectivity analysis of subjects' resting-state imaging sequences using the identified anatomical alterations (i.e., regional volumetric reductions) to guide the functional analysis. The time course of each selected volume of interest (seed) was used as a regressor to be correlated with

the time course of all the voxels throughout the brain. The analysis was based on the method fully described in a recent study by our group (40). The placement of the seeds of interest corresponded to the coordinates showing the maximal between-group anatomical difference obtained in the structural analysis (supplementary Table 1). For each brain location, seeds were defined as 3.5-mm radial spheres using MarsBaR region-of-interest toolbox in Montreal Neurological Institute stereotaxic space ([41], see Figure 6 for a representation of the seeds of interest). Signal values for the seeds were calculated as the average signal of all the included voxels at each data point. In addition to our signals of interest (seeds), we derived estimates of white matter, CSF, and global brain signal fluctuations to include them as non-interest nuisance variables in the linear regression analyses. We selected the SPM5 a-priori templates of gray matter, white matter and CSF, which were normalized to the same (MNI standard) stereotactic space as the subjects' EPI volumes, and thresholded them at 70% percent tissue probability type to compute the binarized masks. Nuisance signal values were then extracted for each mask by calculating the average signal of all the included voxels in the mask at each temporal data point. The global brain signal was also calculated as the mean of the three tissue-type nuisance signals at each temporal data point.

Functional connectivity maps were estimated for each brain region by including the seed and nuisance signals as predictors of interest or no interest in whole-brain, linear regression analyses in SPM5. These first-level single-subject analyses were carried out separately for each seed region. A high-pass filter set at 128 seconds was used to remove low-frequency drifts. Prior to model estimation, each of the 3 nuisance covariates were mutually orthogonalized using an iterative Gram-Schmidt method.

Contrast images were generated for each subject by estimating the regression coefficient between the seed time series and each brain voxel signal. These images were then included in group (second-level) random-effects analyses, using a two-sample t-test model. To assess the magnitude and extent of functional connectivity for each brain seed within groups, we computed one-sample contrasts of interest (Control group: 1 0. Patient group: 0 1) resulting in t-maps (SPMs) that were thresholded using a false discovery rate correction (42) of $P_{FDR}<0.05$ for the whole-brain volume.

Between-group differences in the patterns of functional connectivity gathered by the distinct seed maps were assessed within an implicit mask defined by the global conjunction of the within-group SPMs for both patients and controls. Group differences were considered significant when involving (within the already thresholded network) regions with a minimum cluster extension of 200 voxels at $p<0.01$ uncorrected.

We performed voxel-wise correlation analyses in SPM5 to test for the linear relationship between patients' overall symptom severity (assessed using HAM-17 total score) and the strength of resting-state functional connectivity within each network of interest. These correlation analyses were carried out for regions contained in the implicit mask of the global conjunction of the within-group SPMs for each specific seed-related network. Regional correlations were considered significant when involving (within the already thresholded network) regions with a minimum cluster extension of 200 voxels at $p<0.01$ uncorrected.

Results

Anatomical analysis

Brain structures showing the most evident absolute gray matter volume reductions in MDD patients corresponded to the amygdala-hippocampal region bilaterally, the insulae, the dorsal and ventral medial frontal cortex (MFC), posterior-medial orbitofrontal cortex (OFC), primary visual areas and the cerebellum (Figure 1, Supplementary Table 1). In the analysis assessing relative between-group volume differences (i.e., controlling for total intracranial volumes), a similar anatomical alteration pattern was observed. Such analyses (absolute and relative) did not identify any regions showing significant volume increases in patients compared to control subjects.

Resting-state functional connectivity analysis

In this assessment, we used the regions showing the greatest gray matter volume differences between MDD and comparable healthy subjects (a total of eight anatomical locations) to guide a comprehensive functional connectivity resting-state "seed" analysis.

Figure 2 (and Supplementary Table 2) shows resting-state functional connectivity maps obtained for the right and left amygdala seed analyses depicting a similar functional anatomy, although the right amygdala seed map gathered a more extensive region network. The network included the amygdala-hippocampal region bilaterally, the insulo-operculum-basal ganglia complex, the right parietal operculum-second somatosensory cortex (SII), occipital areas, and part of the cerebellum, midbrain

regions and pons. The patient group additionally showed significant functional coupling between the right amygdala and the subgenual-pregenual ACC region. The between-group comparison analysis for the right amygdala seed map (Figure 2A, Table 2), showed a functional connectivity reduction in the patient group affecting the right parietal operculum, the basal ganglia and a bilateral region involving part of the amygdala, hippocampus and parahippocampal gyrus. In addition, patients showed a significant enhancement of functional connectivity between the right amygdala and the pregenual-subgenual ACC region. For the left amygdala seed map, only patients showed significant functional connectivity reductions, involving the right posterior insula-parietal operculum region (Figure 2B, Table 2).

Figure 3 (and Supplementary Table 2) presents resting-state functional connectivity maps for both insulae. The right insula was seen to be strongly connected with the whole insula-operculum-basal ganglia complex bilaterally, visual areas, a portion of the cerebellum, the pregenual-subgenual ACC and the posterior part of the pons and midbrain. A similar pattern of functional connectivity, albeit less extensive, was observed for the left insular seed map. Reductions in functional connectivity measurements were observed for the patient group (Figure 3A, Table 2) within the right insula seed network involving the insulo-opercular (fronto-parieto-temporal) region bilaterally.

The functional connectivity map originating from the dorsal MFC seed identified a robust functional brain network mainly involving bilateral frontal and parietal neocortex, and a relevant part of the brain medial wall extending to subcortical structures such as the thalamus and basal ganglia (Figure 4A, Supplementary Table 3).

MDD patients specifically showed a reduction in functional connectivity measurements affecting the dorsal-rostral regions of the medial frontal cortex. (Figure 4A, Table 2).

The pattern of resting-state functional connectivity of the ventral MFC seed largely coincides with the so-called *default-mode network* comprising medial frontal areas, the posterior cingulate cortex (PCC) extending to the precuneus and bilateral angular gyri (Figure 4B, Supplementary Table 3). The patient group showed reduced functional connectivity within most elements of the network (Figure 4B, Table 2).

Figure 5A (and Supplementary Table 3) shows the region pattern of functional coupling with the posterior-medial OFC seed mainly gathering pregenual-subgenual portions of the ACC extending to the hypothalamic region, OFC regions and ventral striatum, ventral parts of the insulae and temporal operculum and pole. In the patient group, the pattern additionally included a region within the ventral precuneus and cerebellum. MDD patients showed functional connectivity reductions between the ventromedial posterior OFC seed and surrounding areas of the OFC, whereas a specific increase in functional connectivity was observed involving the most ventral posterior part of the subgenual ACC extending to the hypothalamus. A region within the ventral part of the precuneus also appeared functionally hyper-connected to the ventral medial OFC seed in the patient group (Figure 5A, Table 2).

The last seed involved ventral visual cortex. This seed defined a similar resting-state functional network for both groups (Figure 5B, Supplementary Table 3) including almost the entire occipital lobe extending to the cerebellum and to parietal regions, the medial paracentral lobule, precentral and postcentral gyri extending to dorsal parietal

regions, and portions of the thalamus. No between-group significant differences were obtained in this analysis.

Correlations between clinical severity and fMRI connectivity measurements

Figure 6 (and Supplementary Table 4) illustrates the regional pattern of correlations between patients' clinical severity (HAM-17 total score) and functional connectivity measurements within the assessed networks. Greater MDD severity scores were mostly associated with functional connectivity reductions between cortico-subcortical and neocortical-paralimbic regions (including the subgenual ACC, insula-operculum-basal ganglia regions, lateral OFC, and the PCC and angular gyri). Greater MDD severity scores were additionally associated with greater functional connectivity within the amygdala-hippocampus region bilaterally.

Discussion

As a novel approach, the anatomically-guided assessment of state-dependent functional connectivity in patients with MDD was useful to identify a global pattern of relevant alterations compromising the intrinsic brain dynamic organization in mood-related networks of special relevance to MDD pathophysiology. Areas showing reduced functional connectivity involved dorsal neocortex, paralimbic and limbic regions, which are generally consistent with the overall hypo-functional state characterizing MDD patients. Of particular relevance when considering the broad pattern of dynamic alterations, was the specific observation of functional connectivity enhancement between the right amygdala and the subgenual ACC, and between the ventral-medial OFC and the hypothalamic area in MDD patients, which may be consistent with the neuroendocrine and autonomic stress-related dysfunction extensively reported in MDD. Interestingly, functional connectivity abnormalities within affected networks were partly correlated with the overall symptom severity in patients.

The anatomical analysis assisting the selection of functional seeds of interest in our study showed a distributed pattern of subtle volume reductions, which were however highly consistent with previous literature (3,6,28-33), in MDD patients. The identified anatomical pattern allowed us to globally assess the functional dynamic equilibrium within the networks most relevant to MDD pathophysiology. Previous studies have begun to show possible resting-state functional abnormalities involving part of such networks (23-27), although no comprehensive assessment was previously reported.

A recent study (43) provided the first functional connectivity map using the amygdala as a seed in healthy subjects showing a comprehensive region pattern highly overlapping with the pattern observed in our study. We found significantly reduced functional coupling between the right amygdala and the bilateral amygdala-hippocampal area. Such a result may appear to be in contrast with a previous observation (44) in which the authors instead observed increased correlation between right-left amygdala activity when assessed across subjects. Such an observation when compared to our connectivity measurement (within-subject) may reflect complementary, if not equivalent, underlying phenomena. It is noteworthy, however, that the greater MDD clinical severity in our study was associated with increased functional connectivity within the amygdala-hippocampus related area, an association that had previously been reported using measurements of amygdala metabolic activity (45,46), which may suggest that such connectivity enhancements represent specific markers of depression severity. The amygdala has been regarded as a central pivotal site for emotion processing, showing major roles in detecting and conferring emotional value to sensory stimuli, in emotional memory formation and in guiding behavior towards positive and away from negative goals (47-52). Our study, in agreement with previous observations in a different context (53), showed additional functional disruptions between the amygdala and the bilateral basal ganglia and the posterior insula-SII. Such regions appear to share dense anatomical connections with the amygdala (51,54), and may therefore partly underlie the maintenance of amygdala functions, which have been shown to be partially compromised or negatively biased in MDD patients (55-62).

We observed functional connectivity reductions in MDD patients within the network defined by the right insula seed involving bilateral regions of the insulae extending to frontal and parietal opercula, and temporal regions. A variety of functional resting-state abnormalities have previously been detected within different parts of the insula in MDD patients including both increases (63) and reductions (64) in metabolic activity, which have been shown to significantly modify (i.e., mostly reduce) following antidepressant treatment (65-69). fMRI studies have also reported various insula response abnormalities during task, mostly related to augmented activations during aversive stimulation (70-73), which were not necessarily accompanied by augmented subjective perception in MDD patients. The insular cortex plays a crucial role in the integration, awareness and efferent response to a wide variety of stimuli arising from the internal and external milieu (74). In the light of previously mentioned evidence of insula alterations in MDD, functional connectivity abnormalities reported here may substantially add to current knowledge by informing as to the intrinsic disruption of insula dynamic organization in depression, most probably associated with aberrant processing of ongoing interoceptive signals of somatic and visceral nature frequently reported in such patients (75-78), together with compromised efficient processing of externally-delivered stimulation (76,79).

The network gathered by the dorsal MFC seed mainly involved bilateral frontal and parietal neocortex, medial wall regions and subcortical structures such as the thalamus and basal ganglia. Significant functional connectivity reductions in MDD patients within such a circuit involved a region around the right dorsal-rostral medial frontal wall. Reductions in resting-state metabolic activity within this area in MDD patients have been detected (65,80), in addition to resting-state functional connectivity

disruptions between the rostral ACC and the medial thalamus and pallido-striatum (23,24). Interestingly, the MFC region observed in our study was normally involved, together with dorsolateral prefrontal areas, in the effortful down-regulation of aversive emotions and high-arousal states (2,82). Regulation of affective states is one of the crucial aspects altered in major depression (83). Two studies (84,85) have shown specific impairment (i.e. activation enhancement) in dorsal-rostral ACC response during emotional down-regulation. Moreover, an association between perceived difficulty in down-regulating and symptom severity was observed in patients (84). Additionally, a greater participation of rostral-dorsal medial and lateral frontal areas in depression has also been observed during executive tasks requiring cognitive effort (86-88). All in all, such observations, together with ours, suggest the existence of a multi-context functional inefficiency of brain medial frontal regulatory mechanisms in MDD, which may be primarily observed as connectivity disruption during the resting-state.

The ventral MFC seed gathered the so-called (in previous PET and fMRI resting-state studies) *default mode network* (8,89), which is involved in monitoring and interpreting self-relevant information (90) and normally deactivates in response to attention-demanding processing of external stimulation, two functions that appear to be frequently compromised in major depression (76). Alterations related to the ability to normally deactivate frontal ventral medial wall regions within the network have been commonly reported in MDD, sometimes associated with reduced task performance and symptom severity (88,91-94). Distinct resting-state fMRI connectivity alterations within this network, measured using different methodological approaches or regions of interest, have been initially detected in depression (25,27). We add to previous knowledge by providing evidence of partial functional disconnection between the

anterior ventral MFC seed region and neocortical aspects of the MFC, PCC and angular regions, which were (the latter two) significantly associated with greater clinical severity in patients. Such connectivity disruptions in MDD patients extended through ventral-medial aspects of the OFC, which has been traditionally involved in MDD pathophysiology (28).

In contrast to the predominant pattern of functional connectivity reductions affecting relevant networks in MDD, specific functional connectivity increases were found between the right amygdala and the subgenual ACC, and between the adjacent posterior-medial OFC and the hypothalamus area. Significantly, we found a significant association between greater functional coupling within the subgenual ACC and reduced clinical severity in MDD patients, which may be in agreement with previous studies reporting that enhancements in subgenual-pregenual ACC activity are associated with positive responses to a variety of antidepressant treatment strategies (95-99). The subgenual ACC has been one of the most frequent regions showing alteration in MDD functional neuroimaging (5,28). Its anatomical pattern of connections with the amygdala and proximal structures, the periaqueductal gray and the hypothalamus (100,101) has posited a major role for such region in guiding behavior and affective states through its influence on autonomic, endocrine and visceral function (28,101,102). The observation of functional hyper-connectivity within parts of this circuit (involving subgenual ACC and hypothalamus) would appear to be in agreement with the neuroendocrine and autonomic stress-related dysfunction frequently observed in MDD (103-105).

In our study, greater functional connectivity reductions within networks showing significant alteration in MDD were mostly associated with a greater severity of the depressive episode. Plausibly, functional connectivity alterations within specific networks may be distinctly associated with particular MDD symptoms (e.g., low mood, anhedonia, anxiety, negative rumination, insomnia, anorexia) or symptom domains (affective, cognitive and somato-visceral), which may be fruitfully addressed in future studies. Resting-state functional connectivity assessments in individuals with a high risk of developing the disorder, in remitted asymptomatic patients, during the course of antidepressant treatment and under thoroughly subtype classifications would be of major importance to better define the stability, generalizability and etiological-specificity of resting-state brain-functional biomarkers for major depression.

To our knowledge, this is the first attempt to comprehensively assess possible alterations in the intrinsic brain functional organization underlying the depressive state using brain volumetric abnormalities to guide the study. Our approach successfully detected a predominant pattern of functional connectivity reduction affecting networks relevant to MDD pathophysiology, and specific functional connectivity enhancements in regions of crucial importance for eliciting and maintaining affective states and for triggering stress-related responses. The future study of the interaction of disease-relevant stimuli with a specific baseline functional disposition of the brain may open a new era in the understanding of MDD pathophysiology.

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Figure legends

Figure 1. Brain pattern of gray matter reductions in MDD patients. All depicted clusters survive a minimum of 200 voxels at $p<0.01$ uncorrected. Images are displayed in the neurological convention (R=Right).

Figure 2. Right (A) and left (B) amygdala resting-state functional connectivity networks for Control subjects (C) and MDD patients (P). All clusters depicted in the within-group patterns survive $p<0.05$ FDR corrected and a minimum extension of 200 voxels. The third row in figure A and B represents between-group (C>P, Controls greater than Patients; P>C, Patients greater than Controls) statistical differences in functional connectivity for right and left amygdalar networks. All depicted clusters survive a minimum of 200 voxels at $p<0.01$ uncorrected. Images are displayed in the neurological convention (R=Right).

Figure 3. Right (A) and left (B) insular resting-state functional connectivity networks for Control subjects (C) and MDD patients (P). All clusters depicted in the within-group patterns survive $p<0.05$ FDR corrected and a minimum extension of 200 voxels. The third row in figure A represents between-group (C>P, Controls greater than Patients) statistical differences in functional connectivity for the right insular network (depicted clusters survive 200 voxels at $p<0.01$ uncorrected). Images are displayed in the neurological convention (R=Right).

Figure 4. Dorsal (A) and ventral (B) MFC resting-state functional connectivity networks for Control subjects (C) and MDD patients (P). All clusters depicted in the within-group patterns survive $p<0.05$ FDR corrected and a minimum extension of 200 voxels. The third row in figure A and B represents between-group (C>P, Controls greater than Patients) statistical differences in functional connectivity for the dorsal and ventral medial frontal networks (depicted clusters survive 200 voxels at $p<0.01$ uncorrected). Images are displayed in the neurological convention (R=Right).

Figure 5. Posterior-medial OFC (A) and visuo-cerebellar (B) resting-state functional connectivity networks for Control subjects (C) and MDD patients (P). All clusters depicted in the within-group patterns survive $p<0.05$ FDR corrected and a minimum extension of 200 voxels. The third and fourth row in figure A represent between-group (C>P, Controls greater than Patients; P>C, Patients greater than Controls) statistical differences in functional connectivity for the posterior-medial OFC network (depicted clusters survive 200 voxels at $p<0.01$ uncorrected). Images are displayed in the neurological convention (R=Right).

Figure 6. Maps showing significant correlations between MDD severity scores (HAM-D 17) and functional connectivity within networks showing alteration in MDD patients. (A) Maps showing regions in which greater functional connectivity disruption was associated with greater clinical severity of MDD. (B) Maps showing regions in which greater strength of functional connectivity was associated with greater clinical severity in MDD patients. C) Brain sites where seed regions of interest were placed (sites of maximal between-group anatomical differences, i.e., volumetric reductions in MDD). All depicted clusters survive 200 voxels at $p<0.01$ uncorrected. Images are displayed in the neurological convention (R=Right).

Table 1. Main characteristics of the study sample

	MDD Patients	*Controls*	T/χ^2	*p*
Age at inclusion (mean ± SD years)	44.96 ± 11.47	45.04 ± 10.06	0.25/	0.98
Gender (Females/ Males)	22 / 5	21 / 6	/0.11	0.74
Handedness (Right handed/ Left handed)	26/ 1	26/ 1	/0	1
Years of education (mean ± SD years)	11.93 ± 3.27	13.04 ± 2.90	1.32/	0.19
Age at onset (range, mean ± SD)	19-54, 35.33 ± 10.45	NA	NA	NA
Nº of previous episodes (range, mean ± SD)	0-7, 2.04 ± 1.99	NA	NA	NA
Current episode duration (range, mean ± SD days)	110-1095, 422 ± 307	NA	NA	NA
AD treatment before washout (% Yes / % No)	74% / 26%	NA	NA	NA
AD type before washout (Type, Nº of patients)	SSRI, 16 SNRI, 2 TCA, 2	NA	NA	NA
HAM-D 17*	21.74 ± 2.19	0.07 ± 0.27	50.9/	<0.0001

AD, antidepressant; HAM-D 17, Hamilton Depression Rating Scale, 17-item version; SSRI, Selective Serotonin Reuptake Inhibitor; SNRI, Serotonine-Norepinephrine Reuptake Inhibitor; TCA, Triciclic Antidepressant.

Table 2. Between-group differences in resting-state functional connectivity

Controls > MDD Patients	***X : Y : Z***	***k***	***t***	$P_{Unc.}$
Right Amygdala Network				
Amyg./Hippocamp./Parahip.	-24: -14: -18	264	3.61	<0.0005
	20: -12. -22	283	4.29	<0.0005
Basal Ganglia	30: -8: 0	741	4.71	<0.0005
	-32: -12: -6	222	3.80	<0.0005
SI/Post. Insula/Pariet. Operculum	36: -14: 20	856	4.69	<0.0005
Left Amygdala Network				
Post. Insula/Operculum	42: -22: 22	478	4.29	<0.0005
Right Insula Network				
Insula/Frontal Operculum	50: 16: 6	318	3.58	<0.0005
	-36: 18: -4	200	3.56	<0.0005
Right lateral frontal cortex	54: 2: 22	322	3.83	<0.0005
Parietal Operculum	56: -36: 38	502	5.04	<0.0005
	-60: -24: 20	847	4.42	<0.0005
Temporal Operculum	-40: -20: -2	227	3.66	<0.0005
Dorsal MFC Network				
ACC/pre-SMA/MFC	6: 24: 44	1594	6.27	<0.0005
Ventral MFC Network				
Rostral Medial Frontal Cortex	0: 66: 16	582	3.66	<0.0005
Posterior Cingulate Cortex	-4: -32: 28	337	3.75	<0.0005
Angular Gyri	52: -60: 16	330	4.03	<0.0005
	-48: -64: 18	394	3.19	<0.0005
Posterior-medial OFC Network				
Orbitofrontal Cortex	8: 17: -22	805	4.24	<0.0005
MDD Patients > Controls	***X : Y : Z***	***k***	***t***	$P_{Unc.}$
Right Amygdala Network				
Subgenual-Pregenual ACC	5: 26: -4	365	4.64	<0.0005
Posterior-medial OFC Network				
Subgenual ACC-Hypothalamus	6: 8: -16	491	6.05	<0.0005
Precuneus	-2: -52: 28	346	3.70	<0.0005

Coordinates (X : Y : Z) are given in Montreal Neurological Institute (MNI) Atlas space. *k*, cluster size; Unc., uncorrected; Amyg., amygdala; Hippocamp., hippocampus; Parahip., parahippocampal gyrus. SI, primary somatosensory; Post., posterior; Pariet., parietal; ACC, anterior cingulate cortex; SMA, supplementary motor area; MFC, medial frontal cortex; OFC, orbitofrontal cortex.

Figure 1

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Figure 2

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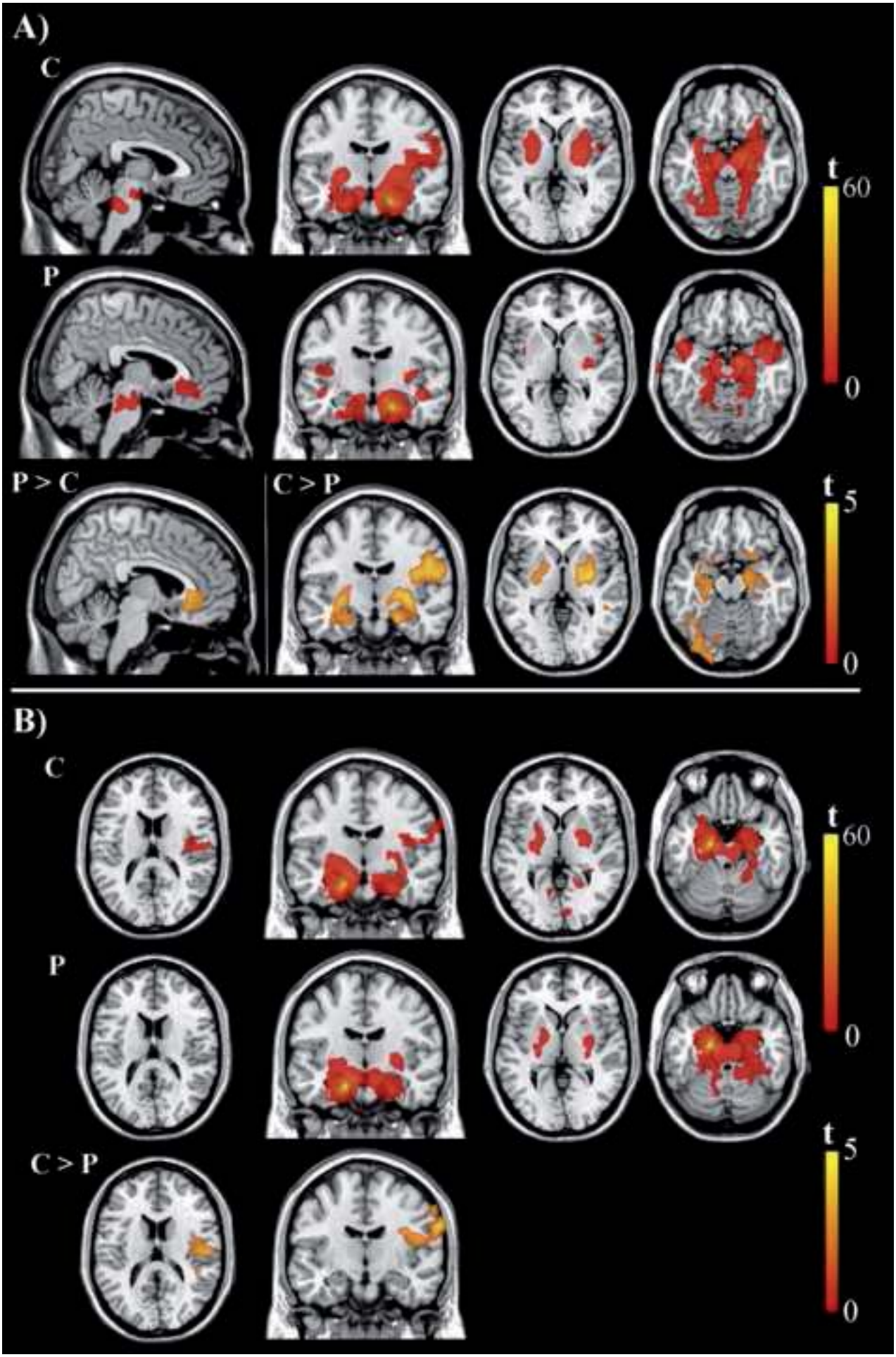

Figure 3

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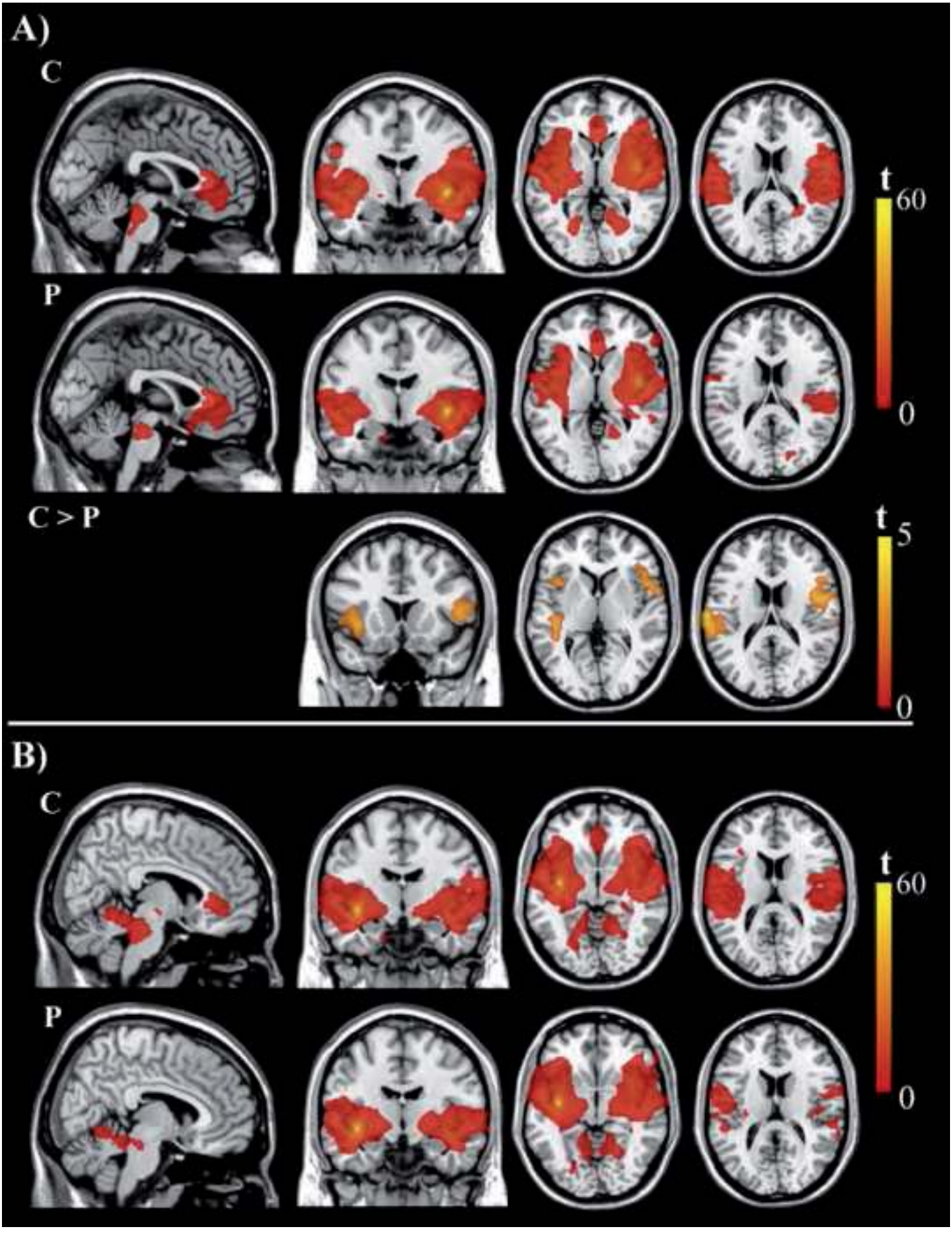

Figure 4

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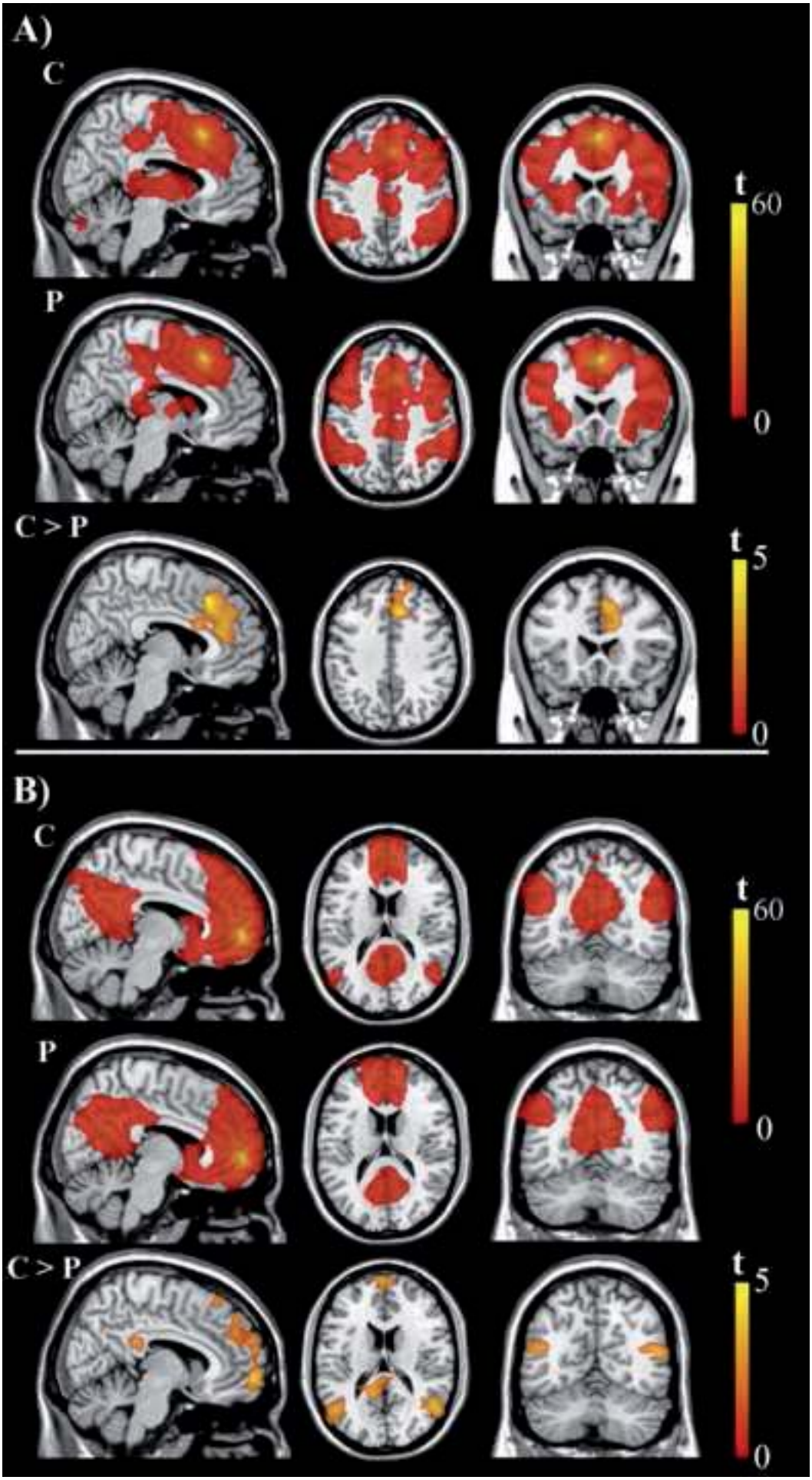

Figure 5

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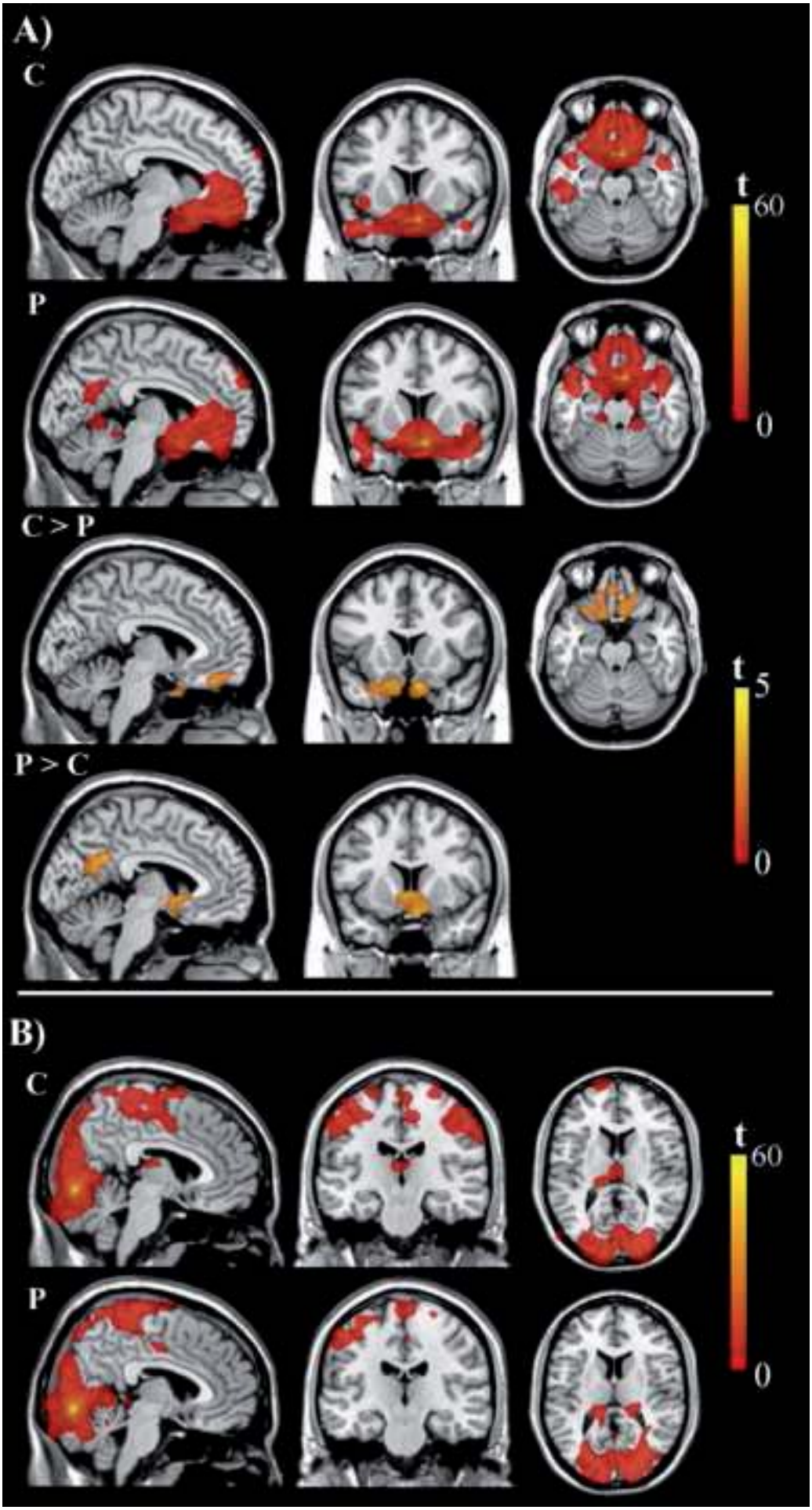

Figure 6
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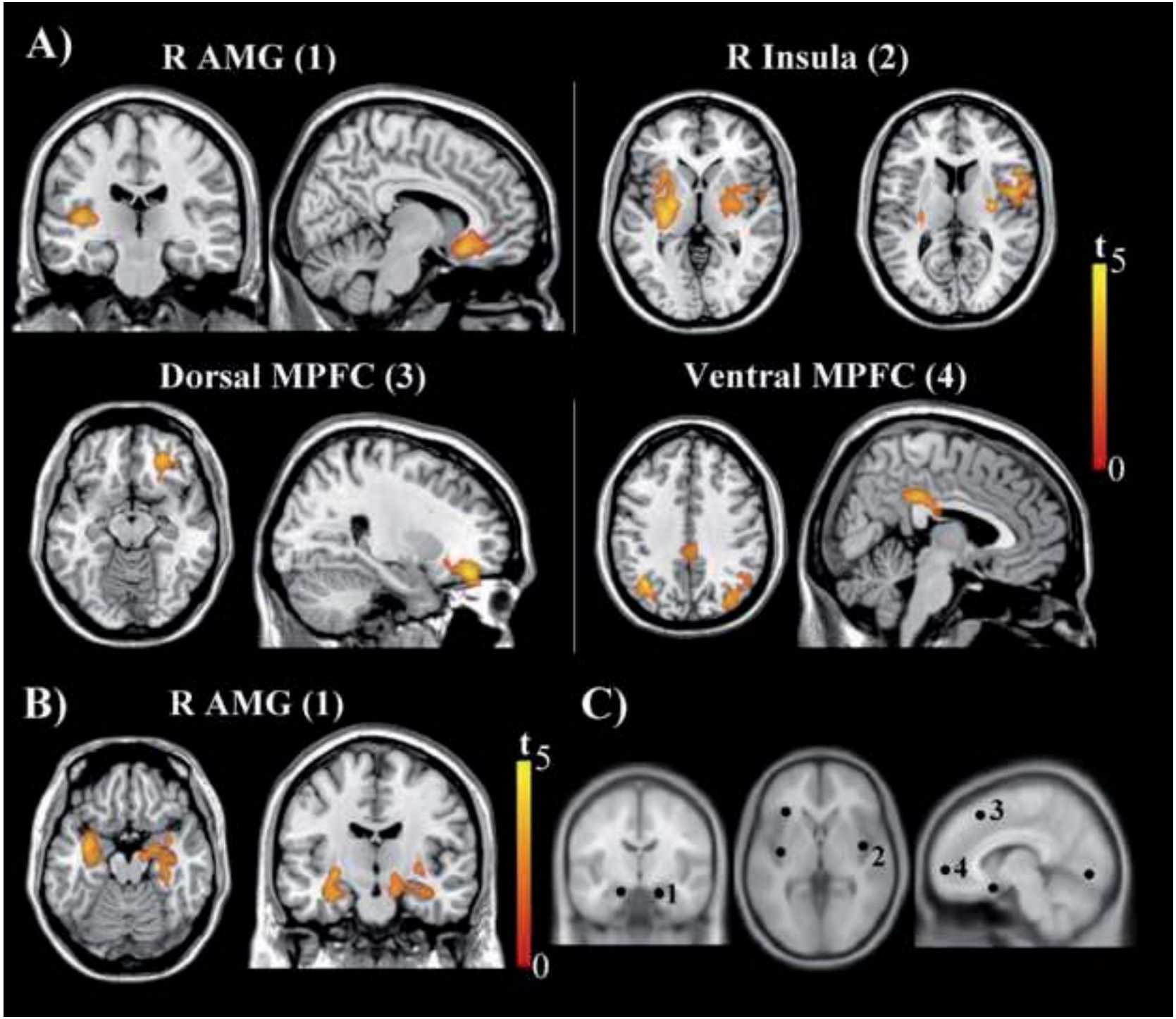

Supplementary Information

Supplementary Table1. Between-group differences in Brain Anatomy (Controls > MDD Patients)

Region	*X : Y : Z*	*k*	*t*	$P_{unc.}$
R Amygdala/Hippocampus/Parahippocampus	18 : -12 : -24	784	3.23	0.001
L Amygdala/Hippocampus/Parahippocampus	-18 : -14 : -22	778	3.35	0.001
R Insula	42 : -4 : 2	827	3.58	<0.0005
L Insula	-38 : -10 : -4	1548	3.64	<0.0005
Dorsal MFC	6 : 22 : 48	2252	3.44	0.001
Ventral MFC	8 : 58 : -2	1349	3.11	0.002
Posterior-medial OFC	6 : 10 : -20	245	2.92	0.002
Visual cortex/Cerebellum	8 : -82 : -6	5976	3.84	<0.0005

Coordinates (X : Y : Z) are given in Montreal Neurological Institute (MNI) Atlas space. *k*, cluster size; *Unc.*, uncorrected; R, right; L, left.

Supplementary Table 2. Within-group resting-state functional connectivity patterns for the Amygdalar (R and L) and Insular (R and L) Networks

	X : Y : Z	*k*	*t*	P_{FDR}
R Amygdala Network	**(Controls; Patients)**	**(C; P)**	**(C; P)**	**(C; P)**
Amygdala/Hippocamp./Parahip.	14: -14: -20; -16: -8: -26	1747; 1030	7.70; 8.42	*; *
	18: -12: -24; 18: -12: -24	2445; 2399	Inf.	*; *
Basal Ganglia	30: -8: 0; ---	1153, ---	7.97, ---	*; ---
	-22: -2: 0; ---	771;---	5.00; ---	*; ---
Insula/Operculum	36: -14: 20; 44: -4: 8	1112; 210	5.94; 4.02	*; .007
	-32: 26: 8; -40: -2: 8	237; 341	3.85; 4.53	.007;.002
Visual Cort./Cerebellum	24: -40: -22; 24: -40: -22	1367; 802	5.54; 6.23	*, *
	-36: -60: -12; -20:-42:-16	1565; 508	5.41; 4.65	*; .001
Pons/ Mesencephalum	0: -28: -28; 2:-28: -26	1985; 1982	4.32; 3.40	.002; .028
Subgenual-pregenual ACC	---; 0:40:-12	---; 343	---; 3.29	---; .030
L Amygdala Network	**(Controls; Patients)**	**(C; P)**	**(C; P)**	**(C; P)**
Amygdala/Hippocamp./Parahip.	20:-12:-20; 16:-14:-20	1778; 2002	6.26; 8.26	*;*
	-18:-14:-22; -18:-14:-22;	2010; 2029	Inf.	*;*
Basal Ganglia	30: -40: 2; 30: -10: 0	432; 237	4.23; 4.46	.005;.002
	-30: -14: -2; -32: 2: -8	407; 501	4.65; 5.37	.001;*
Insula/Operculum	54: -16: 16; ---	297; ---	3.90; ---	.012; ---
Visual Cort./Cerebellum	20: -48: -16; 16: -44: -24	610; 477	4.58; 4.59	.002;.001
	-18: -50: -14; -20:-24:-28	246; 306	3.53; 4.02	.020;.004
Pons	6: -26: -24; 8: -22: -42	750; 1200	4.42; 3.48	.002;.025
R Insula Network	**(Controls; Patients)**	**(C; P)**	**(C; P)**	**(C; P)**
Insula/Operculum/Basal Ganglia	42: -4: -2; 42: -4: -2	10017; 7732	Inf.	*;*
	-44: -6. -4, -46. -6. -4	8441; 5941	10.02; 13.82	*,*
Visual Cort./Parahip./Cerebellum	8:-46:-10; 14:-44:-14	1456; 1211	6.38; 8.42	*;*
	-8: -38: -12; -10: -38: -10	1000; 638	6.02; 5.22	*;*
Subgenual-pregenual ACC	4: 40: 0; 0: 42 :-4	873; 1056	5.28; 4.94	*;*
Pons/Mesencephalum	4: -28: -22; 0: -26: -22	473; 290	7.95; 6.23	*;*
L Insula Network	**(Controls; Patients)**	**(C; P)**	**(C; P)**	**(C; P)**
Insula/Operculum/Basal Ganglia	36: 0: -8; 40: -8: -10	8570; 7690	10.86; 11.49	*;*
	-38: -10. -4; -38: -10: -4	9003; 7826	Inf.	*;*
Visual Cort./Parahip./Cerebellum	12: -52: -8; 16: -44: -14	1430; 1095	5.95; 5.91	*;*
	-10: -40:-14; -10:-54: -10	1030; 1103	4.66; 5.53	*;*
Subgenual-pregenual ACC	2: 40: -2; 0: 22: -4	681; 240	4.41; 4.80	0.001; *
Pons/Mesencephalum	4: -26: -22; -2: -26: -22	647; 212	7.73; 4.92	*;*

* P_{FDR}<0.0005; Coordinates (X : Y : Z) are given in Montreal Neurological Institute (MNI) Atlas space. R, right; L, left; *k*, cluster size; *FDR*, false discovery rate whole-brain corrected; C, Control subjects; P, MDD patients; Hippocamp., hippocampus; Parahip., parahippocampal gyrus; Cort., Cortex; ACC, anterior cingulate cortex. Inf., infinit z score (t > 60), seed location.

Supplementary Table 3. Within-group resting-state functional connectivity patterns for the Dorsal and Ventral MFC, Posterior-medial OFC and Visuo-cerebellar Networks

	X : Y : Z	*k*	*t*	P_{FDR}
Dorsal MFC	**(Controls; Patients)**	**(C; P)**	**(C; P)**	**(C; P)**
R Lateral Frontal Cortex/Ant. Insula	50: 14: 36; 50:14: 38	13669; 13548	10.82; 9.07	*;*
	-38: 2: 48; -36: 48: 8	10247; 10427	8.18; 7.82	*;*
Parietal cortex	56: -48: 42; 40: -46: 44	3691; 3904	7.91; 7.57	*;*
	-56:-50:40; -60: -50: 40	2916; 3231	7.78; 7.10	*;*
ACC/SMA/Pre-SMA/Ant. PCC	6: 22: 48; 6: 22: 48	8820; 8985	Inf.	*;*
Basal Ganglia	12: 10: 6; 14: 6: 10	1649; 1127	7.71; 5.75	*;*
	-10: 10: 2; -10:12:4	1245; 964	7.64; 5.42	*;*
Thalamus	10: -8: 4; 12: 0: 8	1360; 604	6.16; 4.84	*;*
Cerebellum	-32: -74: -32; ---	1435; ---	5.32; ---	*; ---
Ventral MFC	**(Controls; Patients)**	**(C; P)**	**(C; P)**	**(C; P)**
Sup. Frontal G./MFC/ACC	8: 58: -2; 8: 56: -2	17906; 17032	Inf.	*;*
PCC/Precuneus	-2: -64: 24; -2:-64: 22	7984; 7118	11.20; 10.89	*;*
Angular Gyri & extended parietal	50: -72: 32; 50: -74: 36	1856; 1983	7.86; 8.04	*;*
	-46:-74:38; -42: -74: 46	1929; 1546	9.30; 8.44	*;*
Caudate	14: 24: 10; 8: 16: 2	341; 408	5.47; 6.51	*;*
	-12: 24: 4; -12: 16: -4	341; 312	5.47; 5.35	*;*
Posterior-medial OFC	**(Controls; Patients)**	**(C; P)**	**(C; P)**	**(C; P)**
Subg.-preg. ACC/ventral striatum/ MFC/med. & lat. OFC	6: 10: -20; 6: 10: -20	7175; 6709	Inf.	*;*
Insula/Temporal cortex	44: -6: -10; 20: 10: -20	1048; 1765	5.12; 7.28	*;*
	-48: -16: -8; -44: 0: -16	2860; 1856	5.51; 6.88	*;*
Precuneus/Visual	---; 18: -34: -16	---; 635	---; 6.18	*;*
Cort./Parahip./Cerebellum	-16:10:-22; -12: -54: -4	658; 624	7.25; 4.98	*;*
Visuo-cerebellar	**(Controls; Patients)**	**(C; P)**	**(C; P)**	**(C; P)**
Precentral-Postcentral G./Parietal	46: -24: 56; ---	1507; ---	5.59; ---	*;*
Sup.	-40:-14:56; -30: -40: 66	2316; 775	5.21; 4.73	*;*
Paracentral Lobule	-4: -36: 62; ---	1694; ---	5.81; ---	*;*
Visual Cort./ Cerebellum	0: -82: -6; 0: -82: -6	21974; 22062	Inf.	*;*
Thalamus	-20:-30:10; 18:-32:10	394; 221	3.37; 4.50	.009; *

* P_{FDR}<0.0005; Coordinates (X : Y : Z) are given in Montreal Neurological Institute (MNI) Atlas space. *k*, cluster size; *FDR*, false discovery rate whole-brain corrected; C, Control subjects; P, MDD patients; ACC, anterior cingulate cortex; SMA, supplementary motor area; Ant., anterior; PCC, posterior cingulated cortex; Sup., superior; G., gyrus; *MFC, medial frontal cortex; OFC, orbitofrontal cortex;* Subg., subgenual; preg., pregenual; med., medial; lat., lateral; Parahip., parahippocamal gyrus; Cort., cortex; Inf., infinit z score (t > 60), seed location.

Supplementary Table 4. Correlations between MDD severity scores (HAM-D 17) and functional connectivity measurements within altered networks in patients

Less connectivity, greater severity	*X : Y : Z*	*k*	*t*	$P_{Unc.}$
R Amygdala Network				
L Insula	-40: -26: 6	228	4.15	*
Subgenual-pregenual ACC	4: 22: -18	218	3.89	*
R Insula Network				
Basal Ganglia	30: -12: 10	294	4.27	*
	-28: -18: 0	912	4.30	*
Insula/Frontal Operculum	48: 0: 10	417	4.41	*
Dorsal MFC Network				
Ventrolateral OFC	28: 40: -18	293	5.26	*
Ventral MFC Network				
PCC	6: -32: 32	311	4.39	*
Angular gyrus	30: -76: 46	415	3.65	0.001
	-36: -58: 36	307	6.35	*
Greater connectivity, greater severity	*X : Y : Z*	*k*	*t*	$P_{Unc.}$
R Amygdala Network				
Amygdala/Hippocamp./Parahippocamp.	34: -28: -22	337	3.64	0.001
	-34: -2: -18	280	3.79	*

* $P_{Unc.}$<0.0005; Coordinates (X : Y : Z) are given in Montreal Neurological Institute (MNI) Atlas space. *k*, cluster size; *Unc*., uncorrected; R, right; L, left; ACC, anterior cingulate cortex; MFC, medial frontal cortex; OFC, orbitofrontal cortex; PCC, posterior cingulate cortex; Hippocamp., hippocampus; Parahippocamp., parahippocampal gyrus.

3.4. Study 4. Published in the international journal Neuropsychopharmacology

Effects of duloxetine treatment on brain response to painful stimulation in major depressive disorder

Duloxetine effects on brain response to pain in MDD

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Abstract

Major depressive disorder (MDD) is characterized by a constellation of affective, cognitive and somatic symptoms associated with functional abnormalities in relevant brain systems. Painful stimuli are primarily stressful and can trigger consistent responses in brain regions highly overlapping with the regions altered in MDD patients. Duloxetine has proven to be effective in treating both core emotional symptoms and somatic complaints in depression. This study aimed to assess the effects of duloxetine treatment on brain response to painful stimulation in MDD patients. A total of 13 patients and a reference group of 20 healthy subjects were assessed on three occasions (baseline, treatment week 1 and week 8) with fMRI during local application of painful heat stimulation. Treatment with duloxetine was associated with a significant reduction in brain responses to painful stimulation in MDD patients in regions generally showing abnormally enhanced activation at baseline. Clinical improvement was associated with pain-related activation reductions in the pregenual anterior cingulate cortex, right prefrontal cortex and pons. Pontine changes were specifically related to clinical remission. Increased baseline activations in the right prefrontal cortex and reduced deactivations in the subgenual anterior cingulate cortex predicted treatment responders at week eight. This is the first fMRI study addressed to assess the effect of duloxetine in MDD. As a novel approach, the application of painful stimulation as a basic neural stressor proved to be effective in mapping brain response changes associated with antidepressant treatment and brain correlates of symptom improvement in regions of special relevance to MDD pathophysiology.

Key words: Major depressive disorder, fMRI, pain, brain, treatment, duloxetine, antidepressant.

Introduction

Major depression is a frequent and disabling psychiatric disorder (World Health Organization, 2001) characterized by a constellation of mood, cognitive, psychomotor, and somatic symptoms (American Psychiatric Association, 2000). Neuroimaging has contributed to current understanding of MDD pathophysiology suggesting a global misbalance in the neural systems that serve such major aspects of brain function (Drevets et al, 2000; Mayberg, 2003; Sheline, 2003).

Painful stimuli are primarily salient and threatening, and normally provoke rapid withdrawal and stress-related responses (Price, 2000). Functional magnetic resonance imaging (fMRI) has confirmed the involvement of a widespread brain network in processing noxious stimulation including the somatosensory system, limbic and paralimbic regions related to the affective experience of pain and prefrontal regulatory areas modulating the entire brain response (Apkarian et al, 2005; López-Solà et al, 2010; Wiech et al, 2008a). As painful stimulation typically evokes an integrated response involving brain regions that are relevant to MDD pathophysiology (Drevets et al, 2000; Mayberg, 2003; Sheline, 2003), functional neuroimaging using pain paradigms may provide the opportunity to challenge such regions, both for the purpose of investigating MDD abnormalities in response to a basic neural stressor (Bär et al, 2007; Strigo et al, 2008), and also, potentially, for providing objective biological markers of the effects of antidepressant treatment.

The interaction between major depression and pain symptoms appears to be a growing focus of interest in MDD research. Clinical pain complaints have significantly higher mean prevalence in MDD patients (65 % as reviewed by Bair et al, 2003) compared with the general population (24-37%, Regier et al, 1984), and furthermore, the presence of pain complaints in

MDD patients is associated with greater MDD severity and refractoriness (Bair et al, 2003). Additionally, mood symptoms and somatic pain in depression have been shown to improve simultaneously (Blier and Abbott, 2001). Recent evidence suggests that dual serotonine and norepinephrin reuptake inhibitors (such as duloxetine, Trivedi et al, 2008) are effective antidepressants relieving both mood symptoms and somatic complaints in MDD (Gupta et al, 2007). Specifically for the case of duloxetine, several studies have suggested that early symptomatic improvement may already be noticeable after 1-2 weeks of treatment (Hirschfeld et al, 2005; Nemeroff et al, 2002; Shelton et al, 2007). In contrast to the described close clinical association between major depression and pain complaints, it has been suggested that depressed patients are less sensitive to experimentally induced pain on the skin (Bar et al, 2005), although the neural basis for this paradox is still rather unknown (Bar et al, 2007). Additionally, the effect of antidepressant treatment on experimental thermal pain perception and the associated brain responses has not been explored to date.

Neuroimaging techniques have been successfully used to assess the effects of various drugs on baseline brain metabolism and on the cerebral response to specific cognitive and emotional stimuli (Drevets et al, 2008a; Mayberg et al, 2003; Rigucci et al, 2009). Nevertheless, despite its potential interest, it is noteworthy that imaging paradigms based on painful stimulation have not been previously used to investigate the effects of antidepressant agents.

This fMRI study aimed to assess the effects of duloxetine treatment on brain response to heat painful stimulation in MDD patients. The study design included an fMRI assessment at baseline -pretreatment- and assessments following one week and eight weeks of treatment. Brain activations in MDD patients were compared to the activations obtained in a reference group of healthy subjects who were also assessed three times to control for task-repetition

effects. Brain correlates of clinical improvement were investigated for both core and somatic depression symptoms. A specific analysis was also conducted to identify baseline brain imaging predictors of clinical response to treatment. Finally, correlations between experimental pain ratings and fMRI treatment effects on brain responses to pain were also investigated.

Materials and Methods

Subjects

Fifteen patients were consecutively recruited from the Mood Disorders Unit of the University Hospital of Bellvitge. All patients met DSM-IV criteria for MDD with no psychotic features. MDD patients were assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders-Clinician Version (SCID, First et al, 1997) that was conducted by two senior psychiatrists who reached a consensus for all items and also for the compliance of inclusion/exclusion criteria. At inclusion, all patients had a Hamilton Depression Scale (HAM-D 17, Hamilton, 1960; 1967) score equal to or greater than 18. Exclusion criteria included the presence or past history of other Axis I diagnoses and relevant medical or neurological disorders including chronic pain syndromes, and abnormal clinical MRI upon radiological inspection. From the original 15-subject sample, one patient was excluded as she was unable to complete the first fMRI session and another patient voluntarily left the study after the first MRI assessment. The remaining 13 patients underwent both basal and 8-week re-assessment and made up our final study patient sample. One of these 13 patients, however, was unable to complete the week 1 fMRI session as she felt temporarily sick on the assessment day. Table 1 shows the clinical characteristics of the final 13-patient sample.

A comparison group of 21 healthy volunteers also participated in the study. A SCID was carried out in order to discard the presence of Axis I disorders, and also a complete medical interview was performed to exclude subjects with relevant medical or neurological disorders, history of substance abuse and psychiatric illness, and chronic pain syndromes. From the original sample, one subject was excluded due to excessive movement inside the scanner (translation movement in the z-axis > 3 mm) during the baseline fMRI assessment. A total of 20 subjects made up the control sample, although technical issues with the thermal stimulator

compromised the use of one subject's fMRI data from the week 1 assessment. Age, gender, handedness and years of education are reported in Table 1. MDD patients and control subjects did not differ on these variables. All patients and control subjects gave written informed consent to participate in the study, which was approved by the Research and Ethics Committee of the University Hospital of Bellvitge.

Study design

For all patients, the study included an antidepressant medication wash-out of 15 days before treatment onset. Patients received antidepressant treatment with oral duloxetine, a serotonin-norepinephrine reuptake inhibitor, starting at 60 mg/day in a single dosage for 4 full weeks. After week 4, dose increases (up to 120 mg/day) were prescribed on the basis of patients' response when both senior psychiatrists coincided in their clinical judgment. All patients underwent weekly clinical assessments throughout the study period. The following clinical scales were used to assess mood, somatic and general treatment-related response: HAMD-17 (Hamilton, 1960; 1967), Brief Pain Inventory (Cleeland and Ryan, 1994), Symptom Questionnaire- Somatic Subscale (Kellner, 1987) and the Clinical Global Impression of Severity (Guy et al, 1976).

The study consisted of three fMRI assessments, which were carried out at week 0 (before treatment), and following 1 and 8 weeks of treatment. Control subjects also underwent fMRI assessments at baseline, week 1 and week 8, which served to control for task repetition effects on brain responses to painful stimulation.

Stimulus

The Contact Heat-Evoked Potential Stimulator (CHEPS) system was used, which has been designed to provide controlled thermal stimuli (CHEPS, Medoc Ltd., Advanced Medical Systems, Israel). This system is able to provoke pain by direct stimulation of A delta and C nociceptive fibers on a relatively large skin area (via the 27 mm-diameter thermode) through very rapid local heating (70ºC/s rate). In our experiment, painful heat stimulation was applied to the right volar forearm in 10-second blocks each including eleven 50ºC spikes (full-width at half-maximum duration of each spike: 125 ms), starting from a baseline temperature of 32ºC.

In a preliminary session, each subject was trained to rate their perceived pain intensity using a numerical rating scale ranging from 0 (“no pain”) to 100 (“the worst pain”), and perceived unpleasantness using a 9-point verbal descriptor scale ranging from “not at all unpleasant” to “extremely unpleasant” when receiving two full (11 spikes) stimulation blocks.

fMRI pain paradigm

A block design was used consisting of three conditions per stimulation cycle repeated 12 times during a 7-minute run: a rest condition with pseudorandom variable duration (duration range: 12 to 26 s), a 6-second anticipatory condition that began with a brief auditory stimulus (600-ms tone) cuing the subsequent pain condition, and the actual 10-second painful condition (involving the application of the 50ºC spike stimuli). Immediately after the entire fMRI sequence was completed, each subject rated the overall pain intensity and unpleasantness experienced during the 12 painful stimulation cycles.

MRI acquisition

A 1.5 Tesla Signa system (General Electric, Milwaukee, WI) equipped with an eight-channel phased-array head coil and single-shot echoplanar imaging (EPI) software was used. Functional sequences consisted of gradient recalled acquisitions in the steady-state (time of repetition [TR], 2,000 ms; time of echo [TE], 50 ms; pulse angle, 90º) within a field of view of 24 cm, a 64 x 64 pixel matrix, and a slice thickness of 4 mm (inter-slice gap, 1.5 mm). Twenty-two slices parallel to the anterior-posterior commissure line covered the whole-brain. The sequence included 4 additional dummy volumes to allow the magnetization to reach equilibrium.

Image preprocessing

Imaging data were processed using MATLAB version 7 (The MathWorks Inc, Natick, Mass) and Statistical Parametric Mapping software (SPM5; The Welcome Department of Imaging Neuroscience, London). Image preprocessing involved motion correction, spatial normalization and smoothing using a Gaussian filter (full-width at half-maximum, 8 mm). Data were normalized to the standard SPM-EPI template and resliced into 2 mm isotropic voxels in Montreal Neurological Institute (MNI) space.

Statistical analyses

Brain responses to painful stimulation and group comparisons. Our analyses aimed to identify (i) between-group differences in brain activation at baseline (pretreatment); (ii) treatment effects at week 1, (iii) treatment effects at week 8, and (iv) the pattern of correlations between clinical measurements (and experimental pain ratings) and fMRI treatment effects.

Single-subject 1st level analyses were implemented in SPM5 to model fMRI time-series using four box-car regressors; two representing anticipation and painful stimulation periods respectively, and two representing the rest period divided into two parts: a 6-second post-stimulation period and a variable (6-20 s) remaining rest period, considering a hemodynamic delay of 4 seconds. We explicitly modeled the post-stimulation period (6-second interval after each stimulation block), as brain activation may persist during this period (López-Solà et al, 2010; Moulton et al, 2005; Pujol et al, 2009) and may therefore alter the reference baseline with non-controlled remaining activation. A contrast image showing fMRI signal differences between the painful stimulation condition and the second part of the rest period (modeled by the corresponding box-car regressors in SPM5) was calculated for each subject.

The contrast images were then carried forward to subsequent 2nd-level random-effects (group) analyses. One-sample t-statistic maps were calculated to obtain baseline (pre-treatment) activation (and deactivation) patterns for each group, and a two-sample t-test was performed to map between-group baseline brain activation (and deactivation) differences. In order to assess treatment effects on brain response to pain, we performed two separate (week 1 and week 8) 2nd-level mixed ANOVA analyses including the within-subject factor 'time moment' (baseline versus reassessment), and the inter-subject factor 'group' (patient versus control) as independent variables. Group-by-time interaction t-statistic maps were then calculated to identify activation changes that were greater in MDD patients than in healthy control subjects.

For the sake of simplicity we do not report treatment effects on the anticipatory period (before actual painful stimulation onset) as the complete pattern followed the same direction as treatment effects occurring in response to actual painful stimulation, thus adding only marginal information considering the purposes of the study and space limitation.

Correlation analyses between clinical and fMRI treatment effects. Specific correlation analyses were performed in SPM5 to test for linear relationships between clinical improvement in the two symptomatic dimensions of interest, i.e. core emotional and somatic symptoms (measured as reductions in Core [Items 1, 2, 3, 7, 8] and Somatization Subscales [10, 11, 12, 13, 15, 17] of the HAM-D 17) and fMRI brain activation changes at week 1 and week 8 in relation to baseline. These exploratory correlation analyses were carried out for regions showing significant treatment effects at week 1 and week 8 (regions showing significant interaction results from the mixed ANOVA models [reported in table 3]).

An exploratory two-sample t-test analysis was additionally performed to assess treatment-related fMRI activation changes associated with remission (binary factor defined by HAM-D 17 scores below 7 or equal/greater than 7), by comparing the 'baseline *minus* week 8 activation' contrast images between remitting and non-remitting patients (for the regions showing significant treatment effects after 8 weeks).

Imaging predictors of clinical response. To specifically test whether baseline (pretreatment) regional activations were able to predict positive clinical responders to duloxetine (measured as 50% reductions in the HAM-D 17 total score from baseline to week 8), an exploratory two-sample t-test analysis was performed to compare the patterns of baseline activation of clinical responders and non-responders (also for the regions showing significant treatment effects after 8 weeks).

Correlation analyses between pain ratings and fMRI treatment effects. To investigate the relationship between experimental pain perception and brain activation in regions showing significant treatment effects in MDD, additional correlation analyses were

conducted in SPM5. For both intensity and unpleasantness ratings, two models were estimated in MDD patients in order to: (i) correlate changes in subjective pain ratings with changes in fMRI responses from baseline to week 8 and (ii) correlate subjective pain scores and fMRI brain response measurements at week 8. The analyses were carried out for regions showing significant treatment effects at week 8 (regions showing significant interaction results from the mixed ANOVA model [reported in table 3]).

Thresholding criteria. Baseline group activation (and deactivation) results were thresholded at $P_{\text{False Discovery Rate-FDR}} < 0.05$ whole-brain corrected. Between-group differences, interaction effects and correlation analyses were considered significant when involving a minimum cluster extension of 200 voxels (1,600 mm^3) at $p<0.05$ uncorrected. The use of a combined p value/cluster extension thresholding approach has been suggested to provide a more desirable balance between Type I and Type II error rates (Lieberman and Cunningham, 2009). For the brainstem (as it is a small structure), a more lenient extension threshold (cluster >100 voxels) was used.

Results

Clinical response to treatment

Supplementary Table S1 shows patients' response to treatment measured as changes in the selected clinical scales. All in all, a modest, though significant, improvement was observed after one week of treatment in most scales. Differences were robust after eight weeks of treatment in all measurements. At the end of the 8-week treatment period, a total of nine out of thirteen patients met the criterion for clinical response to treatment and six of them met the criterion for clinical remission.

Subjective pain scores during fMRI

MDD patients and control subjects did not differ as to the reported amount of subjective pain intensity (mean ± SD for patients: 5.8 ± 2.4 and control subjects: 6.7 ± 1.8; $t = 1.27$, $p = 0.21$) and unpleasantness (patients, 3.7 ± 1.7; control subjects, 4.6 ± 1.5; $t = 1.6$, $p = 0.13$) during baseline fMRI assessment, although patients showed the lowest values for both measurements. We found no significant group-by-time interaction effect when comparing baseline and week 1 subjective pain scores (intensity $F = 0.72$, $p = 0.40$; unpleasantness $F = 0.04$, $p = 0.85$). Nevertheless, significant group-by-time interactions for pain intensity ($F = 5.21$, $p = 0.03$) and unpleasantness ($F = 7.64$, $p = 0.01$) were found when comparing baseline and week 8 measures. Post-hoc comparisons indicated that although control subjects did not show significant changes from baseline to week 8 re-assessment (intensity: $t = -1.24$, $p = 0.23$; unpleasantness: $t= -1.68$, $p = 0.11$), patients had a tendency to report increased pain intensity ($t = 1.86$, $p = 0.088$) and showed significantly higher unpleasantness scores ($t=2.38$, $p=0.035$) after this period. Interestingly, we found a significant correlation between the reduction in core

emotional MDD symptoms after treatment (baseline – week 8) and the enhancement of pain unpleasantness ratings ($r = -0.59$, $p = 0.03$), and a weaker association for perceived intensity ($r = -0.44$, $p = 0.13$).

Brain response to painful heat stimulation: Baseline assessment

Baseline brain responses to painful heat stimulation are reported in Table 2 and Figure 1. For both study groups, brain response was characterized by significant activation in bilateral insulae extending to basal ganglia, parietal and frontal opercula, ACC-supplementary motor area (SMA), prefrontal cortex and cerebellum. The patient group additionally showed significant activation in the middle temporal gyrus (Brodmann area [BA] 22) and in the hypothalamic-midbrain region. Significant reductions in fMRI signal during painful stimulation compared with rest (deactivations) were only identified in the control subject group and involved the subgenual-pregenual ACC and extended medial prefrontal cortex.

Compared to control subjects, the MDD group showed greater baseline activation in bilateral insulae, frontal and temporal opercula, ventral basal ganglia, hypothalamic region, medial prefrontal cortex, left hippocampus and middle temporal gyrus (Table 2 and Figure 2A). Patients also showed significantly reduced deactivation (i.e., abnormal persistence of activity during stimulation) in a large area involving the subgenual and pregenual ACC and extended medial prefrontal regions (Table 2 and Figure 2B). We found no regions of significantly greater activation in the control group when compared to MDD patients.

Treatment effects on brain response to painful stimulation

Significant group-by-time interactions were observed from baseline to week 1 fMRI assessments revealing a general effect of brain activation reductions in MDD patients and the opposite tendency in control subjects (Table 3, Table S2, Figure 3 and Figure S1). Regions showing a significant interaction effect largely coincided with regions showing enhanced baseline activation (or reduced deactivation) in MDD patients compared with control subjects (bilateral insulae, frontal and temporal opercula, basal ganglia, hypothalamic region, ventromedial prefrontal cortex, left hippocampus, middle temporal gyrus, and subgenual-pregenual ACC regions). Additionally, group-by-time interactions were significant in the dorsolateral prefrontal cortex (BA 9/10).

Significant group-by-time interactions were again observed from baseline to week 8, with group changes showing the direction observed in week 1 interaction analysis and involving a similar region network (Table 3, Table S3, Figure 3 and Figure S1). In addition, an interaction effect was observed along the brainstem including anterior and posterior parts of the pons.

Correlations between clinical and fMRI treatment effects

A specific analysis was carried out to assess the correlation between clinical improvement in both core emotional and somatic symptoms (using the Core and Somatization Subscales of the HAM-D 17, respectively) and fMRI treatment-related changes. This analysis was limited to the regions that showed significant treatment effects. We found that reductions in core MDD symptoms after one week of treatment were significantly correlated with activation reductions (i.e., deactivation increases) in the pregenual ACC region (ventral BA24

and BA32), which showed an abnormal persistence of activity during stimulation at baseline. Reductions in somatic symptoms were significantly correlated with activation reductions in the right dorsolateral prefrontal cortex (BA 9, Table S4 and Figure S2).

Improvement in core MDD symptoms after 8 weeks of treatment correlated with activation reductions in the right dorsolateral prefrontal cortex (BA 9) and left insulo-opercular region, whereas the improvement in somatic symptoms after this period was associated with activation reductions in the pons (Table S4 and Figure S2).

The categorical analysis assessing fMRI treatment-related changes associated with remission showed that remitting (compared with non-remitting) patients had greater activation reductions in the pons after 8 weeks of treatment (Table S4 and Figure S3).

fMRI predictors of clinical response to treatment

We performed an analysis to specifically look for region activations at baseline fMRI capable of predicting treatment responders at week 8. We found that increased baseline activation during painful stimulation in the right dorsolateral prefrontal cortex (BA 9) and reduced deactivation in the subgenual ACC and extended medial prefrontal regions were significantly associated with positive responses to treatment (measured as 50% reductions in the HAM-D 17) (Table S4 and Figure S3).

Correlations between experimental pain ratings and fMRI treatment effects

This analysis allowed us to map the relationship between experimental pain perception and brain activation in regions showing significant treatment effects in MDD. Although no significant results emerged for the unpleasantness correlation analyses, both the increase in perceived pain intensity after treatment (score change from baseline to week 8) and pain intensity scores reported at week 8 were significantly associated with brain regions modulated by treatment in MDD patients (Table S5 and Figure S4). Specifically, (i) increases in experimental pain intensity from baseline to week 8 were associated with treatment-related activation reductions in the right frontal cortex and (ii) greater pain intensity scores at week 8 were significantly associated with greater subgenual ACC (and extended medial prefrontal cortex) deactivation magnitudes.

Discussion

MDD patients treated with duloxetine showed a significant reduction in fMRI pain-related activations (and enhancement of deactivations) when compared to non-treated control subjects. Relevantly, most treatment-related imaging changes occurred in regions showing altered baseline responses to pain in the patient group. Treatment-related deactivation increases in the pregenual ACC, and activation reductions in right prefrontal cortex and pons were associated with MDD symptom improvement. Pontine changes were specifically related to MDD clinical remission. Higher baseline activation in the right prefrontal cortex and lower deactivation in the subgenual ACC predicted clinical response to treatment at week 8. Remarkably, these two brain areas also showed a specific association with the paradoxical increase in experimental pain perception following treatment in the MDD group. Moreover, the treatment-related increase in experimental pain perception was significantly associated with the observed reduction in core emotional MDD symptoms.

The MDD group showed significant enhancement of baseline responses to pain within the insula-operculum-basal ganglia complex bilaterally, hippocampus, the hypothalamic region and the surrounding anterior ventromedial prefrontal. To our knowledge, only two studies have directly explored abnormal brain responses to painful stimulation in MDD patients. Our data are coincident with the results reported by Bar et al (2007) showing increased fMRI activations in MDD patients within several pain-processing regions. In another study, Strigo et al (2008) assessed pain in young adult MDD patients during a continuous performance task and again reported abnormal activations in patients showing increased responses to pain anticipatory cues in limbic and paralimbic regions, but mostly reduced activations during actual pain.

Interestingly, their findings may well suggest a relevant role for attention resources in modulating brain responses to painful stimulation in MDD patients.

Our baseline analysis may provide relevant new findings by also indicating that MDD patients failed to deactivate a relatively large frontal region involving the subgenual-pregenual ACC and adjacent medial prefrontal areas in response to painful stimuli. These ventromedial prefrontal structures, which are a growing focus of interest in MDD research, are normally highly active in resting-state conditions when attention is primarily self-directed and become deactivated in response to attention-demanding external stimulation (Harrison et al, 2008; Raichle et al, 2001). Interestingly, fMRI studies in MDD assessing brain responses during emotion processing, judgment and reappraisal of aversive stimuli (Grimm et al, 2009; Sheline et al, 2009) and during cognitive-executive tasks (Matthews et al, 2009; Mitterschiffthaler et al, 2008; Vasic et al, 2009; Wagner et al, 2006, 2008) have reported a failure to reduce activity in depressed patients in these medial prefrontal regions, which has been linked to symptom severity (Grimm et al, 2009; Matthews et al, 2009). All in all, our findings, together with previous data, may reflect impaired effectiveness for patients in disengaging from self-referential processing.

We observed significant changes in brain responses to pain after one week of treatment manifested as greater activation reductions in treated MDD patients when compared to control subjects, mostly within areas showing abnormally enhanced responses at baseline. The complete eight-week period of duloxetine treatment was associated with significant brain activation reductions in a similar region pattern, together with additional changes in the brainstem. These late (eight-week) brain functional changes were paralleled by robust clinical improvement in core and somatic MDD symptoms, which was fully accomplished up to clinical remission in a relevant proportion of patients. To our knowledge, there is no previous

imaging study to date assessing the effects of duloxetine on brain function. Also, this is the first fMRI study using painful stimulation as a basic neural stressor to map treatment effects on brain activation and their association with clinical outcome (one previous study used painful stimulation to assess the effects of several drug treatments on cerebral blood flow using SPECT [Graff-Guerrero et al, 2008]).

Few studies have previously evaluated short-term effects of antidepressant treatment using neuroimaging tools. A recent SPECT study reported a broad attenuation of cerebral blood flow responses to painful stimulation in a group of MDD patients treated with different antidepressant drugs for two weeks (Graff-Guerrero et al, 2008). Another study using an fMRI paradigm of aversive versus neutral pictures (Davidson et al, 2003) also found significant fMRI BOLD signal changes in the insular cortex after two weeks of treatment using the dual reuptake inhibitor venlafaxine. Both studies also reported significant, albeit mild, reductions in clinical measurements of MDD severity. Mayberg and colleagues (2000) did not report any significant clinical responses after one week of fluoxetine treatment, although metabolic changes were noticed in several cortical and subcortical regions. Our data coincide with previous studies in showing detectable imaging changes at early treatment stages when the clinical effects are typically modest. The potential ability of neuroimaging for the early detection of brain changes could be useful in future drug discovery research, mainly if brain regions relevant to MDD are targeted. In our study, brain activation reductions after one week of duloxetine treatment included activation changes in regions already reported in the previous studies (insula, hippocampus and basal ganglia), but also in the critical subgenual ACC and in the dorsolateral prefrontal cortex.

In contrast with the paucity of studies assessing early brain changes during MDD treatment, imaging assessment of complete treatment effects has been more comprehensive. A

large number of PET and SPECT studies have provided evidence of treatment-related normalization of altered baseline patterns of brain metabolic activity in MDD patients after a variety of treatment strategies (see recent reviews by Frewen et al, 2008; Mayberg, 2009; Padberg and George, 2009; Ressler and Mayberg, 2007; Rigucci et al, 2009; Roffman et al, 2005; Schmidt et al, 2008). Less information, however, is available as to the effects of antidepressant treatment on altered brain systems in MDD when specifically challenged by disease-relevant stimulation. Consistent with our results, fMRI studies using aversive visual stimulation mostly reported treatment-related activation reductions (in regions generally showing abnormally enhanced baseline responses) after eight weeks of treatment with fluoxetine (Fu et al, 2004), sertraline (Sheline et al, 2001) and bupropion (Robertson et al, 2007), and after 16 weeks of cognitive behavioral therapy (Fu et al, 2008). Davidson et al (2003) additionally reported a normalization of abnormal functional deactivations in MDD patients in response to aversive visual stimulation after eight weeks of venlafaxine treatment. All in all, taken with our own findings, these studies appear to suggest a global effect of successful treatment on restoring the equilibrium within the complete pattern of brain activation and deactivation responses to distinct types of aversive stimulation.

Research evidence suggests the participation of the subgenual ACC and surrounding medial regions in both self-referential attention (mentioned above) and in guiding behavior and mood regulation through its influence on autonomic, endocrine and visceral function (Drevets et al, 2008b; Paus, 2001; Price, 1999). The main afferents to this neuroanatomic region come from the amygdala, ventral striatum, thalamus and brainstem monoamine nuclei (Paus, 2001). The subgenual ACC and extended medial prefrontal regions, in turn, send dense projections to the periaqueductal gray matter and, especially, to the hypothalamus, where these projections synapse with neurons projecting to the brainstem and spinal autonomic centers (Barbas et al, 2003). Structural and functional abnormalities of the subgenual ACC have been extensively

reported in depressive patients (reviewed by Drevets et al, 2008b), and have been frequently associated with MDD symptom severity (e. g., Chen et al, 2007; Greicius et al, 2007; Matthews et al, 2009). Previous research has shown the capacity of a wide variety of antidepressant treatment strategies to modulate and normalize functional alterations in the subgenual-pregenual ACC and extended regions within the medial frontal cortex (for relevant examples see: Drevets et al, 2002; Mayberg et al, 2000; 2005; Nahas et al, 2007). The present study shows the association of duloxetine treatment with significant changes in subgenual ACC and adjacent areas involving the normalization of altered baseline responses to aversive painful stimulation. The observed pattern of functional changes suggests that our imaging strategy is also useful in assessing this critical region in MDD.

Symptomatic improvement after eight weeks of duloxetine treatment was associated with treatment-related activation reductions in specific regions and with a paradoxical enhancement in subjective perception of experimental pain. Importantly, functional changes in the right prefrontal cortex, which has been specifically involved in mediating negative affect particularly in the context of MDD (Davidson, 2002a; 2002b; Liotti and Mayberg, 2001), were associated with improvement in core emotion symptoms of depression. Activation reductions in the pons were significantly correlated with improvement in somatic complaints and were specifically associated with clinical remission. In a recent study, Milak and colleagues (2009) reported a specific relationship between baseline metabolic activity in the pons-midbrain region and clinical remission after 12 weeks of pharmacological treatment, which gives greater consistency to this pontine finding. Additionally, Mayberg and colleagues (2002) showed a pattern of metabolic changes in the pons, specifically in positive responders to fluoxetine that did not appear in the placebo responder group, which may suggest that functional changes in the region are more plausibly attributable to specific psychotropic effects of the drug.

The painful stimulation paradigm employed here has successfully provided imaging biomarkers of positive clinical response to duloxetine treatment in MDD patients. Although there is no previously published study on brain functional predictors of positive clinical outcome associated with duloxetine, our findings are highly coincident with imaging literature showing the important role for the subgenual-pregenual ACC and extended medial prefrontal areas and, albeit to a lesser extent, dorsolateral prefrontal regions, in predicting clinical responses to various treatment modalities in MDD (e. g., Brockmann et al, 2009; Chen et al, 2007; Davidson et al, 2003; Dougherty et al, 2003; Keedwell et al, 2010; Little et al, 2005; Mayberg, 1997; Mottaghy et al, 2002; Saxena et al, 2003; Siegle et al, 2006; Wu et al, 1999).

Interestingly, MDD patients at baseline showed a tendency to report reduced subjective experimental pain scores, an effect that was reversed after 8 weeks of treatment. MDD research has provided evidence for the paradoxical phenomena of increased somatic complaints in depressive patients paralleled by decreased pain perception during externally-delivered painful stimulation on the skin (mainly thermal and electrical stimuli) (Bar et al, 2003; 2005; 2007; Dickens et al, 2003; Lautenbacher et al, 1994; 1999). In contrast, MDD patients may show hyperalgesia for deep somatic pain modalities such as muscle ischemia (Bar et al., 2005). Such data may suggest an increased processing of internal somatic and visceral afferent stimulation in MDD congruent with enhanced self-focused attention that may be paralleled by a reduction of brain attentional resources destined to external stimulation, including painful stimuli. Considering the above-mentioned role of the subgenual ACC in maintaining self-focused attention, baseline deactivation reductions in this region fit well with the observed opposite tendencies found for sensitivity to internal and external pain in MDD patients. Coherently, antidepressant treatment with duloxetine was associated with normalization of the abnormally absent subgenual ACC deactivation, and with both an increase in experimental pain sensitivity and a reduction in somatic pain complaints. Moreover, a specific correlation was found at week

8 between greater deactivation magnitudes in this region and higher experimental pain ratings in MDD.

Reductions in right frontal cortex responses to pain from baseline to week 8 in MDD patients were significantly associated with both an increase in subjective experimental pain perception and a decrease in core emotional symptoms of depression. In the context of the discussed paradox, it may indeed be relevant to mention that treatment-related activation reductions (associated with subjective pain score increases) were not observed in brain regions specifically devoted to the encoding of subjective pain perception (which has been mostly attributed to primary somatosensory area, dorsal ACC and specific portions of the insula; Hofbauer et al, 2001; Peyron et al, 2000 and Rainville et al, 1997). Apart from the previously mentioned role of the subgenual ACC in the recovery of sensitivity to externally-induced pain on the skin, the relevant role of the right prefrontal cortex in modulating -i.e., reducing- pain perception in natural and experimentally modified attentional and expectation contexts (e.g. placebo analgesia or distraction while performing another cognitive task) has been widely suggested in previous pain neuroimaging literature (Lieberman et al, 2004; López-Solà et al, 2010; Lorenz et al, 2003; Petrovic et al, 2000; Peyron et al, 1999; Salomons et al, 2004; 2007; Wager et al, 2004; Wiech et al, 2006; 2008b). Coherently, three studies have shown enhanced prefrontal activations associated with reduced experimental pain subjective scores in three distinct psychiatric populations, MDD, adjustment disorder and borderline personality disorder (Bär et al, 2006; 2007 and Schmahl et al, 2006). Our study may further inform as to the neural basis of the inverse relationship between sensitivity to experimental pain and core emotional symptoms of depression by suggesting a crucial role for the right prefrontal cortex (and also partly the subgenual ACC) in mediating both the core antidepressant effects and the changes in subjective pain perception. The experimental animal study by Jochum et al (2007) showed, in agreement with our data, that treatment with the SSRI citalopram for 8 weeks significantly

increased the sensitivity to thermal pain in anxious/depressed rats that had a significant baseline hypoalgesia for this painful stimulation modality.

Although we used a comparison group of healthy subjects to control for task repetition effects over time on brain activation changes, we cannot estimate the influence of placebo effects on the observed results. The absence of a placebo patient group constitutes a relevant limitation of the study, which was considered insurmountable in our clinical context on the grounds of both patient severity and the long period that would have been required without administering effective treatment. Nevertheless, the rate of clinical response and remission achieved following 8 weeks of duloxetine treatment suggest the existence of a significant antidepressant effect beyond placebo (Gupta et al, 2007). Additionally, treatment-related activation changes were observed after 8 weeks of duloxetine in certain regions (pons and anterior insula) previously shown to be targeted by effective fluoxetine treatment, but not modified in positive placebo responders (Mayberg et al, 2002), thus providing our results with greater drug-specificity.

It is relevant to mention that we used a relatively lenient statistical threshold (as in most studies of this type) to test for treatment effects and the correlations between brain response changes and clinical variables. Admittedly, the adopted criteria only partially control for the potential type I statistical error and we should be cautious in the overall interpretation of our results. However, it is also relevant to emphasize that our analyses were comprehensive including three assessments across time and between-group comparisons together with correlation analyses between activation changes and clinical treatment effects in the patient group. The intra-study remarkable coherence between all the results, their consistency with previous research in MDD and the anatomic plausibility of the findings may potentially reduce the overall risk of drawing conclusions based on false positive data. Finally, it would have been

of interest to additionally use a non-painful reference task (such as a sadness induction paradigm) or an fMRI resting-state assessment to perform a direct comparison of the effects of duloxetine on pain-related neural responses with the effects on MDD abnormalities in brain function more primarily associated with core emotional alterations in depressive patients.

As an original approach, painful stimulation as a basic neural stressor proved to be effective in mapping brain response changes associated with antidepressant treatment and brain correlates of symptom improvement in regions of special relevance to MDD pathophysiology. Using this fMRI strategy, the effect of duloxetine in MDD was assessed for the first time by means of neuroimaging. Although placebo effects could not be accounted for, the presented results may further contribute to characterizing the functional brain changes associated with recovery from depression.

Disclosure/Conflict of Interest

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Titles and legends to figures

Figure 1. Brain activation and deactivation patterns for Healthy Controls and MDD Patients in response to painful heat stimulation. A) Activation pattern for Healthy Controls. B) Deactivation pattern for Healthy Controls. C) Activation pattern for MDD patients. D) Deactivation pattern for MDD Patients. All depicted voxels show $p<0.05_{FDR}$ corrected. Images are displayed in the neurological convention (R=Right).

Figure 2. Regions showing greater activation during painful heat stimulation in Patients. A) Patients > Controls, assessed within the activation pattern of the Patient group. B) Patients > Controls, assessed within the deactivation pattern of the Control group. Images are displayed in the neurological convention (R=Right). Extension threshold: 200 voxels. P-voxel level <0.05 uncorrected.

Figure 3. Group by Time interaction effects showing significant activation reductions in the MDD group after 1 (Baseline – 1week) and 8 weeks (Baseline – 8 weeks) of receiving treatment when compared to reassessment effects in the healthy control group. Images are displayed in the neurological convention (R=Right). Extension threshold: 200 voxels. P-voxel level <0.05 uncorrected.

Figure S1. Box-plots illustrate the direction of pain-related activation changes from baseline to week 1 (superior panels) and from baseline to week 8 (inferior panels) fMRI reassessments for the two study groups (C, control subjects; P, MDD patients). Color bars represent the group mean activation (β parameter values showing the relative fMRI signal change from rest to painful stimulation condition) ± standard error of the mean. $W_{0,}$ week 0; W_1, week 1; W_8, week

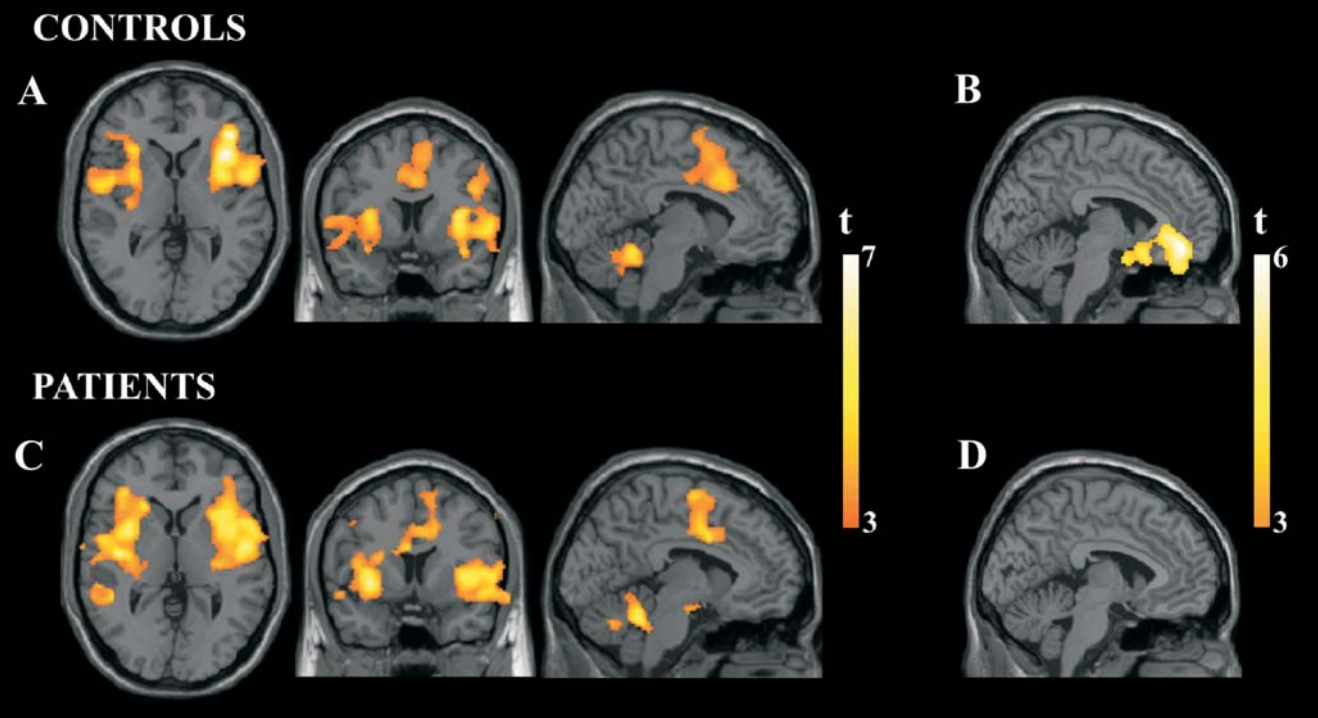
8. Two representative areas (examples of activation and deactivation changes) per week have been selected. Post., Posterior; Operc., Operculum; Preg., pregenual; ACC, anterior cingulated cortex; Prefrontal C., right prefrontal cortex; Subg., subgenual. Supplementary tables S2 and S3 provide the statistics of the within-group repeated-measures analyses assessing the direction of changes for all regions. Data in the boxplots were extracted at peak coordinates (see table 3) of clusters showing significant interaction effects reported in Supplementary Tables S2 and S3.

Figure S2. Maps showing significant correlations between treatment-related activation reductions after 1 and 8 weeks of treatment and reductions in Core subscale and Somatization subscale scores after such periods. Images are displayed in the neurological convention (R=Right). Lateral sagittal images correspond to right hemisphere brain slices. Extension threshold: 200 voxels (100 voxels for the brainstem). P-voxel level <0.05 uncorrected.

Figure S3. Left panel shows greater activation reductions after 8 weeks of treatment in remitter compared with non-remitter patients. Right panel shows regions of higher baseline (pretreatment) activation in responder compared to non-responder patients at week 8. Images are displayed in the neurological convention (R=Right). Extension threshold: 200 voxels (100 voxels for the brainstem). P-voxel level <0.05 uncorrected.

Figure S4. Correlations between experimental pain intensity ratings and fMRI treatment effects in MDD patients. A) Maps showing significant correlations between treatment-related activation reductions after 8 weeks of treatment and increases in subjective pain intensity scores (experienced during fMRI) after such period. B) Maps showing a significant association between greater magnitudes of deactivation in the subgenual ACC at week 8 and greater perception of pain intensity during such fMRI assessment. Images are displayed in the

neurological convention (R=Right). Lateral sagittal images correspond to right hemisphere brain slices. Extension threshold: 200 voxels. P-voxel level <0.05 uncorrected.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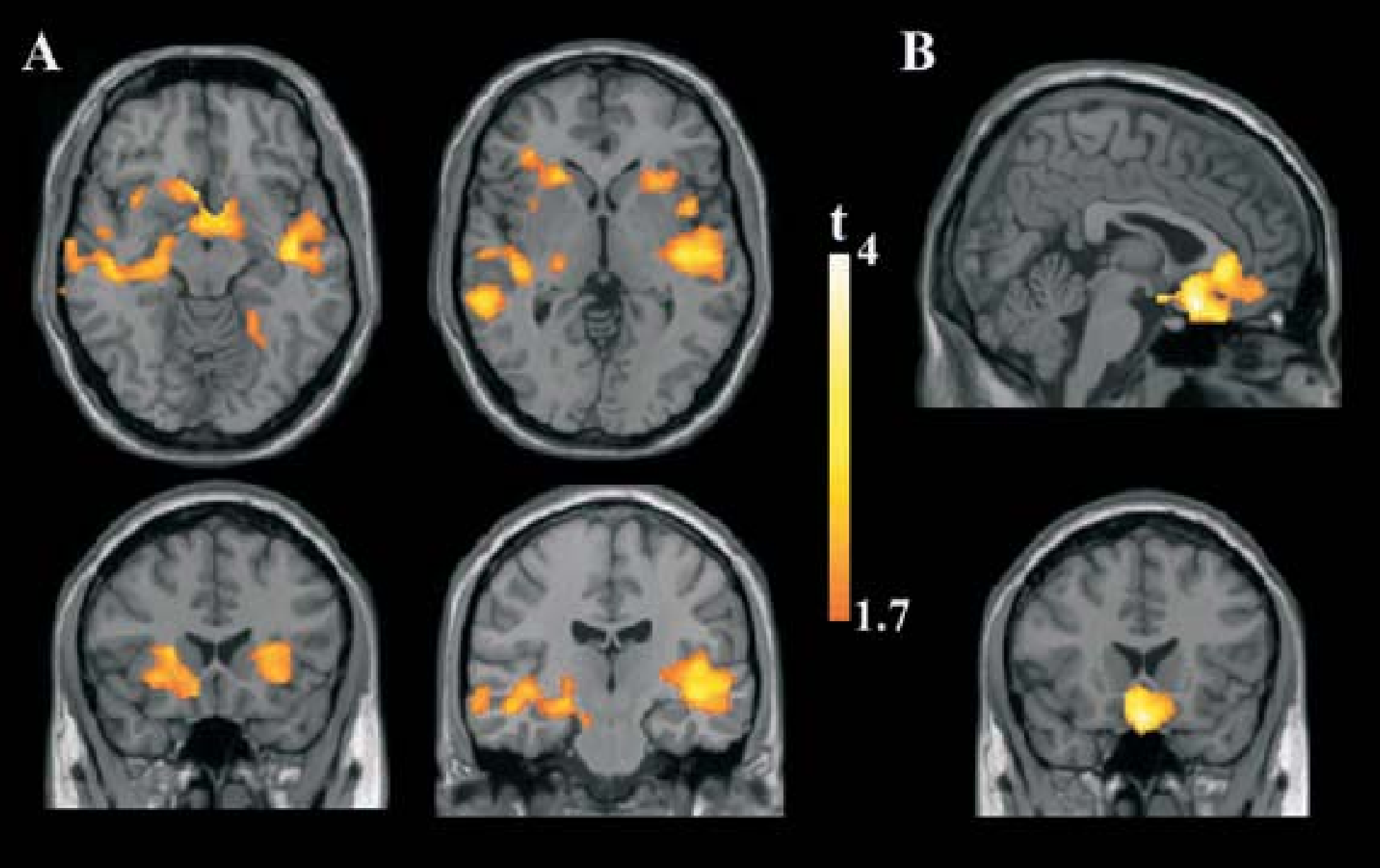
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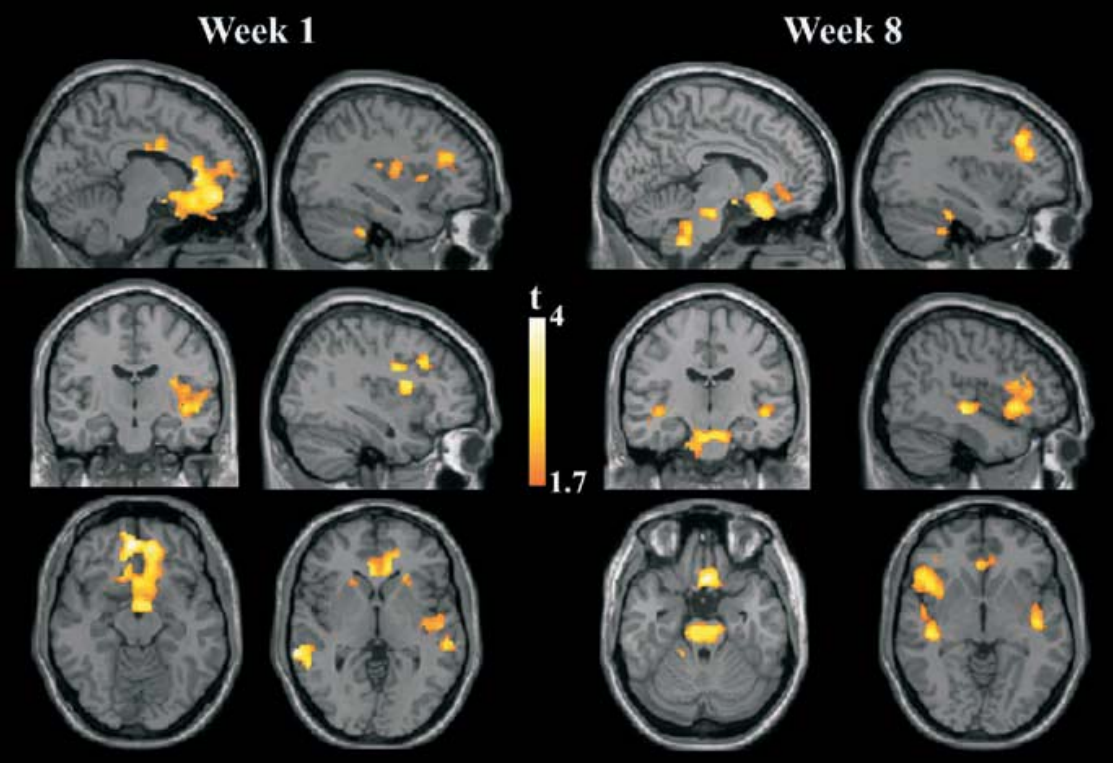
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Table 1. Main characteristics of the study sample

	MDD Patients	*Controls*	T/χ^2	*p*
Age at inclusion (mean ± SD years)	44.6 ± 8.3	47.2 ± 7.7	0.82/	0.41
Gender (Females/ Males)	11/ 2	15/ 5	/0.64	0.52
Handedness (Right handed/ Left handed)	12/ 1	20/ 0	/1.00	0.34
Years of education (mean ± SD years)	11.2 ± 3.2	12.4 ± 2.3	1.16/	0.25
Age at onset (range, mean ± SD)	18 – 47, 36.85 ± 8.53	NA	NA	NA
Total Nº of episodes (range, mean ± SD)	0 – 6, 2.23 ± 2.13	NA	NA	NA
Current episode duration (range, mean ± SD days)	110-1080, 414 ± 340	NA	NA	NA
AD treatment before washout (% Yes / % No)	84.6% / 15.4%	NA	NA	NA
Antidepressant type before washout (Type, Nº of patients)	SSRI, 8 TCA, 2 SNRI, 1	NA	NA	NA
HAM-D 17*	21.3 ± 2.6	0.18 ± 0.5	37.39/	<.0001
BPI*	6.8 ± 1.1	2.4 ± 3.0	5.90/	<.0001
SQ-SS*	15.6 ± 4.0	2.1 ± 1.8	11.50/	<.0001
CGI-Severity *	4.5 ± 0.8	1 ± 0	16.07/	<.0001

AD, antidepressant; SSRI, Selective Serotonin Reuptake Inhibitor; TCA, Tricyclic Antidepressant; SNRI, Serotonin-Norepinephrine Reuptake Inhibitor; HAM-D 17, Hamilton Depression Rating Scale, 17-item version; BPI, Brief Pain Inventory, maximum pain during the week; SQ-SS, Symptom Questionnaire, Somatic Subscale; CGI-Severity, Clinical Global Impression of Severity. NA, non-applicable. *All measurements were obtained at baseline fMRI (before treatment onset).

Table 2. Brain response to painful heat in MDD and Control subjects and between-group differences

Controls Activations	*X : Y : Z*	*k*	*t*	P_{FDR}
Insula – Opercula	-36 0 -8	2252	6.14	0.008
	40 34 2	2071	7.39	0.007
Prefrontal Cortex	-36 32 12	132	4.20	0.022
	46 34 36	581	5.26	0.010
Basal Ganglia	-30 -8 -6	302	5.10	0.011
	30 6 6	233	4.87	0.013
ACC-SMA	6 20 36	1666	4.47	0.018
Cerebellum	4 -50 -18	468	5.28	0.010
Controls Deactivations	*X : Y : Z*	*k*	*t*	P_{FDR}
Subg., Preg. ACC	*-2 46 -14*	*1446*	*5.59*	*0.003*
Patients Activations	*X : Y : Z*	*k*	*t*	P_{FDR}
Insula-Opercula	-42 -10 0	4056	7.37	0.037
	50 -8 4	3990	6.68	0.037
Prefrontal Cortex	-58 -2 42	200	4.36	0.038
	54 0 56	305	5.57	0.037
Basal Ganglia	-30 6 0	354	4.63	0.041
	28 14 2	414	4.43	0.041
ACC-SMA	-8 -12 44	2100	5.75	0.037
Cerebellum	4 -46 -24	1717	6.10	0.037
Middle Temporal Gyrus	-68 -42 -8	298	5.30	0.037
Hypothal. region - Midbrain	-4 -4 -14	132	3.89	0.043
Patients>Controls	*X : Y : Z*	*k*	*t*	$P_{Unc.}$
Increased Activations				
Post. Ins. - Temp. Operc. -HPC	-46 -26 -10	1249	3.18	0.002
	40 -14 -6	2042	4.24	<0.0005
Ant. Ins. - Frontal Operc.	-24 24 6	585	3.01	0.002
Basal G. – Med. PFC - Hypothal. region	-4 16 -16	1146	3.50	0.001
Middle Temp. Gyrus	-62 -42 4	569	3.28	0.001
Reduced Deactivations				
Subg., Preg. ACC	4 18 -18	2185	3.91	<0.0005

Coordinates (X : Y : Z) are given in Montreal Neurological Institute (MNI) Atlas space. *k*, cluster size; *FDR*, false discovery rate whole-brain corrected; *Unc*., uncorrected; ACC, anterior cingulate cortex; SMA, supplementary motor area; Hypothal., hypothalamic; Post., posterior; Ins., insula; Temp., temporal; Operc., operculum; HPC, hippocampus; Ant., anterior; Med., medial. Subg., subgenual; Preg., pregenual.

Table 3. Treatment effects on brain response to painful stimulation

Week 1 treatment effects. Interaction effects (Baseline - Week 1)				
Region	*X : Y : Z*	*k*	*t*	$P_{Unc.}$
Insula - Operc. - HPC	-40 8 14	701	3.46	0.001
	48 -10 -6	761	2.68	0.005
Basal Ganglia	-12 8 22	204	2.71	0.004
	12 4 22	395	2.69	0.005
Hypothal. Region – Med. PFC	6 -2 -16	355	3.88	<0.0001
Middle Temp. Gyrus	-66 -36 0	765	3.55	<0.0001
Subg. Preg. ACC	-6 50 -10	5515	3.86	<0.0001
Dorsolat. PFC	-36 22 34	1097	2.78	0.004
	20 34 26	1014	3.03	0.002
Week 8 treatment effects. Interaction effects (Baseline - Week 8)				
Region	*X : Y : Z*	*k*	*t*	$P_{Unc.}$
Anterior Insula,- Frontal Op.	-46 20 -2	1039	2.94	0.002
Posterior Insula - Temp Op.	-44 -24 -6	281	3.17	0.001
	44 -16 -6	315	2.82	0.003
Hypothal. Region – Med. PFC	4 -2 -16	358	3.05	0.002
Middle Temp. Gyrus	-60 -42 4	473	3.36	0.001
Subg. Preg. ACC	2 22 -24	1368	3.70	<0.0001
Dorsolat. PFC	-30 48 14	219	2.84	0.003
	34 26 34	1002	3.40	0.001
Inferior Pons	2 -38 -36	1717	3.08	0.002
Superior Pons	0 -20 -24	idem	2.92	0.002

Coordinates (X : Y : Z) are given in Montreal Neurological Institute (MNI) Atlas space. *k*, cluster size; Unc., uncorrected; Operc., opercula; Op., operculum; HPC, hippocampus; Hypothal., hypothalamic; Med., medial; PFC, prefrontal cortex; Temp., temporal; Subg., subgenual; Preg., pregenual; ACC, anterior cingulate cortex; Dorsolat., dorsolateral.

SUPPLEMENTARY FILES

Supplementary figures

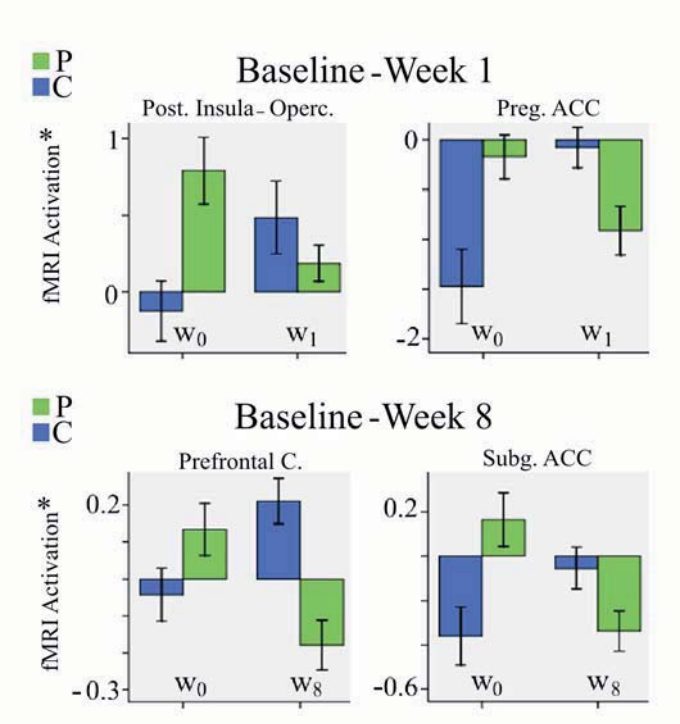

Figure S1. This figure provides box-plots illustrating the direction of pain-related activation changes from baseline to week 1 (superior panels) and from baseline to week 8 (inferior panels) fMRI reassessments for the two study groups.

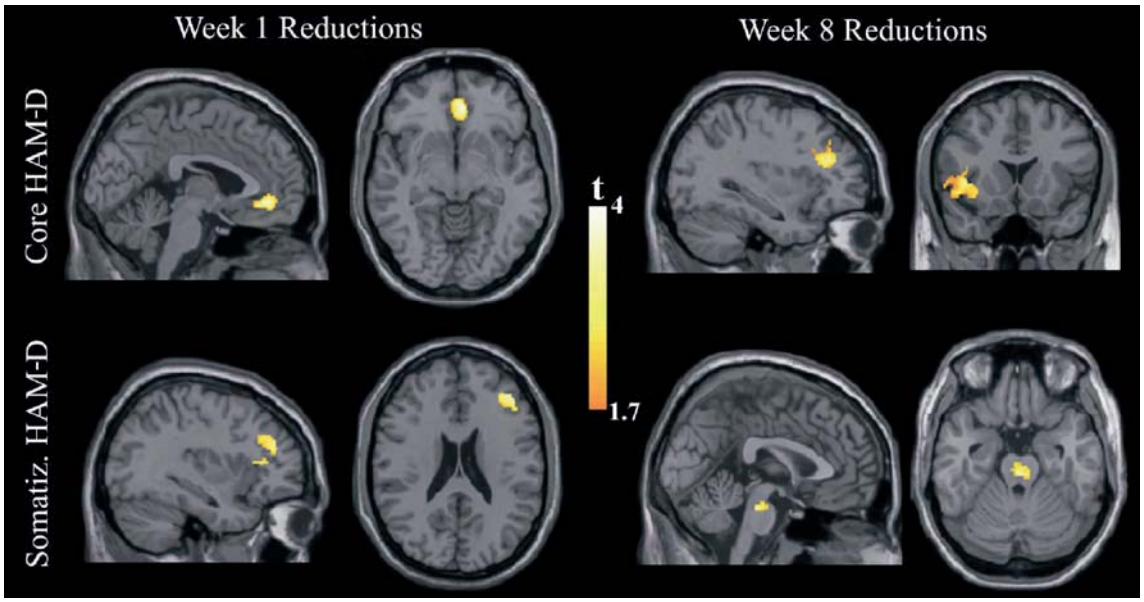

Figure S2. This figure provides the maps showing significant correlations between treatment-related activation reductions after 1 and 8 weeks of treatment and reductions in Core subscale and Somatization subscale scores after such periods.

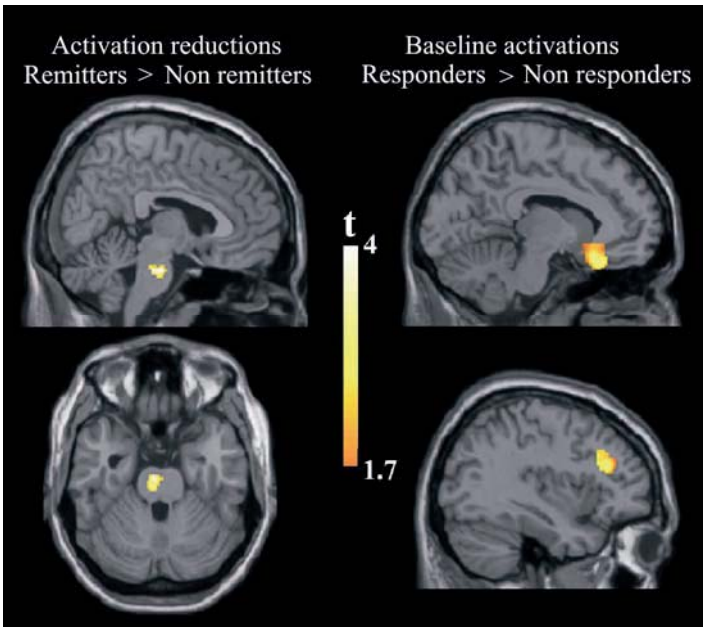

Figure S3. This figure provides (a) the maps showing greater activation reductions after 8 weeks of treatment in remitter compared with non-remitter patients and (b) the maps showing regions of higher baseline (pretreatment) activation in responder compared to non-responder patients at week 8.

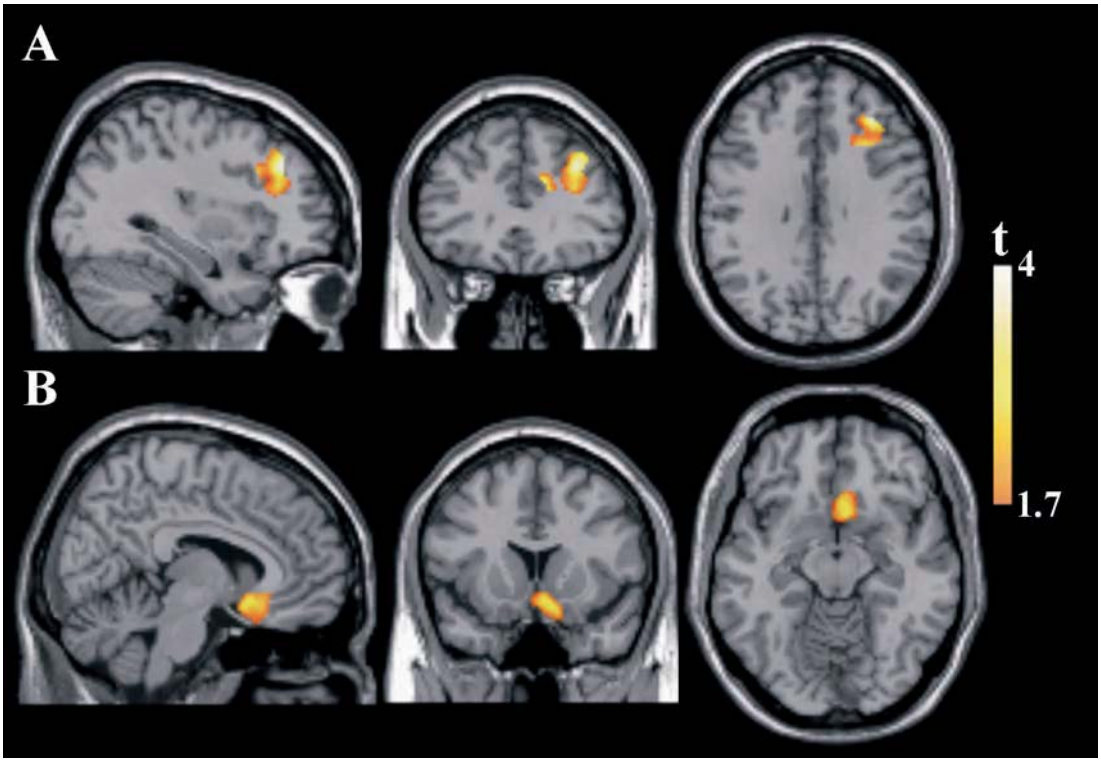

Figure S4. This figure provides the maps of the correlations between experimental pain intensity ratings and fMRI treatment effects in MDD patients.

Supplementary Tables

Table S1. Clinical response to treatment.

	Baseline measures	*Week 1 measures (t; p)**	*Week 8 measures (t; p)***
HAM-D 17 mean ± SD	21.3 ± 2.6	16.7± 3.9 (5.1; p=0.0003)	9.6 ± 5.9 (t=8.6; p<0.0001)
Core HAM-D 17 mean ± SD	7.8 ± 1.6	6.4 ± 2.4 (3.1; p=0.01)	1.7 ± 1.6 (19.4; p<0.0001)
Somat. HAM-D 17 mean ± SD	6.5 ± 1.5	5.8 ± 1.7 (2.2; p=0.05)	2.8 ± 2.3 (5.6; p=0.0001)
BPI mean ± SD	6.8 ± 1.1	5.00 ± 3.1 (2.4; p=0.03)	4.25 ± 3.2 (2.6; p=0.021)
SQ-SS mean ± SD	15.6 ± 3.9	12.9 ± 5.2 (1.4; p=0.17)	10.6 ± 6.3 (2.78; P=0.016)
CGI-Severity mean ± SD	4.5 ± 0.8	3.3 ± 0.5 (4.7; p=0.001)	2.3 ± 1.1 (6.1; p<0.0001)

* t and p-value from the statistical comparison of baseline and week 1 clinical measures. ** t and p-value from the statistical comparison of baseline and week 8 measures. HAM-D 17, Hamilton Depression Rating Scale, 17-item version; Core, Core Subscale. Somat., Somatization Subscale; BPI, Brief Pain Inventory, maximum pain during the week; SQ-SS, Symptom Questionnaire, Somatic Subscale; CGI-Severity, Clinical Global Impression of Severity.

Table S2. Direction of activation changes from baseline to week 1 fMRI assessments for MDD patients and Control subjects

Region (X : Y : Z interaction peak)	*Group*	*Mean Group fMRI Activation* Baseline*	*Mean Group fMRI Activation* Week 1*	*t*	$P_{repeated\text{-}measures}$
L Insula, Operc.	Patients	0.490	-0.038	3.10	0.01
(-40 : 8 : 14)	Controls	0.151	0.463	-2.61	0.02
R Insula, Operc.	Patients	0.790	0.187	2.72	0.02
(48 : -10 : -6)	Controls	-0.126	0.484	-1.90	0.07
Hippocampus	Patients	0.518	-0.254	2.90	0.01
(-26 : -24 : -16)	Controls	-0.105	0.308	-1.39	0.18
L Basal Ganglia	Patients	0.271	-0.285	1.71	0.12
(-12 : 8 : 22)	Controls	-0.038	0.090	-1.28	0.22
R Basal Ganglia	Patients	0.304	-0.158	1.33	0.21
(12 : 4 : 22)	Controls	-0.026	0.191	-1.80	0.09
Hypothal. Region	Patients	0.342	-0.225	2.83	0.02
(6 : -2 : -16)	Controls	-0.156	0.115	-1.69	0.11
Middle Temp. Gyr.	Patients	0.453	-0.366	2.92	0.01
(-66 : -36 : 0)	Controls	-0.027	0.242	-1.87	0.08
Subg. Preg. ACC	Patients	-0.171	-0.909	2.06	0.06
(-6 : 50 : -10)	Controls	-1.473	-0.076	-3.16	0.01
L Dorsolat. PFC	Patients	0.334	-0.107	2.04	0.07
(-36 : 22 : 34)	Controls	0.072	0.308	-1.38	0.19
R Dorsolat. PFC	Patients	0.162	-0.287	1.70	0.17
(20 : 34 : 26)	Controls	-0.064	0.097	-1.42	0.17

* Group mean β parameter value representing the magnitude of activation during painful stimulation (relative fMRI signal change from rest) for the group at the specified time moment (Baseline/Week 1. Coordinates (X : Y : Z) are given in Montreal Neurological Institute (MNI) Atlas space. L, left; R, right; Op., operculum; Hypothal., hypothalamus; Temp., temporal; Gyr., gyrus; Subg., subgenual; Preg., pregenual; ACC, Anterior Cingulate Cortex; Dorsolat., dorsolateral; PFC, prefrontal cortex.

Table S3. Direction of activation changes from baseline to week 8 fMRI assessments for MDD patients and Control subjects

Region (X : Y : Z interaction peak)	*Group*	*Mean Group fMRI Activation* Baseline*	*Mean Group fMRI Activation* Week 8*	*t*	$P_{repeated\text{-}measures}$
L Ant. Ins., Op.	Patients	0.595	0.047	2.49	0.03
(-46 : 20 : -2)	Controls	0.235	1.053	-3.41	<0.005
L Post. Ins., Op.	Patients	0.356	0.111	1.76	0.10
(-44 : -24 : -6)	Controls	-0.039	0.442	-3.23	<0.005
R Post. Ins., Op.	Patients	0.754	0.337	1.63	0.13
(44 : -16 : -6)	Controls	0.040	0.556	-2.22	0.04
Hypothal. Region	Patients	0.291	-0.043	1.89	0.08
(4 : -2 : -16)	Controls	-0.150	0.095	-1.98	0.06
Middle Temp. Gyr.	Patients	0.728	-0.083	4.15	<0.005
(-60 : -42 : 4)	Controls	0.059	0.346	-1.28	0.22
Subg. Preg. ACC	Patients	0.165	-0.337	3.00	0.01
(2 : 22 : -24)	Controls	-0.359	-0.053	-1.53	0.24
L Dorsolat. PFC	Patients	0.448	0.076	1.64	0.13
(-30 : 48 : 14)	Controls	0.113	0.670	-3.01	0.01
R Dorsolat. PFC	Patients	0.134	-0.179	2.34	0.04
(34 : 26 : 34)	Controls	-0.042	0.211	-2.60	0.02
Superior Pons	Patients	0.282	-0.047	1.81	0.10
(0 : -20 : -24)	Controls	0.007	0.408	-2.99	0.01
Inferior Pons	Patients	0.347	-0.203	2.24	0.05
(2 : -38 : -36)	Controls	0.018	0.380	-2.36	0.03

* Group mean β parameter value representing the magnitude of activation during painful stimulation (relative fMRI signal change from rest) for the group at the specified time moment (Baseline/Week 8). Coordinates (X : Y : Z) are given in Montreal Neurological Institute (MNI) Atlas space. L, left; R, right; Ant., anterior; Ins., insula; Op., operculum; Post., posterior; Hypothal., hypothalamus; Temp., temporal; Gyr., gyrus; Subg., subgenual; Preg., pregenual; ACC, Anterior Cingulate Cortex; Dorsolat., dorsolateral; PFC, prefrontal cortex.

Table S4. Brain activation correlates of clinical responses to treatment

Baseline - Week1 activation reductions: Correlation with clinical response					
Clinical Measure	*Region*	*X : Y : Z*	*k*	*t*	P_{unc}
Core Subscale HAM-D 17	Preg. ACC	4 : 42 : -6	203	3.52	0.003
Somatiz. Subscale HAM-D 17	Dorsolat. PFC	36 : 8 : 32	519	4.71	<0.0005
Baseline - Week 8 activation reductions: Correlation with clinical response					
Clinical Measure	*Region*	*X : Y : Z*	*k*	*t*	P_{unc}
Core Subscale	Dorsolat. PFC	28 : 38 : 32	353	3.45	0.003
HAM-D 17	Insula, Op.	-46 : 12 : 0	484	3.95	0.001
Somatiz. Subscale HAM-D 17	Pons	-2 : -24 : -26	171	2.52	0.014
Baseline - Week 8 activation reductions: Remitters versus Non-Remitters					
Clinical Measure	*Region*	*X : Y : Z*	*k*	*t*	P_{unc}
Remission (HAM-D 17 <=6)	Pons	-4 : -18 : -28	103	3.15	0.005
Baseline activation: Responders versus Non-Responders					
Clinical Measure	*Region*	*X : Y : Z*	*k*	*t*	P_{unc}
Response ($HAM\text{-}D\ 17_{8\text{-}weeks}$ <= 50% $HAM\text{-}D\ 17_{basal}$)	Dorsolat. PFC	32 : 28 : 26	205	3.80	0.001
	Subg. ACC	10 : 28 : -22	366	3.61	0.002

Coordinates (*X : Y : Z*) are given in Montreal Neurological Institute (MNI) Atlas space K, cluster size; Unc., uncorrected; Somatiz., Somatization; Preg., pregenual; ACC, anterior cingulate cortex; Dorsolat., dorsolateral; PFC: prefrontal cortex; Op., operculum; HAM-D 17, Hamilton Depression Rating Scale, 17-item version.

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Table S5. Correlations between pain intensity ratings and fMRI treatment effects in MDD

	Region	*X : Y : Z*	*k*	*t*	P_{unc}
fMRI activation reductions vs. pain intensity increases (baseline – week 8)	Dorsolat. PFC	34 : 34 : 34	628	3.78	0.002
fMRI deactivation magnitudes vs. pain intensity scores at week 8	Subg. ACC	8 : 16 : -12	334	3.37	0.003

Coordinates (*X : Y : Z*) are given in Montreal Neurological Institute (MNI) Atlas space; K, cluster size; Unc., uncorrected; Vs., versus; Dorsolat., dorsolateral; PFC: prefrontal cortex; Subg., subgenual; ACC, anterior cingulate cortex.

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5. Discussion

This study emphasizes the role of the dynamic assessment of brain emotion circuits as a potentially useful approach to improve understanding as to how the brain constructs affective experiences in health and disease. Emotion responses and affective states stand out as major determinants in guiding human behavior, in coloring our experiences and determining our life trajectories. The use of pain as a salient and biologically-relevant stimulus generating a complete emotional experience proved useful to our purposes as it was able to evoke, at the group level, in the three populations under study, solid and significant responses within the brain systems of interest. We successfully characterized the existence of different regional response dynamics that were distinctly associated with the final unitary experience of pain, and helped to elucidate pathophysiologically relevant brain disturbances in fibromyalgia patients and major depression. Furthermore, the use of a sustained resting-state fMRI paradigm allowed us to comprehensively depict the alteration in the intrinsic dynamic organization of affect-processing brain networks underlying the sustained depressive state. Finally, our pain paradigm was adequate to capture relevant functional brain changes paralleling the recovery of the euthymic state in major depression. A more complete understanding of how the brain constructs emotions and affective states may be of major relevance to characterize the pathophysiology of psychiatric disorders and, more broadly, of a variety of syndromes involving a "suffering component" from a neuroscientific perspective. The acquirement of evidence-based knowledge on the possible brain mechanisms underlying emotion-related symptomatic states may be helpful in guiding the future development of individually-tailored treatment strategies destined to more efficiently reverse or reduce such "painful" states.

Our first study provided relevant new data on the segregated temporal dynamics of the distinct regions whose combined operations ultimately built up the subjective experience of pain. The movie display of brain activations across a representative stimulation cycle was particularly relevant in conferring the notion of dynamic complexity characterizing brain response to painful stimulation, by showing the progressive engagement of separate brain regions and the different moments at which maxima activation occurred, as well as the variable duration of brain responses.

Moreover, the study suggests that particular components of the brain response to painful stimulation, such as the somato-sensory component, may be processed in a more uniform way across subjects resulting in the highest group activation t-values. Somato-sensory activations more directly depend on the physical features of the painful mechanical stimulus and on the characteristics of the stimulated region, and are therefore less suited to holding high levels of inter-individual response variability such as that observed in pain perception ratings across subjects. Regions prone to contribute in a more relevant way to such variance in subjective pain measurements are those whose processes are not strongly determined by the afferent noxious stimulation *per se* but are more closely associated with the evoked affective response. Good examples of this second type of region would be the anterior cingulate and the lateral frontal cortex, the anterior insula or the periaqueductal gray matter, which, in our experiment, show higher ranges for inter-subject variability resulting in lower group t-statistic values across the whole study period.

Specifically, the dynamic approach successfully captured the existence of distinct temporal dynamics of response in separate regions of the right lateral frontal cortex during painful stimulation and its cued anticipation in healthy subjects, which distinctly contributed to the subjective experience of pain. The right lateral frontal cortex has become one of the most relevant and complex structures in terms of its participation in the generation of efferent elements of the emotional response and in the regulation of affective states (Kalisch, 2009; Wiech et al, 2008a). In particular, it has been traditionally associated with mediating withdrawal behavior from aversive stimulation in a threatening context, in line with early developed scientific dual-system theories of emotion (right hemisphere linked to withdrawal and left hemisphere linked to approach behaviors) (Davidson, 2002a; Downar et al., 2000; Paus, 2000; Paus and Barrett, 2004; Schutter et al., 2008). Also, more recently, a role for the right lateral frontal cortex has been proposed as part of the ventral attention brain network, which is lateralized to the right hemisphere and involves right frontal lateral and the parieto-temporal regions (Corbetta and Shulman, 2002). Such a network has been particularly involved in capturing potentially significant stimulation from the environment in a bottom-up manner. All these functions may appear to be in agreement with the role of our dynamically isolated premotor-prefrontal region that promptly engaged following the auditory tone indicating the arrival of the intense painful stimulus. Such premotor-

prefrontal response was significantly and positively associated with the magnitude of response in a region commonly considered to be one of the most important regions encoding the affective dimension of the pain (i.e., the dorsal anterior cingulate cortex). Moreover, its activation magnitude during the cycle was positively correlated with the overall pain intensity reported by the subject. Another region of the lateral frontal cortex, the frontal operculum was activated during the anticipatory period but reached maximum activation in the middle of the stimulation period. This region represented the overall behavior of most "pain-matrix" regions, whose activation maxima occurred around the middle part of the stimulation condition. Finally, a third region within the anterior prefrontal cortex (BA 46-10) took longer to engage, showing its activation maxima around the last third of the stimulation period. This region appeared to be negatively correlated with the overall unpleasantness experienced by the subject throughout the twelve painful stimuli along the fMRI run. This result is highly consistent with observations by Wiech (2006, 2008b) and Kalish and colleagues (2006) showing the specific contribution of the right lateral aspect of the frontal cortex in mediating regulatory responses ultimately destined to reduce the overall pain or aversive emotion experience (Wiech et al, 2008a).

When specifically applied to the study of brain pathophysiology in fibromyalgia patients, the fMRI dynamic approach proved to be of crucial importance in capturing major abnormalities in brain responses to mild pressure, which were perceived as strongly painful in such patients. Important brain response alterations in FM mostly involved greater magnitude and duration (up to twice the duration of the painful stimulus) of brain responses in a region network particularly relevant to the encoding of affect-related components of the pain experience. Such abnormalities would not have been possible to detect by employing a model-driven ("static" picture of the activations circumscribed to the actual painful stimulation time) analysis approach that would have predicted a similar duration for the painful stimulus as for the actual response elicited by it, which in no way would have reflected the real process. It is noteworthy that the specific abnormalities characterized within the dynamic domain of brain responses in such patients is in striking agreement with the specific observation of augmented levels of temporal summation of second pain and central sensitization effects previously described in FM (Staud, 2002; Staud et al, 2001; 2007), as well as their well-known difficulty in recovering a baseline bearable state once a painful experience has been

fully elicited. Conspicuously, FM patients showed significantly augmented brain responses in affect-encoding regions also when compared to a group of control subjects receiving on average 2.8kg/cm^2 more pressure on the thumbnail (matched by subjective pain perception). In agreement with the largely greater amount of pressure applied to the control group, these subjects showed a tendency to display greater activation magnitudes in somato-sensory regions in response to painful stimulation. On the whole, our findings may be consistent with the notion of augmented brain response to pain in fibromyalgia patients, who showed particularly relevant and robust abnormalities in regions encoding emotion-related (limbic and paralimbic) components of the response.

In the light of previous data, it may be hypothesized that, when normative information is provided as to the dynamics and magnitude of response in brain regions relevant in conferring the subjective experience of pain, the individualized study of brain responses to noxious stimuli may prove to be a valuable tool for obtaining objective measurements of the overall suffering during a painful experience in normal and clinical conditions. Such an application of fMRI tools in the particular case of fibromyalgia syndrome may be valuable when considering its traditionally controversial medical nature due to the fact that patient diagnosis is largely based on subjective symptoms (Smythe, 2000).

The dynamic assessment of resting-state brain functional connectivity in MDD patients proved adequate in capturing a pattern of regional coupling reduction affecting major emotional systems in such patients, part of which were significantly associated with the clinical severity of the depressive state. In contrast, MDD patients showed specific functional connectivity enhancement within regions of crucial importance to the disorder pathophysiology, such as the subgenual anterior cingulate cortex and the hypothalamic region. Our study mostly showed broad inefficiencies (i.e., connectivity deficits) characterizing the brain state of MDD subjects under a severely altered affective state. Relevantly, such a pattern of abnormality, although extensive, was not generalized, and showed right-sided predominance, which would again argue in favor of the traditional view conceding greater importance to the right brain hemisphere in mediating emotion-related behavior and affective states. It is noteworthy that relevant disruptions in connectivity were more commonly observed within regions showing reduced blood flow and metabolism as measured by PET in MDD patients during resting-state (Savitz and Drevets, 2009; Mayberg, 2003; Phillips et al, 2003b), such as

the dorsal-rostral ACC region, frontal, parietal and temporal opercula, and bilateral angular gyri, among others. Connectivity disruptions were additionally detected in limbic regions such as the amygdala and the hippocampus, and around the ventral orbitofrontal cortex and the dorsal basal ganglia. Local alterations involving the amygdala and orbitofrontal cortex may suggest that functional coupling disruptions are also present in basic regions relevant in detecting and conferring emotional value to stimuli. PET findings have mostly, although not always (Drevets et al, 2008; Mayberg, 2003; Phillips et al, 2003b; Savitz and Drevets, 2009), reported augmented metabolism in such regions in MDD patients. Indeed, we observed that more severe patients within our group showed higher connectivity measurements when compared to less severe patients around the amygdala and extended regions. Apart from this specific finding, the rest of the obtained significant correlations showed that greater magnitudes of functional connectivity disruption during the resting-state were associated with greater symptomatic severity of the clinical episode in MDD patients. This was especially so in the case of the insula/operculum, basal ganglia, ventrolateral orbitofrontal cortex, and posterior part of the *default mode network* (Raichle et al, 2001) (including a part of the posterior cingulate cortex and bilateral angular gyri). Such a complete pattern of correlation with clinical severity measurements suggests that greater functional connectivity disruption within a variety of emotion-related brain networks may substantially underlie the multidimensional symptomatic state clinically described in MDD.

Of special relevance is the fact that, in such a broad context of functional connectivity disruptions, our anatomically-guided functional study was able to capture very specific enhancements in connectivity dynamics involving two regions of special relevance to MDD pathophysiology (i.e., the subgenual ACC and the hypothalamic region). Significantly, the former abnormality seemed to be associated with a protective effect in MDD patients showing that the higher the functional coupling between the amygdala seed and the subgenual ACC, the lower the severity of the depressive episode. This observation appears to be in agreement with a wide variety of studies suggesting that an enhancement of resting-state baseline PET metabolic activity in this region extended to the pregenual ACC is a specific and robust predictor of positive clinical responses to treatment in such patients (Chen et al, 2007; Dougherty et al, 2003; Keedwell et al, 2010; Mayberg et al, 1997b; Saxena et al, 2003). Our dynamic approach was able to

capture abnormalities within such a specific region, which appears to be a difficult target for most activation paradigms commonly applied in MDD fMRI studies. Considering the role of the subgenual ACC in regulating autonomic and vegetative function (Drevets et al, 2008; Paus, 2001; Price, 1999), and the hypothalamus involvement in stress-related neuroendocrine responses (particularly in the context of MDD, Carney et al, 2005; Carroll et al, 1976; Pariante and Miller, 2001), the specific enhancement in connectivity observed here may to some extent have a "bridge" role between different aspects of the altered self-centered state in MDD patients including unpleasant bodily sensations arising from the viscera and the limbs, sustained stress-related suffering sensations, ruminative and pervasive self-related thoughts, etc.

On the whole, such findings may well suggest that the dynamic approach applied to the study of the "default" functional organization of the brain may provide relevant information relating to the substrates characterizing a pathologically altered state of the self such as the depressive episode. The employed resting-state fMRI paradigm may be used as a helpful and simple way to explore abnormalities within basic emotion-related brain systems of special relevance in affective disorders.

Our last time-related study was specifically designed to effectively capture relevant brain functional changes occurring in parallel to the positive effects of antidepressant treatment following one and eight weeks of the SNRI duloxetine in MDD. In this study we again used painful stimulation as a basic neural stressor to target all major emotion circuits in a context of potential external threat. Our approach proved to be effective in mapping brain response changes associated with antidepressant treatment and brain correlates of symptom improvement in regions of special relevance to MDD pathophysiology. Specifically, MDD patients treated with duloxetine showed a significant reduction in fMRI pain-related activations (and an enhancement of deactivations) when compared to non-treated control subjects, and, relevantly, most treatment-related imaging changes occurred in regions showing altered baseline (before treatment) responses to pain in the patient group. Moreover, we found important correlations between the magnitude of treatment-related response changes in the pregenual-subgenual ACC, right prefrontal cortex and pons, and MDD measurements of symptomatic improvement. Finally, the approach proved to be effective not only in detecting specific changes characterizing clinical remission in MDD, but also in

successfully mapping significant regions whose pain-related activation at baseline was capable of predicting potential clinical responders to antidepressant treatment.

As an original approach, painful stimulation used as a primary salient aversive stimulus, proved to be effective in mapping brain response changes associated with antidepressant treatment and brain correlates of symptom improvement in regions of special relevance to MDD pathophysiology. Using this fMRI strategy, the effect of duloxetine in MDD was assessed for the first time by means of neuroimaging. Although placebo effects could not be accounted for, the presented results may further contribute to characterizing the functional brain changes associated with recovery from depression and may therefore contribute in delineating the functional state-dependent neuroanatomy of the depressive episode.

All in all, the four studies presented in the PhD thesis constitute a step forward in the dynamic characterization of how the brain constructs emotion perception and sustained affective states in the context of both health and disease. As a final conclusion, we should not forget the conceptualization of the brain as a complex inter-connected organ, which is highly active and organized even during passive rest conditions, and which reorganizes in response to both external and internal salient afferent stimulation and during sustained affective states. In agreement with such a perspective, we may also emphasize that emotion perception, albeit being described in terms of a unitary experience of the self, is constructed as the result of a complex combination and interaction of processes occurring in separate and interconnected regions, which appear to be characterized by different temporal response dynamics supporting functional specialization, and which sometimes contribute as opposing forces in determining the final quality of the experience.

Future lines of work:

Taken as a whole, the present study suggests that future research dealing with the study of brain and emotions from a neural system perspective may relevantly consider the essentially dynamic nature of the assessed process. Alternatively, crucial information may be lost or misinterpreted on the basis of using un-informed *a-priori* designed inaccurate analysis models.

It may be worth considering that some types of emotional responses may not be possible to elicit with the same level of intensity on multiple occasions during a short period of time (as would be required in a block design fMRI paradigm) due to the occurrence of single-event habituation mechanisms. This may be the case for "surprise", "startle" and sudden threat responses, which may completely change when elicited on several occasions in a short time window. Therefore, specific studies showing the complete functional neuro-anatomy and the dynamic picture of brain response to single events of a salient affective nature may be especially relevant in further understanding the neural basis for biologically-relevant basic and rapidly-habituating emotion responses, which to date remain mostly unexplored in affective neuroimaging studies. Also, using a similar approach, it may be useful to study the progressive habituation (and spontaneous response recuperation) or sensitization mechanisms characterizing different emotion responses, by analyzing, through a series of repetitive stimulation events, how the dynamic picture of brain response evolves from event to event.

The idea presented in the previous paragraph may prove to be of special relevance in improving our understanding of the pathophysiology underlying specific pain or psychiatric conditions characterized by the presence of sustained hyper-responses to certain stimulation types, which may be (the hyper-responses) particularly difficult to re-conduct to baseline once they have been evoked. Good examples of abnormalities that may be more sensitively detected using a dynamic study approach may include those occurring in fibromyalgia or other forms of chronic pain under mechanical stimulation, sensory hyper-sensitization syndromes in response to chemicals/electromagnetic/auditory stimuli, obsessive-compulsive disorder when facing specific disease-relevant stimulation or social phobia, major depression and anorexia nervosa in response to various types of self-referential stimuli. It may also be relevant for the study of schizophrenic patients, considering the abnormal nature of their startle-response habituation.

Additionally, the whole-brain study of functional reorganization during sustained affective states induced by the continuous presentation of symptom-provoking stimuli in psychiatric conditions may prove to be of special interest in providing relevant and specific brain functional fingerprints underlying different symptomatic affective states.

Finally, studying the action of various treatment strategies on such aberrant dynamic processes in disorders of the affective sphere or involving a "suffering" component may be particularly relevant in further understanding the action mechanisms, which are still widely unexplored, of such strategies from a neural system dynamic perspective. By understanding how the dynamic abnormalities in emotion-related brain processing reverse, and how such normalization accompanies symptomatic improvement, valuable knowledge will be acquired as to how the brain constructs emotion and affective states and to discover the most common mechanisms underlying the brain's pathologic disequilibrium under psychiatric and chronic pain conditions.

5. Conclusions

1. fMRI was able to identify distinct behaviorally relevant temporal courses of activation in response to painful mechanical stimulation in the whole brain and within specific regions of the right lateral frontal cortex in healthy subjects, which demonstrated distinct modulatory roles in subjective pain perception.

2. The dynamic assessment of brain response to painful stimulation significantly increased fMRI sensitivity to detect brain activation alterations in fibromyalgia patients. The results further suggest aberrant and durable nociceptive processing in fibromyalgia patients involving higher order brain systems related to the affective dimension of pain.

3. Depressive (MDD) patients showed altered brain activity during the resting-state with predominant functional connectivity decreases consistent with the patients' general hypo-functional brain state, and particular increases in specific limbic and subcortical structures relevant in eliciting affective, somatic and visceral responses. The pattern of results suggests a comprehensive alteration of the intrinsic functional equilibrium in major emotional systems in depression.

4. fMRI was able to identify (i) the temporal evolution of brain responses to painful stimulation in emotion-related systems associated with the improvement of MDD patients' affective state following antidepressant treatment (ii) brain imaging correlates of symptomatic improvement in specific clinical dimensions of interest (iii) baseline brain response measurements predicting clinical responders following 8 weeks of antidepressant treatment.

6. Summary of the Thesis (Resumen de la Tesis. Resum de la Tesi)

Resumen de la Tesis

"The dynamic dimension of the emotional experience assessed during painful stimulation and in the resting-state using functional magnetic resonance imaging"

Esta tesis tiene por objeto la caracterización de la dimensión dinámica o temporal de la experiencia emocional estudiada mediante resonancia magnética funcional. Se ha analizado la respuesta cerebral a estímulos emocionales primarios, así como la actividad cerebral en el estado anímico basal en sujetos sanos y en pacientes con trastornos afectivos. Los avances técnicos introducidos recientemente en el ámbito de la adquisición y análisis de los datos de resonancia magnética funcional han hecho posible esta aproximación al estudio de la "experiencia" emocional humana, considerada como el resultado de la interacción de distintas operaciones neurales ubicadas en diferentes sistemas cerebrales.

Específicamente, en una primera aproximación, se ha utilizado la provocación de dolor como estímulo emocional esencialmente aversivo y biológicamente primario con el fin de caracterizar las dinámicas de respuesta de las diferentes regiones y sistemas cerebrales que configuran la experiencia emocional unitaria del ser humano. Se ha estudiado una muestra de individuos sanos, así como un grupo de pacientes con fibromialgia y otro con depresión mayor, en base a que estos sujetos presentan síntomas primariamente relacionados con la esfera afectiva. En una segunda aproximación, se ha caracterizado la dinámica de la organización funcional en los distintos sistemas cerebrales que modulan las emociones durante el estado anímico basal en el cerebro sano y en pacientes deprimidos, nuevamente.

El trabajo presentado se compone de cuatro estudios con los siguientes objetivos y resultados particulares:

(I) El primer estudio tenía como objetivo específico analizar la especialización funcional de distintas regiones de la corteza frontal derecha, en base a la predicción de la existencia de diferentes dinámicas de respuesta al estímulo doloroso mecánico en un

grupo de sujetos sanos. Es conocido el hecho de que dicha región tiene un papel relevante y a la vez multifacético en la modulación de los estados afectivos en general y de aquéllos inducidos mediante estimulación dolorosa en particular, sin embargo, la especialización funcional de las distintas áreas frontales no ha sido investigada hasta la fecha. La metodología aplicada en este estudio fue capaz de detectar la existencia de distintas respuestas funcionales en diferentes regiones de la corteza frontal lateral derecha, identificando tres regiones cuyo inicio y máxima amplitud de respuesta ocurrían en momentos temporales diferentes y cuya contribución a la percepción final de dolor descrita por el sujeto era cualitativamente distinta.

(II) Teniendo en cuenta los resultados del estudio previo, que mostraban la diferente contribución de distintas regiones cerebrales, con diferentes dinámicas de respuesta, a la experiencia subjetiva de dolor descrita por el individuo, se planteó el segundo estudio. Su objetivo residía en comprobar la utilidad potencial de contemplar la dimensión temporal de la respuesta cerebral al dolor para caracterizar anomalías en el procesamiento cerebral de dicho estímulo en pacientes con fibromialgia. Estos pacientes, que presentan dolor crónico generalizado sin causa orgánica definida, normalmente muestran una dificultad específica para recuperarse de la experiencia de dolor (y del sufrimiento asociado) una vez ésta ha sido desencadenada (mecanismos de sensibilización temporal). Teniendo en cuenta esta alteración, esperábamos que el acoplamiento temporal entre el estímulo doloroso y la correspondiente respuesta cerebral desencadenada por éste se hallara distorsionada en pacientes fibromiálgicos, especialmente en aquellas áreas que son relevantes para la construcción de la experiencia afectiva. La evaluación dinámica de la respuesta cerebral al dolor mecánico en dichos pacientes aumentó significativamente la sensibilidad de la resonancia magnética funcional para detectar patrones de respuesta aberrante en pacientes fibromiálgicos. Específica y novedosamente, los resultados mostraron una alteración robusta en la dinámica de respuesta (i.e., duración de la respuesta patológicamente incrementada) de las regiones encargadas de construir la dimensión emocional de la experiencia dolorosa en los pacientes. Dicha alteración se observó significativamente asociada al aumento patológico de la percepción de dolor.

(III) Existe un amplio solapamiento entre los sistemas cerebrales que construyen la experiencia afectiva asociada al dolor y los sistemas que se hallan alterados en la

depresión mayor (ejemplo paradigmático de trastorno de la esfera afectiva). El objetivo del tercer estudio consistió en evaluar las alteraciones funcionales existentes en el equilibrio dinámico basal de los sistemas cerebrales emocionales en el cerebro del paciente deprimido. Este estudio permitió identificar una disminución significativa de la conectividad funcional (o acoplamiento temporal de la actividad cerebral regional) en la mayor parte de sistemas implicados en la fisiopatología de la depresión mayor, que es congruente con el estado cerebral hipo-funcional característico de dichos pacientes. Las alteraciones dinámicas detectadas se mostraron asociadas, en parte, a la severidad clínica del episodio depresivo. Asimismo, fue especialmente relevante la observación de un aumento específico en el acoplamiento temporal de la actividad cerebral de regiones implicadas en la función neuroendocrina y autonómica, las cuales se encuentran frecuentemente alteradas en la depresión mayor y en estados caracterizados por la presencia de estrés sostenido. Este trabajo constituye una aportación relevante por la exhaustiva caracterización del patrón de alteración en la organización dinámica intrínseca (disposición básica en reposo) de los sistemas cerebrales emocionales durante el estado depresivo.

(IV) Las experiencias emocionales en general y la experiencia subjetiva de dolor en particular varían de forma importante en intensidad y en cualidad en función del estado receptivo del individuo. En este sentido, es esperable que tanto la experiencia de dolor como las subyacentes respuestas cerebrales relevantes a la hora de construirla muestren un cambio paralelo a la mejoría de la sintomatología depresiva tras recibir tratamiento médico efectivo. El último estudio estaba dirigido a detectar los cambios a lo largo del tiempo en las respuestas a estimulación dolorosa en circuitos cerebrales relevantes para el procesamiento emocional al cabo de una y ocho semanas de tratamiento antidepresivo. La aproximación dinámica mediante resonancia magnética funcional permitió en este caso identificar la normalización de las alteraciones en la respuesta cerebral que dichos pacientes presentaban antes de iniciarse el tratamiento, en plena expresión sintomática del episodio depresivo. Asimismo, el análisis detectó una correlación significativa entre la respuesta clínica y los cambios en la activación cerebral después del tratamiento. Por último, este estudio se mostró eficaz para identificar regiones cuya respuesta al dolor durante el episodio depresivo era capaz de predecir la presencia de una respuesta clínica positiva al cabo de ocho semanas de tratamiento.

La dimensión temporal de la respuesta emocional ha sido hasta ahora ampliamente ignorada en el ámbito de la investigación en neuroimagen, tanto en situaciones normales como en condiciones patológicas con repercusión sobre la esfera afectiva. En su conjunto, los resultados de esta tesis sugieren que el enfoque dinámico del estudio de la actividad cerebral permite una caracterización más completa de los aspectos básicos de la experiencia emocional. La aproximación dinámica propuesta en este trabajo podría contribuir a elucidar aspectos importantes de la fisiopatología de las condiciones clínicas que cursan con sufrimiento y a entender mejor el fenómeno general de la emoción, tan fascinante como complejo y hasta ahora difícilmente objetivable.

Resum de la Tesi

"The dynamic dimension of the emotional experience assessed during painful stimulation and in the resting-state using functional magnetic resonance imaging"

La present tesi té per objecte la caracterització de la dimensió dinàmica o temporal de l'experiència emocional estudiada a través de la ressonància magnètica funcional. S'ha analitzat la resposta cerebral a estímuls emocionals primaris, així com l'activitat cerebral en l'estat anímic basal en subjectes sans i en pacients amb trastorns afectius. Els avenços tècnics introduïts recentment en l'àmbit de l'adquisició i anàlisi de les dades de ressonància magnètica funcional han fet possible aquesta aproximació a l'estudi de l'"experiència" emocional humana, considerada como el resultat de la interacció de diferents operacions neurals ubicades a diferents sistemes cerebrals.

Específicament, en una primera aproximació, s'ha emprat la provocació de dolor com a estímul emocional essencialment aversiu i biològicament primari amb la finalitat de caracteritzar les dinàmiques de resposta de les diferents regions i sistemes cerebrals que configuren l'experiència emocional unitària de l'ésser humà. S'ha estudiat una mostra d'individus sans, així com un grup de pacients amb fibromialgia i un altre amb depressió major, en base a què aquests subjectes presenten símptomes primàriament relacionats amb l'esfera afectiva. En una segona aproximació, s'ha caracteritzat la dinàmica de l'organització funcional en els diferents sistemes cerebrals que modulen les emocions durant l'estat anímic basal en el cervell sa i en pacients deprimits, novament.

El treball presentat es composa de quatre estudis amb els següents objectius i resultats particulars:

(I) El primer estudi tenia com a objetiu específic analitzar l'especialització funcional de diverses regions de l'escorça frontal dreta, en base a la predicció de l'existència de diferents dinàmiques de resposta a l'estímul dolorós mecànic en un grup de subjectes sans. És conegut el fet de què l'esmentada regió té un paper rellevant i a la vegada multifacètic tant en la modulació dels estats afectius en general com en aquells induïts mitjançant estimulació dolorosa en particular. Tanmateix l'especialització funcional de les diferents àrees frontals no havia sigut previament investigada. La metodologia

aplicada fou capaç de detectar l'existència de diferents respostes funcionals en diverses regions de l'escorça frontal lateral dreta, identificant tres regions per les quals l'inici i la màxima amplitut de resposta tenien lloc en moments temporals diferents. Aquestes regions caracteritzades per presentar una dinàmica de resposta temporal diferent, van mostrar, a més a més, una contribució qualitativament oposada a la experiència final de dolor descrita pel subjecte.

(II) Tenint en compte els resultats de l'estudi previ, que mostraven la diferent contribució de diverses regions cerebrals, amb diferents dinàmiques de resposta, a l'experiència subjectiva de dolor descrita per l'individu, es va plantejar el segon estudi. L'objetiu d'aquest residia en comprovar la utilitat potencial de contemplar la dimensió temporal de la resposta cerebral al dolor per tal de caracteritzar anomalies en el processament cerebral de l'esmentat estímul en pacients amb fibromialgia. Aquests pacients, que presenten dolor crònic generalitzat sense causa orgànica definida, normalment mostren una dificultat específica per recuperar-se de l'experiència de dolor (i del patiment associat) un cop aquesta s'ha desencadenat (mecanismes de sensibilització temporal). Tenint en compte aquesta alteració, esperàvem que l'acoblament temporal entre l'estímul dolorós i la corresponent resposta cerebral desencadenada per aquest es trobés distorsionada en pacients fibromialgics, especialment en aquelles àrees que es mostren rellevants per a la construcció de l'experiència afectiva. L'avaluació dinàmica de la resposta cerebral al dolor mecànic en aquests pacients va augmentar significativament la sensibilitat de la ressonància magnètica funcional per a detectar patrons de resposta aberrant en pacients fibromialgics. Específicament i de forma novedosa, els resultats van mostrar una alteració robusta de la dinàmica de resposta (i.e., duració de la resposta patològicament incrementada) de les regions encarregades de construir la dimensió emocional de l'experiència dolorosa en els pacients. Aquesta alteració es trobà significativament associada a l'augment patològic de la percepció de dolor.

(III) Existeix un ampli solapament entre els sistemes cerebrals que construeixen l'experiència afectiva associada al dolor i els sistemes que es troben alterats a la depressió major (exemple paradigmàtic de trastorn de l'esfera afectiva). L'objetiu del tercer estudi va consistir en avaluar les alteracions funcionals existents en l'equilibri dinàmic basal dels sistemes cerebrals emocionals al cervell del pacient deprimit. Aquest

estudi va permetre identificar una disminució significativa de la connectivitat funcional (o acoblament temporal de l'activitat cerebral regional) en la major part de sistemes implicats en la fisiopatologia de la depressió major, essent congruent amb l'estat cerebral hipo-funcional característic d'aquests pacients. Les alteracions dinàmiques detectades es van veure associades, en part, a la severitat clínica de l'episodi depressiu. Així mateix, fou especialment rellevant l'observació d'un augment específic en l'acoblament temporal de l'activitat cerebral de regions implicades en la funció neuroendocrina i autonòmica, les quals es troben freqüentment alterades a la depressió major i en estats caracteritzats per la presència d'estrès sostingut. Aquest treball constitueix una aportació rellevant per l'exhaustiva caracterització del patró d'alteració en l'organització dinàmica intrínseca (disposició bàsica en repòs) dels sistemes cerebrals emocionals durant l'estat depressiu.

(IV) Les experiències emocionals en general i l'experiència subjectiva de dolor en particular varien de forma important en intensitat i qualitat en funció de l'estat receptiu de l'individu. En aquest sentit, és esperable que tant l'experiència de dolor com les subjacents respostes cerebrals rellevants a l'hora de construir-la mostrin un canvi paral·lel a la milloria de la simptomatologia depressiva després de rebre tractament mèdic efectiu. L'últim estudi estava dirigit a detectar els canvis al llarg del temps en les respostes a estimulació dolorosa en circuits cerebrals rellevants per al processament emocional al cap d'una i de vuit setmanes de tractament antidepressiu. L'aproximació dinàmica mitjançant ressonància magnètica funcional va permetre identificar en aquest cas la normalització de les alteracions en la resposta cerebral que aquests pacients presentaven abans d'iniciar-se el tractament, en plena expressió simptomàtica de l'episodi depressiu. Així mateix, l'anàlisi va detectar una correlació significativa entre la resposta clínica i els canvis en l'activació cerebral després del tractament. Per últim, aquest estudi es va mostrar eficaç per identificar regions que mostraven una resposta al dolor durant l'episodi depressiu capaç de predir la presència d'una resposta clínica positiva al cap de vuit setmanes de tractament.

La dimensió temporal de la resposta emocional ha estat fins ara àmpliament ignorada en l'àmbit de la recerca en neuroimatge, no només en situacions normals sinó també en condicions patològiques amb repercussió sobre l'esfera afectiva. En el seu conjunt, els resultats d'aquesta tesi suggereixen que l'enfocament dinàmic de l'estudi de l'activitat

cerebral permet una caracterització més completa dels aspectes bàsics de l'experiència emocional. L'aproximació dinàmica proposada en aquest treball podria contribuir a dilucidar aspectes importants de la fisiopatologia de les condicions clíniques que cursen amb patiment i a entendre mejor el fenomen general de l'emoció, tan fascinant como complexe i, fins ara, difícilment objectivable.

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