Development of Neuroscience-Based Methods for Detection of Consciousness in Severely Brain Injured Patients

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Abstract

Neurologists routinely use visual, auditory, tactile, or noxious stimuli to examine the purposeful motor responses indicating awareness in patients with disorders of consciousness (DOC), however with a high rate of misdiagnosis. Previous studies have shown that functional neuroimaging can be used to detect conscious awareness in this patient group because some patients fulfilling the clinical criteria for a diagnosis of vegetative state [VS; recently renamed as unresponsive wakefulness syndrome (UWS)] exhibited purposeful activity manifested in cerebral activation, rather than in the overt motor responses. For example the UWS patient in the study of Owen et al. (2006) was obviously able to follow instructions. It is possible, however, that patients who cannot follow instructions (e.g., due to limited memory/attention capacities) are nevertheless conscious and retain emotional abilities to feel pain and pleasure. With this in mind, a hierarchical functional neuroimaging approach was developed in the present study, starting with the simplest form of processing within the domain of pain perception, then progressing to emotional processes of pain cries from other persons and ending with the more complex and demanding mental imagery task.

Brain responses were acquired from a group of 54 non-traumatic brain-injured patients with a clinical diagnoses of UWS (N = 30) or minimally conscious state (MCS; N = 24). A range of patterns of activations was observed: while some patients did not show significant responses in any of the three experimental paradigms, others, including patients diagnosed as being in UWS, demonstrated significant responses in appropriate neuroanatomical locations, that were virtually indistinguishable from those obtained from a group of 15 healthy volunteers performing the same task (Responder in patients: 59.3% in pain experiment, 46.3% in emotional experiment, 13% in mental imagery task).

The data indicate that some DOC patients retain islands of residual function and that in the absence of behavioural response, functional neuroimaging provides an additional tool for the assessment team. More importantly, the present study provides clear evidence that the higher cognitive functions such as attention, working memory or language comprehension do not make the whole of human consciousness. There are more basic and probably simpler cognitive functions which processes not only the own pain, but also the pain of others. This so called affective consciousness can survive even very severe brain damage, suggesting that for many DOC patients, an emotional contact with caregivers might be established to improve their quality of life.

Zusammenfassung

Neurologen verwenden routinemäßig visuelle, auditive, taktile oder noxische Reize bei Patienten mit Bewusstseinsstörungen, um die gezielten motorischen Reaktionen auszulösen, von denen man annimmt, dass diese Bewusstsein widerspiegeln. Diese Methode ist leider sehr fehleranfällig und somit werden Patienten häufig falsch diagnostiziert. Studien in der Vergangenheit haben gezeigt, dass die funktionelle Bildgebung zur Untersuchung des aktuellen Bewusstseinszustand in dieser Patientengruppe eingesetzt werden kann weil Patienten im "vegetativen Zustand" [VS; neu eingeführte Bezeichnung "unresponsive wakefulness syndrome" (UWS)] aufgabenspezifische Gehirnaktivitätsmustern zeigten. Kürzlich beschrieb Adrian Owen in seiner Studie eine UWS Patientin, die offensichtlich in der Lage war, Anweisungen zu befolgen, was ein eindeutiger Hinweis auf ein intaktes Bewusstsein ist. Allerdings müssen vor allem negative Ergebnisse der funktionellen Bildgebung mit großer Vorsicht interpretiert werden. Eine fehlende Aktivierung heißt nicht zwangsläufig dass der Patient kein Bewusstsein mehr aufweist, sondern es ist durchaus möglich dass der Patient, der Anweisungen nicht folgen konnte, z.B. aufgrund begrenzter Gedächtnisleistung oder Aufmerksamkeitskapazitäten, dennoch die Fähigkeit besitzt, emotionale Reize wie Schmerz und Freude zu empfinden. Deshalb wurden im Sinne eines hierarchischen Ansatzes eine Reihe von Experimenten entwickelt: einmal die einfachste Form der Verarbeitung der Schmerzreize, dann die emotionalen Reaktionen auf schmerzvolle Schreie anderer Personen und zuletzt eine komplexe und anspruchsvolle Aufgabe der mentalen Vorstellung.

In einer Gruppe von 54 nicht traumatischen Patienten mit einer klinischen Diagnose von UWS (N = 30) oder minimal bewusstem Zustand (MCS; N = 24) wurde die Gehirnaktivität gemessen. Es zeigten sich unterschiedliche Aktivierungsmustern: Einige Patienten zeigten

keine signifikanten Reaktionen bei allen drei Experimenten, hingegen andere, darunter auch Patienten, die als UWS diagnostiziert wurde, wiesen ähnliche Reaktionen wie die gesunden Probanden (Anzahl positiver Ergebnisse bei Patienten: 59,3% beim Schmerzexperiment, 46,3% beim Emotions-Experiment, 13% bei der mentalen Vorstellungsaufgabe) auf.

Die Ergebnisse zeigen, dass einige Patienten residuale kognitive Funktionen besitzen, obwohl sie diese nicht motorische ausdrücken können. Außerdem zeigen die Daten der vorliegenden Arbeit, dass die funktionelle Bildgebung eine zusätzliche Methode für die klinische Untersuchung ist. Darüber hinaus liefert die vorliegende Studie klare Belege dafür, dass die höheren kognitiven Funktionen wie Aufmerksamkeit, Arbeitsgedächtnis oder Sprachverständnis nicht notwendigerweise das ganze menschliche Bewusstsein widerspiegeln. Es gibt grundlegende und wahrscheinlich auch einfachere kognitive Funktionen, die nicht nur den eigenen Schmerz, sondern auch den Schmerz von anderen verarbeiten. Dieses so genannte affektive Bewusstsein kann trotz schweren Hirnschäden vorhanden sein, was darauf hindeutet, dass für viele Patienten einen emotionalen Kontakt mit dem Betreuungsteam hergestellt werden könnte, um ihre Lebensqualität zu verbessern.

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Outline

1 Introduction

1.1 Consciousness

"Consciousness: The having of perceptions, thoughts and feelings; awareness. The term is impossible to define except in terms that are unintelligible without a grasp of what consciousness means. Many fall into the trap of confusing consciousness with self-consciousness - to be conscious it is only necessary to be aware of the external world. Consciousness is a fascinating but elusive phenomenon: it is impossible to specify what it is what it does or why it evolved. Nothing worth reading has been written about it."

The International Dictionary of Psychology (ed. N.S. Suderland, 1989)

Questions about the phenomenon of consciousness have been asked for many decades. Although conscious experience is the most familiar thing to all of us, it is one of the biggest mysteries in the universe. On the surface, we know consciousness far more intimately than we know the rest of the world, because it is so fundamental. However, from the objective viewpoint we understand the rest of the world far better than we understand consciousness, since explicit verbalisation of an intuitive understanding of consciousness requires the transformation of the subjective first-person experience into an objective third-person description. The International Dictionary of Psychology really hedges the issue, as the British psychologist Stuart Sutherland (1989) argued that it is impossible to define consciousness. Indeed, numerous attempts have been made to define consciousness but none present definition is acceptable to all scientists. There seems to be no single answer that explains the nature of consciousness. While philosophy has dealt with the interaction between mind and body (Chalmers, 1996), psychology has focused on explanation mental phenomena with the subjective and objective experiences (Zeman, 2001), and neuroscience has explored

consciousness in terms of neural events occurring within the brain (Zelazo et al., 2007), cybernetics has programmed the computer to simulate consciousness (Russell & Norvig, 2010). The various views of philosophy, psychology, neuroscience, and computer science all come up with different interpretations of conscious experience.

Despite the lack of any agreement upon theory of consciousness, many experimental scientists believe that consciousness consists in the broad access to the whole system of brain processes and thus can be operationally defined by the dual aspects of wakefulness and awareness (Plum & Posner, 1980; Baars, 1988; Zeman, 2001; Laureys et al., 2005a; Kotchoubey, 2005a, 2008). Wakefulness, characterized by open eyes and responses to external stimuli, is provided by the brainstem ascending reticular activating system indentified by Morruzi and Magoun (1949) and its projections to a network of structures in the thalamus, basal forebrain and cerebellum which regulate the activation of more regions of the cerebral cortex (Garcia-Rill, 1997). Wakefulness defines the level of consciousness which may be decreased, increased, or roughly the same as that experienced in normal wakefulness.

Awareness refers to the sub-state that permits subjective dimension of human experience of sensations, perceptions, thoughts, memories, emotions, desires and intentions (Zeman, 2003). Awareness encompasses two components: awareness of the environment and awareness of self. Awareness of the environment refers to the conscious perception of one's environment through the sensory modalities (e.g. visual, auditory, and somatosensory). Awareness of self can be defined as a mental process without requirement of the mediation of the senses and is not related to external stimuli for its presence (e.g., mind wandering, daydreaming, inner speech, mental imagery, etc.). Awareness of self also refers to the knowledge of our own social and cultural history as well as our family membership (Gosseries et al., 2011a). Awareness defines the content of consciousness and requires a functioning reticular activating

system, but mainly relies on a functioning thalamus, cerebral cortex, and their white matter connections (Bernat, 2006). However, cortical activity (e.g. activity in a small, isolated cortical network) does not always give rise to awareness (Baars, 2002; Rees et al., 2002). Although wakefulness and awareness are intimately correlated in normal psychological states (e.g., one needs to be awake in order to be aware), it is possible to identify circumstances under which they are disconnected: in complex partial seizures it is possible to be awake but not aware; in rapid eye movement sleep and lucid dreaming awareness can occur without wakefulness (Gawryluk et al., 2010). Therefore, consciousness is not a black-or-white, all-or-nothing type of phenomenon, but varies along several states (Dennett, 1995). The boundaries between these states are not always sharp but often are gradual and progressive transitions (Gosseries et al., 2011a).

1.2 Disorders of consciousness

Whereas understanding consciousness is one of the major unsolved problems in science, the medical approach focuses on disorders of consciousness (DOC), such as brain damage leading to three main pathological states on a continuum ranging from coma with no awareness and arousal at the low end to minimally conscious state with full arousal and fluctuating awareness at the high end (Kotchoubey et al., 2011a). Since Bjorn Ibsen in Denmark invented the artificial respirator in the 1950s, it has become possible to sustain circulation function in individuals who previously didn't survive their severe brain injury. After an acute traumatic or non-traumatic brain damage, patients may lose consciousness and fall into a coma. Although some of these patients permanently lose all brain functions (brain death), others go on to make recoveries from coma within the first days after the acute brain insult. From the moment the patients open their eyes, they move out of a coma and progress to a condition of "wakefulness without awareness", also known as vegetative state (VS). Roughly one-half of

VS patients gradually recover awareness, entering a minimally conscious state (MCS), while others may awaken from their coma and evolve directly to MCS. It should be stressed that besides these three major kinds of DOC (coma, VS and MCS), there is a wide range of conditions and disorders that alter consciousness, including certain psychiatric disorders, pathophysiological conditions, temporary or chronic states and mild or severe impairments. Although these three main pathological states do not describe DOC completely, they provide a useful classification starting point that is commonly accepted in the literature (Gawryluk et al., 2010).

1.2.1 Coma

Coma is a pathological state of eyes-closed non-responsiveness in which patients cannot be aroused to wakefulness even when intensively stimulated (Plum and Posner, 1980). A state of coma typically follows bilateral hemispheric damage or white matter damage or occurs as a consequence of focal lesions to brainstem affecting the reticular activating systems and its thalamic projections (Bateman, 2001). Individuals in coma are characterized by complete loss of sleep–wake cycles, showing, at most, reflexive responses to painful stimulation and have neither wakefulness nor awareness. Comatose patients have reduced autonomous functions such as breathing and thermoregulation and require respiratory assistance. Coma hast to last for at least one hour to be distinguished from syncope, concussion or other states of transient unconsciousness. Persistent coma longer than a month is extremely rare in the absence of complicating metabolic, infectious, or toxic factors (Bernat, 2006). Patients begin to awaken and recover within 2 - 4 weeks and then progress to VS or MCS, or more rarely to locked-in syndrome or they die (Kotchoubey, 2008; Laureys et al., 2008). General slowing of the electroencephalogram (EEG) with theta activity and 50-70% decrease of global brain metabolism are reported in comatose patients (Laureys, 2005b).

1.2.2 Vegetative state

After a few days or weeks of coma, some patients may regain their sleep-wake rhythm, as indexed by cyclic eye opening and closing, start to breathe without assistance, show preserved autonomic function and exhibit spontaneous or stimulus-induced reflexive motor activity. These clinical signs typically indicate their progression to a vegetative state. Although the patients are awake, having roving eye movements, non-purposeful facial and limb movements, and retaining sufficient brainstem and hypothalamic functions for survival, they are considered, by definition, to be unaware of themselves and their surroundings (Jennett, 2002). Following criteria are used to characterize the vegetative state according to the guideline of the The Multi-Society Task Force on Persistent Vegetative State (1994):

- No evidence of awareness of self or environment
- Incapable of interaction with others
- No evidence of sustained, reproducible, purposeful, or voluntary behavioural responses to visual, auditory, tactile, or noxious stimuli
- No evidence of language comprehension or expression
- Presence of sleep-wake cycles
- Preserved hypothalamic and brain stem autonomic functions to permit survival with medical and nursing care
- Bowel and bladder incontinence
- Variably preserved cranial-nerve reflexes and spinal reflexes

This condition was first discovered by Ernst Kretchmer (1940) who described it as “apallic syndrom”, meaning a complete lack of cortical activity. Calvet and Coll (1959) called it later

"coma vigil" and Jennet and Plum (1972) coined the term "persistent vegetative state" in a classic paper in the lancet. The name was chosen because the patient's mental life is like the life of a plant (Kotchoubey, 2005a). All most important vegetative functions (respiration, thermoregulation, and metabolism) and even sleep-wake cycles are preserved. However, there is no experience. In 1994, the Multi-Society Task Force on PVS defined the temporal criteria for irreversibility and introduced the notion of permanent vegetative state. If patients are still in a vegetative state a month after brain injury, they are considered to be in a persistent vegetative state. If patients show no sign of awareness even longer than a year with a traumatic etiology or 3 months with non-traumatic injury, they are regarded as in a permanent vegetative state (The Multi-Society Task Force on PVS, 1994; Jennet, 2005a). These time ranges are based on the assumption that the chance of recovery decreases remarkably at these time points. In case of a permanent declaration, the ethical and legal end-of-life issues, of withholding and withdrawal of life sustaining treatment, can be discussed (Celesia , 2000; Jennet, 2005b). Although it is important to differentiate between persistent and permanent vegetative state, these two terms are often both abbreviated as PVS and cause unnecessary confusion. Moreover, although the probability to regain consciousness for patients defined as "permanent VS" is low, it is not zero (e.g., Dyer, 1997; Faran et al., 2006). Therefore, it is preferable to avoid the use of the terms and to describe a patient simply using the etiology and duration (Gosseries et al., 2011b).

70 years after Kretchmer's description, the European Task Force on Disorders of Consciousness proposed a new name for VS: "Unresponsive Wakefulness Syndrome (UWS)" (Laureys et al., 2010). This more descriptive and neutral term was chosen in order to solve the issue of the negative associations intrinsic to the word "vegetative" as well as the diagnostic errors and their potential effect on the treatment and care for the patients. "Unresponsive" refers to the fact that the patients are unable to show non-reflex movements or command

following. “Wakefulness” stress that they open their eyes spontaneously or upon stimulation. “Syndrome” means a number of clinical signs (Laureys et al., 2010). To be in line with the European Task Force on Disorders of Consciousness, the term UWS is used in the following text to describe the clinical and pathological condition of diffuse cortical damage that features wakefulness without awareness.

UWS is caused typically by critical damage to the thalami (Kampfl et al., 1998a; Adams et al., 2000), cerebral cortex, or its white matter connections (Owen & Coleman, 2008), and in few untypical cases by midbrain lesions (Matsuda et al., 2003; see also comment by Kotchoubey, 2004a). In contrast to coma, brainstem functions are mostly intact (Celesia, 1997; Giacino, 1997). UWS patients show substantial changes in electrical brain activity (general slowing) as well as in brain metabolism (40–50% of normal values). Somatosensory cortical potentials and brainstem auditory evoked potentials are often preserved (Laureys et al., 2004a). The prevalence of this condition is estimated to be between 56 and 140 per million in the USA (Multi-Society Task Force on the Persistent Vegetative State, 1994) and 19 per million in Vienna (Stephan et al., 2004), while in the Netherlands it lies about only 2 per million (Lavrijsen et al., 2005). The reason for the large variance in the estimation of prevalence is not clear, but could be explained by home care of some UWS patients or by withdraw decisions made earlier in the illness (Bernat, 2006), or by different inclusion criteria (Kotchoubey, 2008). In addition, these prevalence reports may suffer from poor psychometric characteristics (i.e. insufficient validity and reliability) (Beaumont and Kenealy, 2005).

1.2.3 Minimally conscious state

Some patients may continue to recover from the UWS, progressing to a condition in which consciousness is severely impaired, but some very weak and inconsistent evidence of

purposeful or voluntary responses can be observed (i.e. wakefulness with significantly diminished awareness). This borderline condition is coined “minimally conscious state” (MCS) (American Congress for Rehabilitation Medicine, 1995; Giacino & Kalmar, 1997; Giacino et al., 2002). In contrast to the UWS, MCS patients demonstrate reproducible, but extremely limited awareness of self or environment, and limited means of communication. The diagnostic criteria for the MCS were recently proposed by Aspen Neurobehavioral Conference Workgroup (Giacino et al., 2002):

- Global impaired responsiveness
- Command following
- Gestural or verbal yes/no response (regardless of accuracy)
- Intelligible verbalization
- Purposeful behaviour (movements or affective behaviours that occur in contingent relation to relevant environmental stimuli and are not due to reflexive activity) such as
  – smiling or crying in response to verbal or visual emotional (but not neutral) stimuli
  – vocalization or gestures that occur in direct response to the linguistic content of questions
  – reaching for objects that demonstrates a relationship between object location and direction of reach
  – touching or holding objects in a manner that accommodates the size and shape of the object
  – sustain visual pursuit to moving stimuli

Like the UWS, use of the term MCS does not necessarily imply irreversibility of the disease process, the condition may be transient, chronic or sometimes permanent. However, because the MCS is a more recently introduced entity and the knowledge about it is more limited than

that of the UWS, there is no well- established criterion of the time intervals for permanent MCS. Patients emerging into MCS after weeks or months in UWS may continue to recover slowly, regaining further meaningful behavioural functions over a period of months or years (Cranford, 2002). Bruno and colleagues (2011a) suggested a sub-categorization for the MCS condition with MCS+ for the display of high-level behavioural functions (e.g. command following or intelligible verbalizations) and MCS- for the evidence of low-level behavioural responses (e.g. visual pursuit, localization of noxious stimulation or purposeful behaviour such as appropriate smiling or crying to emotional stimuli). Since this entity is more recent than the MCS, validation and further research of other diagnostic criteria are still required. Unlike the coma and UWS, MCS hasn't been recognized by the World Health Organization in its International Statistical Classification of Diseases diagnosis codes. Some proposed to have a separate code for this condition because the lack of clear distinction between UWS and MCS might encumber scientific studies and international analyses in the challenging field of DOC (Gosseries et al., 2011; Charland & Laureys 2011), while others remained sceptical to the usefulness and wisdom of creating such a new diagnostic category (Coleman, 2002; Shewmon, 2002).

From a neurologic standpoint, MCS patients are thought to function at the level of a patient with severe dementia (Cranford 2002). General slowing of the electrical brain activity and an overall decrease of cerebral metabolic activity by 20–40% were observed in MCS patients (Laureys et al., 2004b). According to the Aspen Neurobehavioral Conference Workgroup, the prevalence of adult and paediatric cases of MCS in the United States is estimated to be between 112,000 to 280,000 based on operationally defined diagnostic criteria extracted from a large state registry (Giacino et al., 2002).

1.2.4 Traumatic versus non-traumatic aetiology

UWS and MCS are clinical syndromes that can be caused by head injury, brain anoxia, hemorrhage (particularly subarachnoid hemorrhage), less frequently by encephalitis or toxic brain lesion (Kotchoubey et al., 2005b). These various causes can be categorized into three main types: acute traumatic brain injuries, non-traumatic damage (include acute hypoxic-ischemic neuronal injury suffered during cardiopulmonary arrest, stoke and meningoencephalitis), and other causations like neurodegenerative disorders, metabolic dysfunctions and developmental malformations (Kinney and Samuels, 1994). Traumatic and non-traumatic brain injuries typically produce acute coma and from there to either death or DOC. The very rare neurodegenerative or metabolic diseases can gradually lead to the UWS or MCS without an acute coma phase (Kotchoubey, 2008).

Patients with traumatic brain injuries typically exhibit diffuse axonal injuries that disrupt white matter connections (Kinney and Samuels, 1994). It is caused by a shearing effect on the axons exerted by different torques induced in dense grey matter and less dense white matter (Adams et al., 1989; Bernat, 2006). Small haemorrhages followed in the corpus callosum, the upper brainstem, fronto-parietal white matter tracts and the corona radiata can be observed in this type of injury (Tong et al., 2004; Kotchoubey, 2008). Traumatic brain injury damages the white matter tracts more than the grey matter (Bernat, 2006). Gray matter lesions are in most cases restricted to the medial thalamus and the basal ganglia. Patients with non-traumatic etiology show various degrees of cortical and thalamic neuron death, while the brainstem neurons remain intact in most cases because their relatively low metabolic demands make them less susceptible (Dougherty et al., 1981). Non-traumatic injuries lead to extensive multifocal or diffuse laminar thalamo-cortical necrosis. One of the most striking characteristics of the Schiavo case was his 615 g brain, as a consequence of cortical necrosis

as well as total loss of basal ganglia and thalamic neurons (Bernat, 2006). In general, non-traumatic injury damages the grey matter more than the white matter (Kinney and Samuels, 1994).

Previous studies showed that adults in a coma immediately after a non-traumatic injury have a poorer prognosis than those in a coma after a traumatic injury, with 85 percent or more dying within the first month after the insult or remaining in a UWS (Levy et al., 1985; Bates, 1991). Prognosis for recovery from UWS and MCS is limited. According to the Multi-Society Task Force on PVS (1994), 33% of UWS patients with traumatic injury for one month recovered consciousness within three months, and 52% recovered within one year. After a non-traumatic injury, 11% percent of UWS patients for one month recovered consciousness within three months, and 13% recovered within one year. The same tendency that the traumatic etiology has a better prognosis than non-traumatic injury was also found in MCS patients (Giacino et al., 2002). The prevalence of traumatic UWS is estimated to be between 1 and 10 per 100,000, and non-traumatic UWS is at least as frequent (Grossman and Hagel, 1996).

1.3 Assessment of consciousness

For many years, the majority of mainstream scientists did not accept consciousness as a research topic due to the general belief that a phenomenon defined in subjective terms could not properly be studied using objective experimental methods (Hendriks-Jansen, 1996). In medicine, clinicians tried to quantify consciousness by observing a patient's arousal and responsiveness, and by disentangling automatic responses from volitional movements or command following (Bruno et al., 2011b). However, the interpretation of exhibited behaviours can be very challenging. In the absence of any agreed definition of consciousness, there remains considerable debate as to whether certain behaviours should be classified as

conscious as opposed to unconscious processes (Coleman et al., 2009). By this fact, the line between conscious and unconscious must ultimately rely on the pragmatic principle that a person can only be considered to be unequivocally conscious if he or she can signal that this is the case (Monti et al., 2009), as Passingham and Lau (2006) noted "The operational index of consciousness is the ability to report". The challenges are particularly difficult when the assessment is associated with patients in DOC because of a degree of diagnostic uncertainty ranging from unaware to fully conscious, and also because that motor responses in these patients may be minimal, inconsistent, easily exhausted, or may be undetectable (Owen and Coleman, 2007). A patient who is unable to perform reproducible, purposeful or voluntary behavioural responses due to inability to speak or blink an eye or move a hand, may retain partial levels of conscious processing, thus cannot be designated unconscious. Furthermore, a MCS patient can be wrongly diagnosed as being in UWS, just as a patient in locked-in syndrome (LIS) can be easily confused with a UWS. Cases like Julia Tavalaro, Gary Dockery and Terry Wallis sadly represent the real-world examples of the problem (Tavalaro and Tayson, 1997; Doyle, 2001).

Indeed, assessment of wakefulness and awareness by behavioural observation leads to a high rate of misdiagnosis. Clinical studies performed in the early 1990s by Childs et al. (1993) in Texas and Andrews et al. (1996) in London found around 40% of patients referred to rehabilitation centres with a diagnosis of UWS were in fact misdiagnosed. Many patients were able to communicate when assessed by a specialist team, even though most of them were found to have severe visual impairments and joint contractures. It should be stressed that these study were conducted before the international agreed diagnostic criteria were published and therefore might be overly pessimistic (Laureys et al., 2010). However, a recent study by Schnakers et al. (2009) confirmed the unchanged high rate of diagnostic error despite the publication of the Multi-Society Task Force on PVS (1994) criteria as well as the publication

of clinical guidelines on the diagnosis criteria for MCS (Giacino et al., 2002). Such a high rate of diagnostic error suggests the need for greater diagnostic precision for DOC patients because the diagnosis may have an impact on prognosis and treatment (Owen and Coleman, 2008). In this perspective, many studies have suggested that additional techniques, such as electrophysiological and brain imaging assessment tools, which does not rely upon an overt motor response, might be valuable in the assessment of DOC patients with preserved cognitive functions that are undetectable using standard clinical methods (Kotchoubey, 2005c; Laureys, 2006; Schiff, 2006; Owen, 2008).A multimodal assessment approach including both standard clinical behavioural methods and instrumental measures may help to reduce the risk of misdiagnosis.

1.3.1 Behavioural assessments

Numerous behavioural rating scales have been developed and validated for the assessment of consciousness in brain injured patients (Majerus et al., 2005). A general consensus has been reached that the behavioural measurement should explore each sensory modality in turn through a series of stimuli which scale in complexity (Coleman et al., 2009). There is a large variability in the psychometric integrity of the various tools (Bernat, 2006). Below are some sophisticated assessment methods that are used most frequently in clinical practice.

Glasgow Coma Scale

For many years, the "gold standard" for testing consciousness has been the Glasgow Coma Scale (GCS), which is widely used in acute trauma and intensive care settings. Since Teasdale and Jennett (1974) firstly reported and validated the GCS, it has been used internationally because of its brief and simple administration in routine clinical care. The GCS has three subscales that measure arousal level, verbal abilities and motor behaviours. The total score

can range from 3 to 15. Brain damage is considered to be “moderate” if a patient scores between 9 to 12 and “serious” with a score less than 9 (Deuschl and Eisen, 1999). Despite its widespread use, the GCS has been criticized as insufficient for the assessment of UWS and MCS due to its crude measurement of awareness and its omission of relevant neurological functions (Howard and Hirsch, 1999). The Glasgow Liege Scale (GLS) is an extended version of the GCS which allows additionally a standardized evaluation of brainstem reflexes (Born, 1988).

Sensory Modality Assessment and Rehabilitation Technique

The Sensory Modality Assessment and Rehabilitation Technique (SMART) (Gill-Thwaites, 1997) was designed especially for VS patients to identify minimal and fluctuating signs of consciousness that can be missed by using the GCS. The SMART correlates with an appropriate rehabilitation programme for each level of function and has been validated in a group of 60 UWS and MCS patients (Gill-Thwaites and Munday, 2004).The SMART is a hierarchical scale including five response levels (Level 1 = no response; Level 2 = reflex response; Level 3 = withdrawal response; Level 4 = localization response; Level 5 = discriminative response) (Giacino et al., 2009).

Wessex Head Injury Matrix

The Wessex Head Injury Matrix (WHIM) was developed to set up a transition between the assessments of acute coma and the later applicable realization of neuropsychological tests (Shiel et al., 2000). The 62 items of WHIM covering a wide range of daily life functions were created on the basis of a longitudinal observation of 97 severely brain-injured patients recovering from coma and then ordered according to the sequence of recovery observed in these patients (Gosseries et al., 2011a). The WHIM evaluates arousal level and verbal communication, visual-motor modalities, cognitive skills, and social behaviours (Majerus and

Van der Linden 2000) and offers the advantage of particularly sensitive detection of improvements in MCS patients.

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Coma Recovery Scale-Revised

The Coma Recovery Scale-Revised (CRS-R), which is originally developed by clinicians from the JFK Johnson Rehabilitation Institute (Giacino et al., 1991), is a recent clinical tool that has been specifically developed to differentiate MCS from UWS patients, evaluate prognosis and establish treatment plans (Giacino et al. 2004). The CRS-R is considered to be the only behavioural scale incorporating the international diagnostic criteria of UWS and MCS. Psychometric studies have confirmed the reliability and diagnostic validity of the CRS-R across raters with varying levels of experience indicating that CRS-R as an index of neurobehavioral function can be used to semantically search for signs of voluntary behaviour by trained examiners and produces reasonably stable scores in interdisciplinary medical rehabilitation (Schnakers et al., 2008a). Indeed, the CRS-R is regarded as the new gold standard for the behavioural assessment in DOC patients. The 23 items of CRS-R were selected to constitute six subscales addressing auditory, visual, motor, oromotor, communication, and arousal functions. The items are hierarchically arranged according to their degree of complexity with the lowest item on each subscale representing reflexive response and the highest item reflecting meaningful and purposeful behaviours. Scoring is standardized and based on the presence or absence of operationally defined behavioural responses to specific sensory stimuli (Giacino et al., 2009; Gosseries et al., 2011a). Depending on the patient's responsiveness, it takes about 10 to 60 minutes to perform the assessment. Despite the relatively high level of reliability and validity, CRS-R results can strongly vary as a function of the experience of the diagnostician (Løvstad et al., 2010). CRS-R is freely available in different languages (see http://www.comascience.org).

1.3.2 Electrophysiological assessments

Electroencephalography (EEG) records directly the spontaneous electrical brain activity induced by neuronal functional activity in the cortex through electrodes placed on the scalp. It provides a unique and non-invasive measurement to obtain information about how the cortex processes signals and prepares actions (Picton & Hillyard, 1988; Kotchoubey, 2002a; Kotchoubey, 2005a). This technique has been used for a long time to evaluate both sensory and cognitive functions in patients with severe brain damage (Reuter et al., 1989; Kotchoubey et al., 2001; Kotchoubey et al., 2006a; Wu et al., 2011). Most UWS patients demonstrated generalized slowing of background electrical brain activity in the delta range and the patients in the most sever form showed electrocerebral silence (Hansotia, 1985). Kulkarni et al. (2007) reviewed a series of EEG performed in UWS patients to assess the diagnostic value of EEG and found that the EEG findings in UWS showed a large variety including normal, continuous generalized slowing, intermittent generalized slowing, background slowing, background suppression, alpha, generalized periodic pattern, PLEDS, and triphasic waves. Therefore the EEG findings in UWS may be too heterogeneous and too variable to be of diagnostic value (Kulkarni et al., 2007). Evidence in MCS patients of diffuse slowing in the theta or delta range has also been shown in several studies. Most MCS patients have diffuse slowing in the theta or delta range (Giacino, 2005), or in the theta or slow alpha (7.5–8 Hz) range (Kotchoubey et al., 2005). In accordance with the results in UWS patients (Kulkarni et al., 2007), the spectrum of EEG findings in MCS is also exceedingly variable (Young, 2000). The diagnostic value of individual classifications has not been adequately addressed, although in some cases certain EEG features are associated with a poor outcome and provide useful information in predicting eventual survival (Wu et al., 2011)

Bispectral Index

The EEG bispectral index (BIS) is a variable statistically and automatically derived from the EEG, which is originally developed and validated as an assessment tool for depth of anaesthesia and sedation. The BIS is normalized on a scale of 0 (isoelectric signal) to 100 (patient fully aroused). Low BIS values ranging from 40–55 indicate that a patient is unconscious during general anaesthesia or other diminished states of consciousness such as natural sleep (Schnakers et al., 2008). Pandit et al. (2002) reported that the BIS profile of a UWS patient during anaesthesia and surgery was similar to that of a normal subject. Schnakers et al. (2008) found in 43 DOC patients a higher correlation between behavioural scales and BIS as compared to other EEG parameters. Higher BIS values were found in patients who recovered at 1 year post-insult showed higher BIS values as compared to patients who did not recover. Moreover, BIS values differentiated levels of consciousness and distinguished VS from MCS while other EEG parameters did not (Schnakers et al., 2008). EEG-BIS recording is an interesting additional method that enables to predict recovery of consciousness in patients with severe brain injury (Fabregas et al., 2004).

Short-Latency Event-Related Potentials

Sensory and cognitive event-related potentials (ERPs) provide an objective method for assessing preserved brain functions at the patient's bedside by averaging the EEG activity according to the onset of a repeated stimulus (Kotchoubey, 2006b). Short-latency ERPs (up to 100 ms after a stimulus), or exogenous ERPs (e.g., brainstem auditory, somatosensory and visual evoked potentials) depend on the physical properties of the external stimulus (i.e., mainly on stimulus qualities) to detect whether is any delay in propagation of sensory signals from receptors via ascending pathways to the cortex (Coleman et al., 2009). Short-latency ERPs are routinely used in intensive care and offer valuable information if a particular sensory pathway is functioning. Short-latency ERPs are known to persist even in unconscious states (Laureys et al., 2006). Previous studies of short-latency ERPs have confirmed that the

absence of cortical somatosensory evoked potentials such as N20 indicates poor outcome of recovery from coma (Robinson and Micklesen, 2004, Carter and Butt, 2005). To sum up, short-latency responses possess a high false positive rate but a low false negative rate and can, therefore, be predictors of a poor prognosis but not for a good prognosis.

Long-Latency Event-Related Potentials

Long-latency ERPs (obtained after 100 ms), or endogenous ERPs (i.e., mainly on the task and subject's state) are more important and reliable since they reflect cognitive neuronal activity. Long-latency ERPs such as P300, mismatch negativity (MMN), P600, readiness potential, enable the identification of individual physiological components that contribute to a particular cognitive process (e.g. detecting an infrequent event in an auditory paradigm) (Coleman et al., 2009). Although studies using short-latency ERPs have proved helpful in informing the diagnostic decision-making process, long-latency ERP studies using more complex stimulation provide more beneficial information in DOC patients (Neumann and Kotchoubey, 2004). Long-latency ERP components can be classified according to whether they reflect the processing of physical stimulus features (e.g., pitch, intensity, duration), or the analysis of stimulus meaning (e.g., semantics) (Kotchoubey, 2005c).

MMN is a short-duration auditory cognitive ERP response typically elicited after approximately 100–200 ms by acoustical deviations in frequency, intensity, duration, timbre, or more complex stimulus patterns (Kotchoubey, 2005c). MMN indexes operation of the auditory sensory memory system (Javitt et al., 1998). It is a good predictor of awakening and it precludes comatose patients from moving to UWS (Naccache et al., 2005; Fischer et al., 2006; Daltrozzo et al., 2007). However, a recent study performed in a group of 27 UWS and 11 MCS patients showed that the ERP is not related to the time from coma onset and cannot differentiate MCS patients from UWS patients (Fisher et al., 2010).

The auditory evoked potentials P300 (around 300 ms after the stimulus) is another ERP response elicited by detecting a rare, perceptually salient and unpredictable stimulus in a regular train of standard stimuli (Sutton et al. 1965). It assesses the integrity of acoustic and semantic discrimination. Reuter et al. (1989) were probably the first who observed a P3-like wave to rare stimuli in three UWS patients, a finding replicated by Rappaport et al. (1991), who examined eight severely disabled patients, five of whom were in UWS. Later two larger studies were performed by Witzke and Schoenle (1996) who evaluated the ERP components of 66 UWS patients on the basis of mere visual inspection of the waveforms, and by Kotchoubey et al. (2001, 2003) who assessed a large population of DOC patients and applied, for the first time, strict statistical criteria based on the analysis of single trials. Since that time ERP studies have identified aspects of preserved speech processing in patients considered to meet the clinical diagnostic criteria defining UWS and MCS (Kotchoubeyet al., 2005b; Schnakers et al., 2008c). The P300 response was also found when the DOC patients hear their own name in a sequence of unfamiliar names in a passive condition (Kotchoubey et al., 2004b; Perrin et al. 2006). In MCS patients, Schnakers et al. (2008c) found a larger P300 response to the subject's own name (SON) in both a passive condition and an active condition in which patients were asked to perform a cognitive task such as counting the number of times they heard their own name. In UWS patients no such an effect was observed in both the passive and active conditions.

Mismatch negativity (MMN) and the P300 components have also shown some useful prognostic utility in identifying those patients who might go on to recover consciousness or progress to a UWS following severe brain injury (Wijnen et al., 2007; Fischer et al., 2008). In 50 severe comatose patients, MMN to deviants was found in 14/50 patients and a central–parietal P3 to SON was found in 21/50 patients (Fischer et al., 2008). The use of P300 elicited

by SON increases the prognostic value of MMN alone and improves the assessment of comatose patients by demonstrating the activation of higher-level cognitive functions in some of them (Fischer et al., 2008). A meta-analysis of Daltrozzo et al. (2007) indicated that MMN and P300 appeared to be reliable predictors of awakening in low-responsive patients with stroke or haemorrhage, trauma and metabolic encephalopathy aetiologies.

EEG offers a great advantage that it can be applied at patient's bedside. Although in EEG technique only cortical processes can be measured, for UWS and MCS patients the issue of interest is whether and to what extent cortical information processing takes place (Kotchoubey et al., 2002b; Kotchoubey et al., 2005b). However, ERP studies may have some limitations. First, the presence of cortical ERPs does not necessarily imply consciousness (Yingling, 2001). Perrin et al. (2006) argued that the P300 is not a reliable marker of consciousness but rather reflects automatic processing, because it could be recorded in well-documented UWS patients who never recovered. Second, most of the EEG patterns are not specific and do not allow reliable differentiation between conscious and unconscious brain processing (Kotchoubey et al., 2005b). Third, the interpretation of raw EEG signals also requires considerable expertise and training (Gosseries et al., 2011). Nevertheless, ERPs would appear to represent an objective assessment tool, which enables identifying residual cognitive functions in DOC patients and would benefit from further investigation using brain imaging techniques (Coleman et al., 2009).

1.3.3 Brain imaging assessments

Like electrophysiological assessments, brain imaging method such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), are another instrumental technique which offers an objective index of brain activity at rest and during

active cognitive processing independent on the patient's ability of demonstrating overt behavioural responses. Therefore, these techniques are well equipped to identify covert cognitive functions in DOC patients who are otherwise incapable of intelligible or sustained behavioural expression, and provide complementary information to clinical examinations (Giacino et al., 2009). While functional brain imaging discloses how consciousness is mapped in the brain, anatomical brain imaging reveals features of diagnostic specificity for DOC patients (Bernat, 2006). Anatomical magnetic resonance imaging (MRI) sequences and computer tomography (CT) showed a widespread cortical and thalamic atrophy in UWS patients that increases in severity after months to years (Jennett, 2002). Kampfl et al. (1998a, 1998b) assessed 42 UWS patients after closed head injury using anatomical brain imaging and found that the degree of atrophy is a poor predictor of later improvement. Other studies using diffusion-weighted MRI sequences obtained within a week after a hypoxic-ischaemic injury showed that the morphology is a better predictor for recovery from coma: the presence of large, symmetrical areas of restricted diffusion, particularly in the hemispheric white matter, is associated with poor outcome (Chalela et al., 2001; Els et al., 2004). At the same time, the advances in functional neuroimaging have allowed great progress not only in our understanding of the brain structure, but also in understanding of the function and connectivity as well as its relationship to the emergence of consciousness (Charland and Laureys, 2011). There are currently several functional neuroimaging methods, some of them measure brain activity at rest and others use passive sensory stimulation or active paradigms to observe the cortical responses.

Functional neuroimaging at rest

In the last twenty years, various functional neuroimaging techniques such as fluorodeoxyglucose (FDG), positron emission tomography (PET) and single-photon emission computerized tomography (SPECT) have been used to measure resting-state metabolic

function (Levy et al., 1987; Beuthien-Baumann et al., 2003) and its change over time relative to clinical improvement (Voss et al., 2006). PET studies showed that the grey matter metabolic rates are reduced to 50-70% in comatose patients (Tommasino et al., 1995). This baseline decrease of cerebral metabolism is similar to the level of healthy individuals during deep sleep and general anaesthesia (Maquet et al. 1997; Alkire et al. 1999). In UWS patients, a globe decrease in metabolic function of 50-60% was observed (Laureys et al., 2004a), whereas in MCS patients the metabolic pattern differs significantly with a reduction of global hypometabolism to 20–40% of normal value (Schiff et al., 2005). Some brain regions appear to be more important than others for the recovery of consciousness. In UWS patients certain cortical regions in the frontoparietal network, including bilateral prefrontal regions, Broca's area, parieto-temporal and posterior parietal areas as well as precuneus, are particularly impaired (Laureys et al., 2004a). In MCS, these regions remain relatively well preserved and their functional connectivity is generally retained (Laureys et al., 2000). Precuneus and posterior cingulate cortex are supposed to be the most activated regions during wakefulness and the least activated under general anaesthesia or during deep sleep, the metabolic activity of MCS patients in these two regions is significantly greater as compared to the values noted in UWS patients (Laureys et al., 2005a). Indeed, in a single case study recovery from UWS was shown to be paralleled by the restoration of metabolic functions in the two regions (Laureys et al., 1999).

Another functional neuroimaging approach to measure brain activity at rest became recently possible by means of fMRI. In contrast to PET, fMRI resting state measurement obtains data typically within 10 minutes and provides a more indirect assessment of neural functions (i.e. evaluating hemodynamic changes). Resting state fMRI showed that the default mode network consisting of the posterior cingulate cortex / precuneus, temporo-parietal junctions, medial prefrontal cortex and the parahippocampal gyri, which is considered to represent internal or

self awareness (Soddu et al., 2009; Vanhaudenhuyse et al., 2010a), is critically impaired in UWS patients (Boly et al., 2009). Another study in MCS patients by Vanhaudenhuyse et al. (2010b) demonstrated an intermediate pattern with a higher functional connectivity of the posterior cingulate cortex and precuneus as compared to UWS patients. In addition, the degree of connectivity within this network identified with fMRI appears to be quantitatively related to the behavioural CRS-R scores (Gosseries et al., 2011b). Although neuroimaging studies at rest are useful, they can only identify functionality at the most general level rather than relating cerebral activity to specific cognitive processes (Owen and Coleman, 2007)

Functional neuroimaging with external stimulation

Functional neuroimaging studies employing auditory, visual and somatosensory stimuli are of greater relevance since the so-called "activation" studies can capture precisely regional physiological changes of cerebral activity and link them to specific cognitive processes. In the late 1990s, De Jong et al. (1997) used PET in a posttraumatic UWS patient to measure regional increases in cerebral blood flow in response to a story told by his mother. Increased blood flow in the anterior cingulate and temporal cortices was found in comparison to non-word sounds, possibly reflecting emotional processing of the contents, or tone, of the mothers speech. Meanwhile, another PET study was performed by Menon et al. (1998) to investigate covert visual processing in response to familiar faces. Robust activity was observed in the right fusiform face area when pictures of the faces of family and close friends were presented to an UWS patient. Both studies gave some information of the utility of brain imaging to explore preserved cognitive function, however, both studies only reported single cases and it was unclear whether the utility of these tests would extend to groups of patients (Coleman et al., 2009).

Two years later, Schiff et al. (2000) used combined recordings of PET and magnetoencephalography (MEG) together with anatomical MRI and found some correlations between neurophysiologic and behavioural indices of residual cortical functions in 5 UWS patients. In another PET study of 15 vegetative state patients, Laureys et al. (2002) found that high-intensity electrical stimulation activated the midbrain, the contralateral thalamus and the primary somatosensory cortex in every patient but not in the secondary somatosensory, insular, posterior parietal or anterior cingulate cortices. The greatest limitation of PET is the low power to detect statistically significant responses. Group studies are often required to satisfy standard statistical criteria. Given the heterogeneous nature of DOC and the clinical need to define each patient in terms of their diagnosis, residual functions and potential for recovery, such limitations are of paramount importance in the evaluation of these patients (Owen and Coleman, 2007).

Recently there has been a significant development in the field of functional neuroimaging: the relative shift of emphasis from PET to fMRI (Owen and Coleman, 2008). Compared with PET, fMRI is more widely available, provides increased statistical power, improved spatial and temporal resolution and has no associated radiation burden (Owen et al., 2001). fMRI has since been used to detect different aspects of preserved cognitive, sensory and auditory function (Bekinschtein et al., 2005; Schiff et al., 2005; Owen et al., 2005, 2006; Staffen et al., 2006; Di et al., 2007; Coleman et al., 2007). As compared with UWS patients, MCS patients demonstrated a more striking and extensive activation of distributed cortical neuronal networks that are normal or close to normal values, suggesting a much more integrated information processing (Laureys and Boly, 2007). For example, both study by Laureys et al. (2004b) using baby cries and study by Schiff et al. (2005) employing story read by the patient's mother showed that auditory stimuli with emotional valence elicit significantly

extended brain activation in MCS in contrast to sounds without meaning (Charland and Laureys, 2011).

Among the most challenging problems in DOC is the question whether neuroimaging can provide unequivocal evidence of awareness. Owen et al. (2006) presented remarkable findings in a carefully reported case based on mental imagery tasks. A 23 years old UWS patient who suffered a traumatic brain injury 5 months earlier was asked to imagine playing tennis and moving around the rooms in her house. The results showed brain activation similar to those obtained from a group of healthy subjects performing the same task. This study demonstrated clear evidence of conscious awareness in the absence of overt behavioural responses.

A lot of recent studies have made a strong case that fMRI has also the power to assess high-level components of linguistic comprehension based solely on brain activation patterns (Monti et al., 2009). Coleman et al. (2007) compared cortical responses to sentences containing semantically ambiguous words (e.g., there were "dates/pears" in the fruit bowl) with matched unambiguous sentences. A subset of UWS and MCS patients tested on this task showed activations in posterior temporal and inferior frontal cortices that were indistinguishable from those have been observed in healthy subjects. In another study, Yu et al. (2011) found that hearing of factually incorrect sentences (e.g., primary school students learn "statistics/mathematics") induced higher fMRI signals in two classical language areas: Broca's and Wenicke's areas and their homologues in the right hemisphere as well as the thalamus. The authors argued that the paradigm may be more suited to identify signs of consciousness in non-communicative patients, because a finding of a consistent brain response to factually incorrect sentences would be a stronger argument for the presence of conscious awareness than the response to semantically incongruent sentences.

It has been argued that fMRI studies in DOC patients should be applied hierarchically, starting with the simplest form of processing within a particular domain (e.g. primary perceptual responses) and then progressing sequentially through more complex cognitive functions (Owen and Coleman, 2008; Kotchoubey and Lang, 2011c). Although no single paradigm achieves all these goals, when combined they might have the ability to provide valuable information which informs and may even change a patient's diagnosis (Coleman et al., 2009). Kotchoubey et al. (2011b) described an interesting case in a 61-year-old woman, who had a brainstem infarction including the pons cerebri due to a basilar thrombosis. An intra-arterial lysis was performed, which resulted in a subarachnoidal and left parietal intracerebral haemorrhage. The primary infarct indicated a possible LIS, while the two additional haemorrhages could have resulted in a DOC such as UWS or MCS. To help in disentangling possible disorders, a battery of functional tests using fMRI was performed including pain, trace conditioning and mental imagery paradigms. The positive fMRI results indicated that the patient's condition would gradually evolve towards LIS or another state of severe immobility rather than UWS or MCS. This conclusion resulted in a decision for more aggressive behavioural therapy, later in combination with pharmacological treatment. On the basis of the fMRI data, the authors were able to obtain funding to prolong the patient's stay in the rehabilitation unit. The prognosis was confirmed, and several weeks later the patient improved above the level of MCS.

All these results provide compelling cases that technological developments in the field of instrumental assessments have greatly increased our ability to detect preserved cognitive processes in the absence of any overt behavioural response in DOC patients, by simply observing patterns of brain activation.

2. Aim of the current research

From a diagnostic point of view, recovery from coma, a deep state of unconsciousness, is indicated by the return of signs of wakefulness. However, differentiating UWS from MCS patients can be very challenging. Existing clinical criteria often emphasize the evidence of voluntary behavioural responses, but it is not clear what type of evidence is sufficient to certify that a specific motor output is purposeful or meaningful because movements that appear to be voluntary my actually be reflexive in nature and vice versa (Laureys et al., 2006). This difficulty may reflect a number of problems including discrepancies in the diagnostic guidelines between countries and a lack of consistency in patient assessment. There is a need for using additional evidence-based techniques, such as functional neuroimaging, which does not rely upon a motor action to identify preserved cognitive function, as a supplement to current clinical assessments (Monti et al., 2009).

To date, only few neurophysiological studies have demonstrated unequivocal, objective evidence of consciousness in behavioural defined DOC patients using a motor imagery paradigm (Kotchoubey et al., 2003; Owen et al., 2006; Monti et al., 2010). This approach relies upon the nature and self-evident criteria of consciousness and thus may encounter a serious problem of addressing higher-level consciousness (Kotchoubey and Lang, 2011d). Performance of the imagery task developed by Kotchoubey et al. (2003) and Owen et al. (2006) requires not only language comprehension, but also other complex cognitive functions such as selective attention, concentration on the task, short-term memory to remember the instruction, and the translation of the instruction into an imagination. Lacking only one of these abilities would lead to failure and result in a significant rate of false negative, not to mention that this situation could be further complicated when patients have underlying

deficits in communication functions, such as aphasia, agnosia or apraxia. Therefore, it is still unclear whether this technique will be applicable to a wide range of patients.

Panksepp (2005) suggested that a different kind of awareness, putatively called affective awareness, being a comparatively intrinsic function of the brain, is the easiest variant of consciousness. Affective awareness is simple and more stable and can thus survive even a very severe brain damage that is incompatible with cognitive conscious functions (e.g., attention, language, imagination). Since the intrinsic coherence of emotional responses can be demonstrated by activation of specific brain zones in response to external stimulation, one may ask whether DOC patients can also exhibit the corresponding changes in neural activity. If the idea of affective awareness, which is more robust toward brain trauma than cognitive awareness, is correct, one can expect that relevant brain activations in DOC patients would be found more frequently than in active command following tasks.

The aim of the present study was, therefore, a systematic examination of several levels of consciousness using the fMRI technique. We report the performance of a group of 15 age-matched healthy controls and a group of 54 non-traumatic DOC patients on sensory affects (pain perception), emotional feelings (hearing pain cries of other persons) as well as on a more complex cognitive task (mental imagery). These three experimental paradigms comprise a hierarchical approach to the fMRI assessment of patients with impaired consciousness, which can be very helpful by providing two advantages. First, they can offer a level of internal consistency when the three experiments all reveal positive findings. Second, they also present valuable information about cognitive specificity (Monti et al., 2009). For example, if activation is found in pain and emotional experiments, but not in mental imagery paradigm, then it would suggest that the patient may retain preserved function in pain and emotion processing, whereas nothing can be concluded about more complex cognitive functions.

The present study should answer the question how prevalent such residual aspects of cognitive processes can be found in UWS and MCS patients, and based upon reliable quantitative evaluation methods rather than subjective expert judgments. Our hypotheses were that: (1) in DOC patients, hemodynamic BOLD responses to emotionally relevant stimuli (pain, pain cries of others) will be more frequently observed than responses in the mental imagery task and (2) there is a significant difference in residual cognitive functions between MCS and UWS patients.

3. General methods

3.1 Healthy controls

Fifteen subjects (aged 29 to 62, mean = 43.60, SD = 10.37; 7 females) participated in the study. All participants were right-handed native speakers of German. They gave informed written consent and were paid for their participation. All reported no history of psychiatric or neurological disorders, and no current use of any psychoactive medications. The study was approved by the local Ethics Committees (University of Tuebingen) and conducted in accordance with the Declaration of Helsinki.

3.2 Patients

As stated above in Section 1.2.4, there are substantial differences in terms of morphology and functional characteristics between traumatic and non-traumatic DOC patients. Because the number of traumatic DOC patients in Germany is steadily decreasing due to the progress in the acute neurosurgery, non-traumatic aetiologies (e.g., brain anoxia, stroke) can be regarded as constituting the main problem now. For this reason, the present study included only non-traumatic DOC patients.

Fifty-four patients (aged 16 to 75, mean = 49.26, SD = 15.35; 24 females) were examined, of whom, thirty patients met the diagnostic criteria of UWS (Laureys et al., 2010), and twenty-four patients fulfilled the diagnostic criteria of MCS (Giacino et al., 2002). The UWS group contained twenty-five anoxic, four hemorrhage and one encephalopathy patients. The MCS cohort comprised of nine anoxic, nine hemorrhage, two stroke patients and three patients with encephalopathy. The diagnosis of UWS or MCS was based on extensive clinical testing and

(in 47 patients) also on a standardized behavioural assessment using the Coma Recovery Scale-Revised (CRS-R; Giacino et al. 2004). All patients had preserved auditory brainstem potentials at least on one side. The study was performed according to the declaration of Helsinki and approved by the local ethics committee (University of Tuebingen). Patients' legal representatives had given their written informed consent.

Patients' morphologic information provided by T1-weighted scans was assessed using a visual rating scale developed by Galton et al. (2001) and Bekinschtein et al. (2009) (0 = no atrophy, 1 = very low, 2 = mild, 3 = sever, 4 = highly severe atrophy). The degree of cortical and subcortical atrophy was evaluated by experienced raters who were blind concerning the identity of patients. The T1 anatomical images were inspected without any transformation and normalisation process. The time interval between measurement and disease onset correlated significantly with the degree of atrophy (Spearman $\rho = 0.761$, $p < 0.001$) (see Figure 1), but none of these variables correlated with the CRS-R score. The relevant clinical characteristics are listed in Table 1.

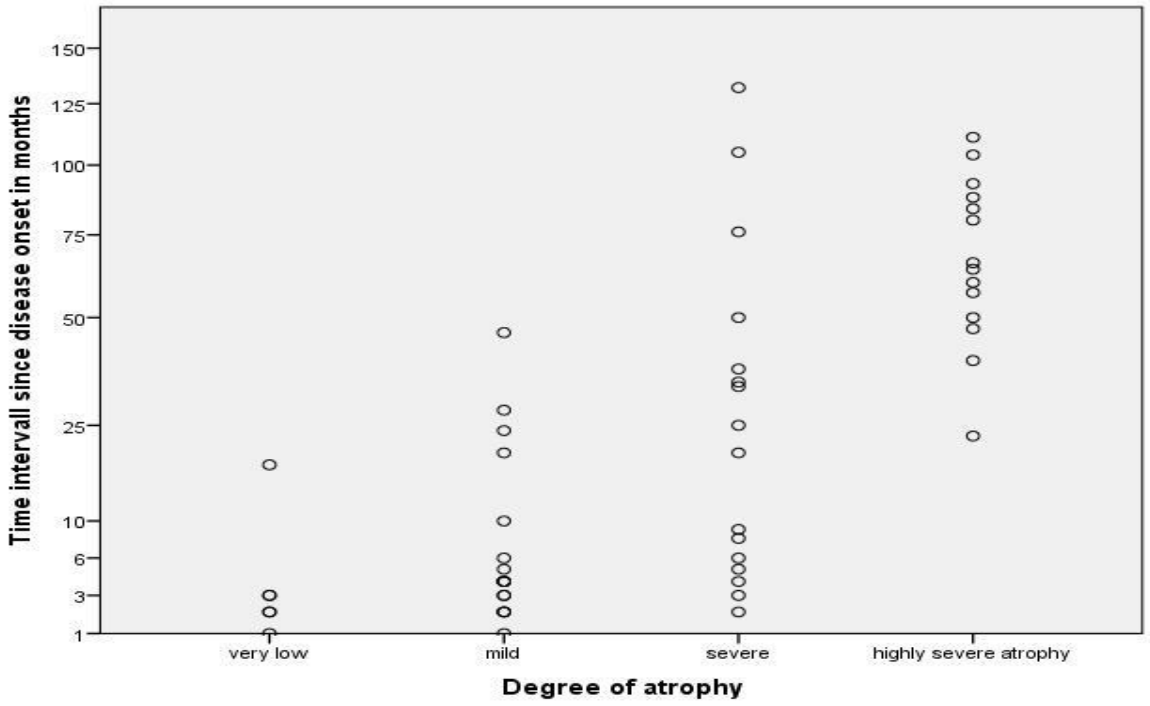

Figure 1. Correlation between duration since ictus and degree of atrophy

Table 1 **Clinical Characteristics of the Patients**

No.	Location	Sex	Age	Diagnosis	Etiology	Interval since Ictus in month	CRS	Atrophy
1	B.A.	F	16	UWS	Anoxic	20	5	2
2	B.A.	M	36	UWS	Anoxic	3	6	3
3	B.A.	F	64	UWS	Anoxic	104	6	4
4	B.A.	F	69	UWS	Hemorrhage	39	5	4
5	B.A.	F	38	UWS	Anoxic	25	5	3
6	B.A.	F	52	UWS	Anoxic	60	8	4
7	B.A.	F	71	UWS	Anoxic	2	5	2
8	B.A.	M	36	UWS	Anoxic	9	6	3
9	B.A.	F	56	UWS	Hemorrhage	33	6	3
10	B.A.	M	44	UWS	Hemorrhage	23	4	4
11	B.A.	M	29	UWS	Anoxic	34	6	3
12	B.A.	F	63	UWS	Anoxic	2	2	2
13	B.A.	M	19	UWS	Anoxic	4	7	2
14	B.A.	M	40	UWS	enceph	3	7	2
15	B.A.	M	36	UWS	Anoxic	50	4	4
16	B.A.	F	62	UWS	Hemorrhage	4	6	2
17	B.A.	F	30	UWS	Anoxic	1	5	2
18	B.A.	M	57	UWS	Anoxic	57	5	4
19	B.A.	M	44	UWS	Anoxic	50	7	3
20	B.A.	F	62	UWS	Anoxic	66	7	4
21	B.A.	M	25	UWS	Anoxic	3	6	2
22	B.A.	M	51	UWS	Anoxic	1	5	1
23	TÜ	M	64	UWS	Anoxic	111	*	4
24	TÜ	M	55	UWS	Anoxic	80	6	4
25	TÜ	M	47	UWS	Anoxic	64	5	4
26	TÜ	M	75	UWS	Anoxic	20	*	3
27	TÜ	F	53	UWS	Anoxic	84	*	4
28	TÜ	F	54	UWS	Anoxic	93	5	4
29	TÜ	F	45	UWS	Anoxic	287	2	4

Table 1 (Continued)

No.	Location	Sex	Age	Diagnosis	Etiology	Interval since Ictus in month	CRS	Atrophy
30	TÜ	M	59	UWS	Anoxic	88	*	4
31	B.A.	F	73	MCS	Hemorrhage	2	14	2
32	B.A.	M	23	MCS	Encephalopathy	3	11	1
33	B.A.	M	66	MCS	Hemorrhage	6	12	3
34	B.A.	F	50	MCS	Hemorrhage	8	10	3
35	B.A.	F	69	MCS	Hemorrhage	4	8	3
36	B.A.	M	71	MCS	Anoxic	37	11	3
37	B.A.	M	57	MCS	Anoxic	105	7	3
38	B.A.	M	46	MCS	Anoxic	2	11	1
39	B.A.	M	55	MCS	Hemorrhage	6	13	2
40	B.A.	M	31	MCS	Encephalopathy	28	11	2
41	B.A.	F	54	MCS	Hemorrhage	2	9	1
42	B.A.	F	69	MCS	Stroke	4	8	2
43	B.A.	M	48	MCS	Stroke	2	7	3
44	B.A.	M	65	MCS	Anoxic	132	8	3
45	B.A.	M	45	MCS	Anoxic	3	9	1
46	B.A.	F	35	MCS	Anoxic	10	7	2
47	B.A.	F	50	MCS	Anoxic	5	9	3
48	B.A.	F	62	MCS	Hemorrhage	4	9	2
49	B.A.	M	50	MCS	Hemorrhage	47	11	4
50	B.A.	F	57	MCS	Hemorrhage	46	10	2
51	B.A.	F	19	MCS	Anoxic	5	10	2
52	TÜ	M	41	MCS	Anoxic	24	*	2
53	TÜ	M	47	MCS	Encephalopathy	18	*	1
54	TÜ	M	25	MCS	Anoxic	76	*	3

Notes: No., patient number; location (B.A., Bad Aibling; TÜ, Tuebingen); Sex (F, female; M, male); UWS, Unresponsive Wakefulness Syndrome; MCS, Minimally Conscious State; CRS, Coma Recovery Scale-Revised (, no CRS-R assessment available); Atrophy (0, no atrophy; 1, very low; 2, mild; 3, sever; 4, highly severe atrophy)*

Table 2 lists the baseline characteristics of all the subjects. There were no significant differences between the groups for age and sex. In the UWS and MCS groups, there was no significant difference with respect to the time since ictus, but there were significant differences for CRS-scores [$t(45) = -9.903$, $p < 0.001$], degree of atrophy [$t(52) = 3.59$, $p = 0.001$] and aetiologies [$\chi 2(3) = 10.82$, $p<0.013$].

Table 2 Baseline characteristics of the patient and control group

Characteristics	UWS N = 30	MCS N = 24	Healthy control N = 15
Age (years)[a]	48.4 ± 15.5	50.3 ± 15.4	42.4 ± 11.8
Sex[b]			
Male	16 (53.3)	14 (58.3)	7 (46.7)
Female	14 (46.7)	10 (41.7)	8 (53.3)
Time since ictus (months)[a]	47.3 ± 57.0	27.1 ± 34.9	
CRS[a]	5.4 ± 1.4	9.8 ± 1.9	
Atrophy[a]	3.1 ± 0.9	2.3 ± 0.8	
Aetiology[b]			
Anoxic	25 (83.3)	10 (41.7)	
Hemorrhage	4 (13.3)	9 (37.5)	
Encephalopathy	1 (3.3)	3 (12.5)	
Stroke	0 (0)	2 (8.3)	

[a] Values in cells are mean ± standard deviation; [b] Values in cells are frequency (percentage).

3.3 Data acquisition

MRI scans were acquired at two imaging centers (Bad Aibling & Tuebingen, Germany) in order to avoid the unnecessary patient transportation. In Bad Aibling, where forty-three patients were examined, data were collected using a 1.5 T Siemens Symphony MR Scanner (Siemens, Erlangen, Germany). Functional images were acquired using a T2*-weighted echo-planar imaging (EPI) sequence with the following parameters: repetition time = 3410 ms,

echo time = 50 ms, Field of View = 192 mm, flip angle = 90 °, 64 x 64 matrix, 36 slices covering the whole brain, slice thickness 3 mm, no gap, voxel size 3 x 3 x 3 mm. An anatomic MRI for superimposition with functional images was acquired using a T1-weighted three-dimensional multiplanar reconstructed sequence (repetition time = 1860 ms, echo time = 3.57 ms, 176 slices, slice thickness = 1 mm, voxel size 0.9 x 0.8 x 1.0 mm). The patient's vital parameters (heart rate, oxygen saturation) were monitored by an experienced physician throughout the measurement. In Tuebingen, imaging was performed on a 3 T Siemens Trio scanner. After a T2*-weighted acquisition (repetition time = 2380 ms, echo time = 25 ms, Field of View = 210 mm, flip angle = 90 °, 64 x 64 matrix, 40 slices covering the whole brain, slice thickness 3 mm, no gap, voxel size 3.3 x 3.3 x 3.0 mm), anatomical images were obtained using the MP-RAGE sequence (repetition time = 2300 ms, echo time = 2.98 ms, 160 slices, slice thickness = 1 mm, voxel size 1.0 x 1.0 x 1.1 mm).

Head movement was minimized by using a form cushion. Before functional images preprocessing, the first four volumes were discarded to allow for magnetic saturation effects and the subjects' adaptation to the environment.

3.4 Data preprocessing

Image processing was carried out using SPM8 software package (Wellcome Department of Cognitive Neurology, London, England, U.K.), implemented in Matlab R2010a (Mathworks, Inc., Sherborn, MA). Data were first manually reoriented and then preprocessed. Preprocessing included realignment, coregistration of the high-resolution scans with the functional images, segmentation into grey and white matter, normalization to the template of the Montreal Neurological Institute, and spatial smoothing with an 8-mm full-width half-maximum isotropic Gaussian kernel.

4. Pain experiment

4.1 Theoretical and empirical background

Pain represents a very distinctive class of conscious experiences, beloved by philosophers due to the fact that pain is difficult to map directly onto any structure in the world or in the body, although it is usually associated with some part of the body (Chalmers, 1996). Pain perception is a multidimensional phenomenon, based on integration of sensory, affective, cognitive and homeostatic information that are processed in parallel brain networks. The sensory-discriminative component of pain provides information on the modality (mechanical, chemical, thermal), the site, intensity, and time-course of a painful stimulus, which allows suitable behavioural responses for avoiding injury to be made (Brooks et al., 2005). The affective component of pain comprises the unpleasant character of pain perception with a wide range of negative emotions in various degrees, such as anger, fear and sadness (Craig, 2003). The cognitive component refers to the attention, anticipation and memory of past experiences and interacts with the other components giving rise to modulation of pain (Valet et al., 2004).

Numerous imaging studies with PET and fMRI have revealed that pain experience cannot be reduced to activity in a single isolated brain region but rather involves a well-formed neural network, the so-called pain matrix (PM) (Jones et al., 1991; Peyron et al., 2000; Iannetti and Mouraux, 2010). More specifically, two distinct cerebral subsystems are considered to be involved in pain perception: a lateral sensory network encompassing the primary (SI) and the secondary somatosensory (SII) cortex, the lateral thalamus, as well as the posterior insula cortex (PI) (Mutschler et al., 2011); and a medial affective network constituting of the anterior insula (AI), the anterior cingulate cortex (ACC), and the prefrontal cortex (Wiech et al., 2001;

Medford and Critchley, 2010). In addition, motor-related areas (cerebellum and the supplementary motor area) are involved in pain perception and processing. Their participation can be regarded as a motor counterpart in the sensory-discriminative component (Vignemont and Jacob, 2012), although some cerebellar structures are, probably, involved in affective-cognitive pain responses (Moulton et al., 2010)

The management of pain in DOC patients is challenging because they cannot communicate their feelings. The perception of pain is defined as conscious experiences but the definition of UWS excludes this subjective phenomenon. If a patient never show any sign of volitional movement in response to noxious stimuli in a clinical examination, it may be concluded that the patient does not experience pain. However, as noted by the International Association for the Study of Pain (1986), the inability to communicate verbally or non-verbally does not rule out the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment. Given the limitation that the behavioural assessment of pain may have critical consequences, several authors have stressed the necessity of a better knowledge of pain and suffering in DOC patients using functional neuroimaging methods (Bernat, 2006; Laureys and Boly, 2007; Demertzi et al., 2009).

Pandit et al. (2002) reported in a case of a 55-year-old woman in UWS for 10 years after traumatic brain injury that her BIS profile during anaesthesia and surgery was similar to that of a normal subject, implying the possibility that UWS patients might sense noxious stimuli at a cortical level. Laureys et al. (2002) used PET to measure cortical processing of noxious stimulation of the median nerve patients and found significant brain activations in the brainstem, contra-lateral thalamus and SI in every examined patient. However, the observed SI activation was functionally isolated and disconnected from the other brain areas and the higher-order cortices that are proposed to be necessary for consciousness. This finding was

confirmed by Boly et al. (2005) in 15 UWS patients compared to 15 controls. In sharp contrast to the UWS patients, one MCS patient showed a close to normal neural activation including the brainstem, the thalamus, S1 and S2, the insula, the posterior parietal, superior temporal and anterior cingulate cortices in response to noxious stimulation. Furthermore, Boly et al. (2008) reported in a group of 15 UWS and 5 MCS patients that all areas of the pain matrix were less active in UWS than in MCS and controls. In another study, Kassubek et al. (2003) recorded PET responses during painful stimulation and found additional higher-order activation of SII and insula cortex even in long-term UWS patients, indicating the possibility of affective experiences of pain.

It should be stressed that the pain perception in MCS patients was not sufficiently investigated with only two studies in very small samples (1 to 5 patients). Moreover, the fMRI assessment has not been performed in UWS and MCS despite the fact that fMRI offers higher statistical power, improved spatial and temporal resolution than PET. The present study aimed at using fMRI to investigate the neural processes involved in pain perception in both healthy subjects and DOC patients.

4.2 Methods

4.2.1 Stimuli and experimental design

Electrical stimuli (5mA, duration=2ms) were presented to the subjects in a block design. The stimulation block lasted approximately 60 seconds and included the presentation of 60 painful stimuli with an interstimulus interval (ISI) of 1 second. The stimulation block was followed by a 60 seconds baseline blocks in which no painful stimulation was presented. In total, there were three stimulation blocks and three baseline blocks. The task sequence was controlled by a PC running "Presentations" software (Neurobehavioral Systems, Albany, CA, USA).

The pain stimulation was an electrical stimulus applied on the left index finger by a finger electrode, delivered by an electrical stimulus generator (Digitimer, DS7A, UK). The intensity was set at 5 mA to ensure that the shocks were painful, but not harmful. After the scanning sessions, healthy participants were asked to rate the pain stimulation using the visual analog scale (VAS, from 0 = non pain at all to 10 = worst pain imaginable). The average score was 3.93 (SD=1.28). In another unpublished study with sixteen healthy controls (7 males, mean age 25.63 [SD 4.41]), the stimuli were evaluated as highly-arousing (mean 7.31, SD 1.54 on the scale from 1 to 9) and rather unpleasant (mean 7.81, SD 0.91 on the scale from 1=very pleasant to 9=very unpleasant).

4.2.2 Data analyses

Preprocessed data were analyzed subject-by-subject using the general linear model in SPM with hemodynamic response function modelled as boxcar predictors. The movement parameters and their derivatives were added as additional regressors in the design matrix. In

healthy controls, first-level contrasts were introduced in a second-level analysis, corresponding to a random effects model in which subjects are considered as random variables. Main effects were computed using one-sample t tests, including all subjects for the contrast of interest, which yielded a statistical parametric map of the t statistic. All the statistical maps were thresholded at $p<0.001$ uncorrected to identify differential activations between conditions in the whole brain analysis, and only clusters surpassing a corrected p value of <0.05 on cluster level were considered as significantly activated. Results in patients were tested at the single-subject level. The whole-brain statistical map was thresholded at $p<0.001$ uncorrected with a minimum of five contiguous voxels to identify differential activations between conditions at the single-subject level.

In addition, a region of interest (ROI) analysis was performed by taking into account previous neuroimaging studies that have examined pain perception. ROIs of the anterior cingulate cortex (ACC), insula, primary and secondary somatosensory cortices (SI, SII), thalamus and cerebellum were drawn using automated anatomical labelling masks (Tzourio-Mazoyer et al., 2002) and the WFU Pickatlas (Maldjian, Laurienti, Kraft, & Burdette, 2003). The ROIs were superimposed onto each patient's T1 image with manual adjustments to those anatomical landmarks if necessary (Bekinschtein et al., 2011). As a first step of the ROI analyses, results were thresholded at $p<0.005$, uncorrected. Then the significance levels and cluster sizes within the anatomically defined ROIs were computered. Only activations surviving a small-volume correction for family wise error (FWE) at $p<0.05$ were considered significant.

4.3 Results

4.3.1 Signal changes during pain stimulation in healthy subjects

A direct statistical comparison between stimulation and baseline conditions revealed that all areas of the PM showed greater activation in the former condition than in the latter. The areas are listed in Table 1 according to anatomical regions, cluster sizes, MNI coordinates, Z-score and significance levels of activations. The majority of the activated voxels were located bilaterally in the primary and secondary somatosensory cortex, the insula cortex, the anterior cingulate cortex, the thalamus and the supplementary motor area. Additionally, pain stimulation caused contralateral activation in the right precentral gyrus and in the right inferior frontal gyrus. Significant activations were also observed in cerebellum at an uncorrected voxelwise p-value of 0.001. The areas are also shown in Figure 2, projected on a standard anatomical template.

Individual results are reported in Table 4. As summarized in Table 5, all 15 healthy subjects demonstrated significant activation in at least one considerable component of the PM. Taking into account that the central pain matrix consists of approximately two parallel subsystems (sensory-discriminative & affective-cognitive), the sensory part was activated in all volunteers and the affective part was activated in nine (60%) participants. The activations of higher-order processing regions, such as insula, ACC, SII and cerebellum were found in all subjects, the lower-order activations (SI and thalamus) in 12 subjects (80%).

In addition, correlation analyses revealed significant relationships between age and number of activated brain areas in the PM ($r = -0.893$, $p < 0.001$) (Figure 3).

Table 3 **Regions more active during the stimulation condition than during the baseline condition in pain experiment**

Region	L/R	BA	p value	Cluster size (voxels)	Peak in MNI x	y	z	z score
supplementary motor area	L+R	6	<0.001	704	-6	17	52	5.36
anterior cingulate cortex	L+R	24						
thalamus	L+R		<0.001	285	-15	2	1	5.00
insula	R	13	<0.001	705	45	-28	19	4.63
inferior parietal lobule	R	40			51	-31	25	4.45
postcentral gyrus	R	2						
inferior parietal lobule	L	40	<0.001	464	-42	-34	22	4.21
insula	L	13			-42	-16	7	4.21
postcentral gyrus	L	2						
precentral gyrus	R	6	<0.001	176	39	-10	55	4.05
inferior frontal gyrus	R	44	0.014	54	51	5	16	4.08

Clusters identified with a threshold of p < .05 Family-Wise-Error corrected for multiple comparisons.

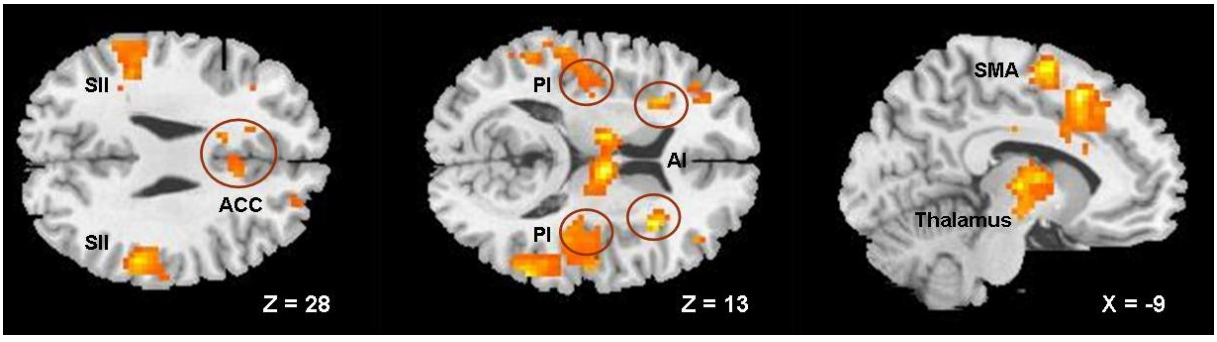

Figure 2. Significant clusters from the random-effects contrast of stimulation versus baseline. The statistical threshold employed was an uncorrected p value of 0.001 for illustrative purposes.

Table 4 **Individual results of the pain experiment for each of the chosen ROIs in healthy controls**

Subject number	Sex	Age	ACC	AI	SII	SI	Thalamus	PI	Cerebellum
1	F	52	No	No	+	+	No	No	+
2	F	29	No	+	+	+	+	+	+
3	M	46	No	No	+	No	+	No	+
4	M	29	+	+	+	+	+	+	+
5	F	31	+	+	+	+	+	+	+
6	F	35	+	+	+	No	No	+	No
7	M	32	+	+	+	+	+	+	No
8	M	62	No	No	+	No	No	+	No
9	F	47	No	No	No	+	No	+	No
10	M	52	No	+	+	+	No	+	No
11	F	58	No	No	+	+	No	No	No
12	M	48	+	+	+	+	No	No	No
13	F	28	+	+	+	+	+	+	+
14	M	33	No	+	+	+	No	+	+
15	M	54	No	No	+	No	No	No	No

Results thresholded at small volume correction corrected $p < 0.05$. Sex (F, female; M, male); ACC, anterior cingulate cortex; AI, anterior insula; SII, secondary somatosensory cortex; SI, primary somatosensory cortex; PI, posterior insula; +, significantly positive BOLD signal in the pain stimulation condition compared with baseline condition; No, no significant response.

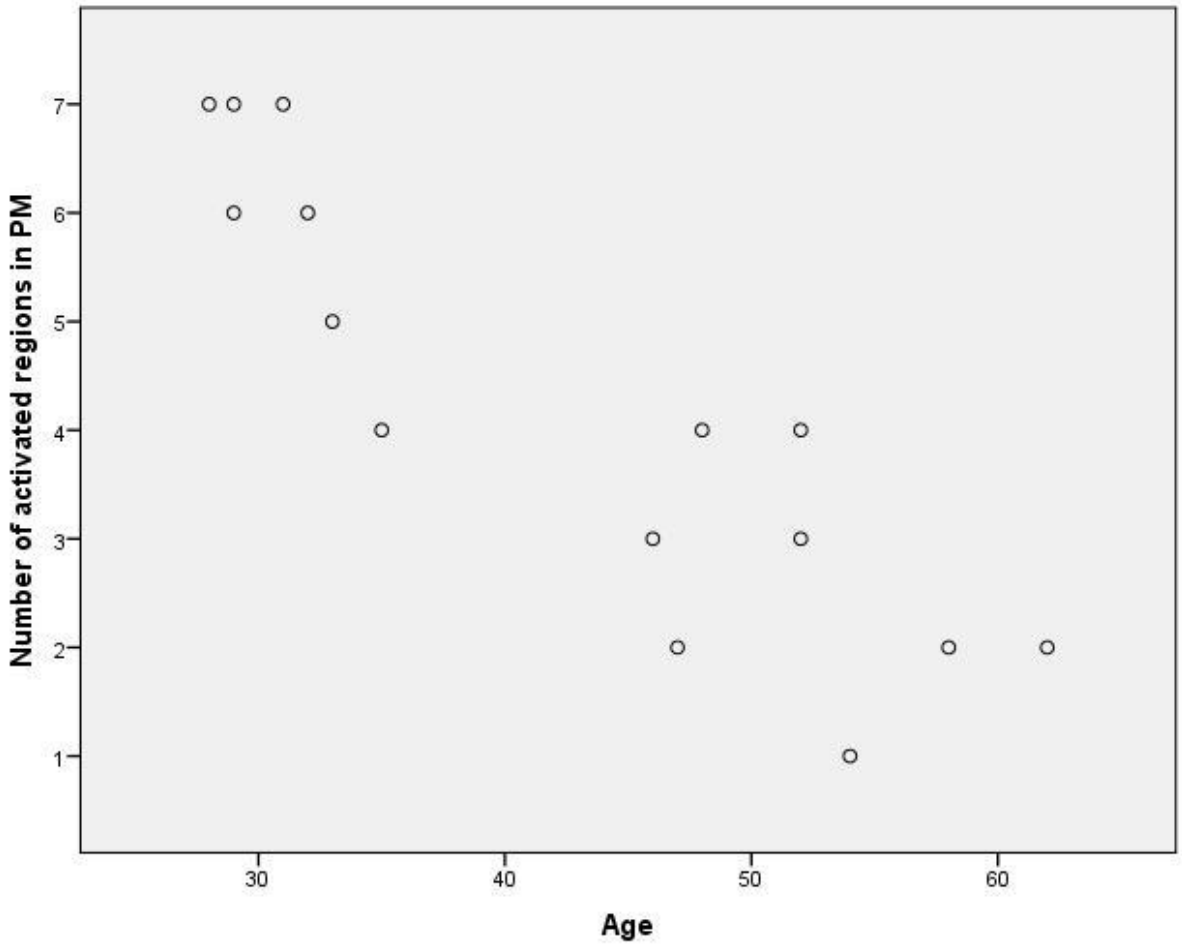

Figure 3. Correlation between age and brain responses in healthy controls

4.3.2 Signal changes during pain stimulation in DOC patients

Applying the ROI Analysis in the DOC patients yielded the following results on a single subject level (Table 6, Figure 4): 16 of 30 UWS (53.3%) and 16 of 24 MCS patients (66.7%) showed signal changes during pain stimulation in at least one of the PM regions. Sensory activations were observed in 15 UWS (50%) and 15 MCS patients (62.5%); Affective activations were found in nine UWS (30%) and seven MCS patients (29.2%). Eight UWS (26.7%) and six MCS patients (25%) demonstrated activations in both sensory and affective PM subsystems. Higher-order processing regions were activated in 15 UWS (50%) and 15 MCS patients (62.5%), and lower-order activations were observed in four UWS (13.3%) and

nine MCS patients (37.5%). When MCS patients were then compared with UWS patients, significantly higher frequencies of lower-order activations were found in MCS than UWS patients ($\chi2(1) = 4.26$, $p<0.05$). The number of non-responders appeared to be higher in UWS than MCS patients. However, this difference did not reach statistical significance ($\chi2(1) = 0.982$, $p = 0.322$). These results are also displayed in Table 5.

Table 5 Frequencies of significant activations in pain experiment (in %)

	Sensory Responders	Affective Responders	Sensory AND Affective Responders	Non-responders	Lower-order	Higher-order
UWS N = 30	50	30	26.7	46.7	13.3*	50
MCS N = 24	62.5	29.2	25	33.3	37.5*	62.5
Controls N = 15	100	60	60	0	80	100

The asterisks indicate significant differences between UWS and MCS; Subjects were regarded as "affective responders" if at least one of the two ROIs strongly involved in the affective stimulus processing (ACC and anterior insula) was significantly activated; "sensory responder", significant activation in at least one of the "sensory" ROIs (primary and secondary somatosensory cortices, posterior insula, thalamus and cerebellum); Lower-order processing regions, SI and thalamus; Higher-order processing regions, insula, ACC, SII and cerebellum.

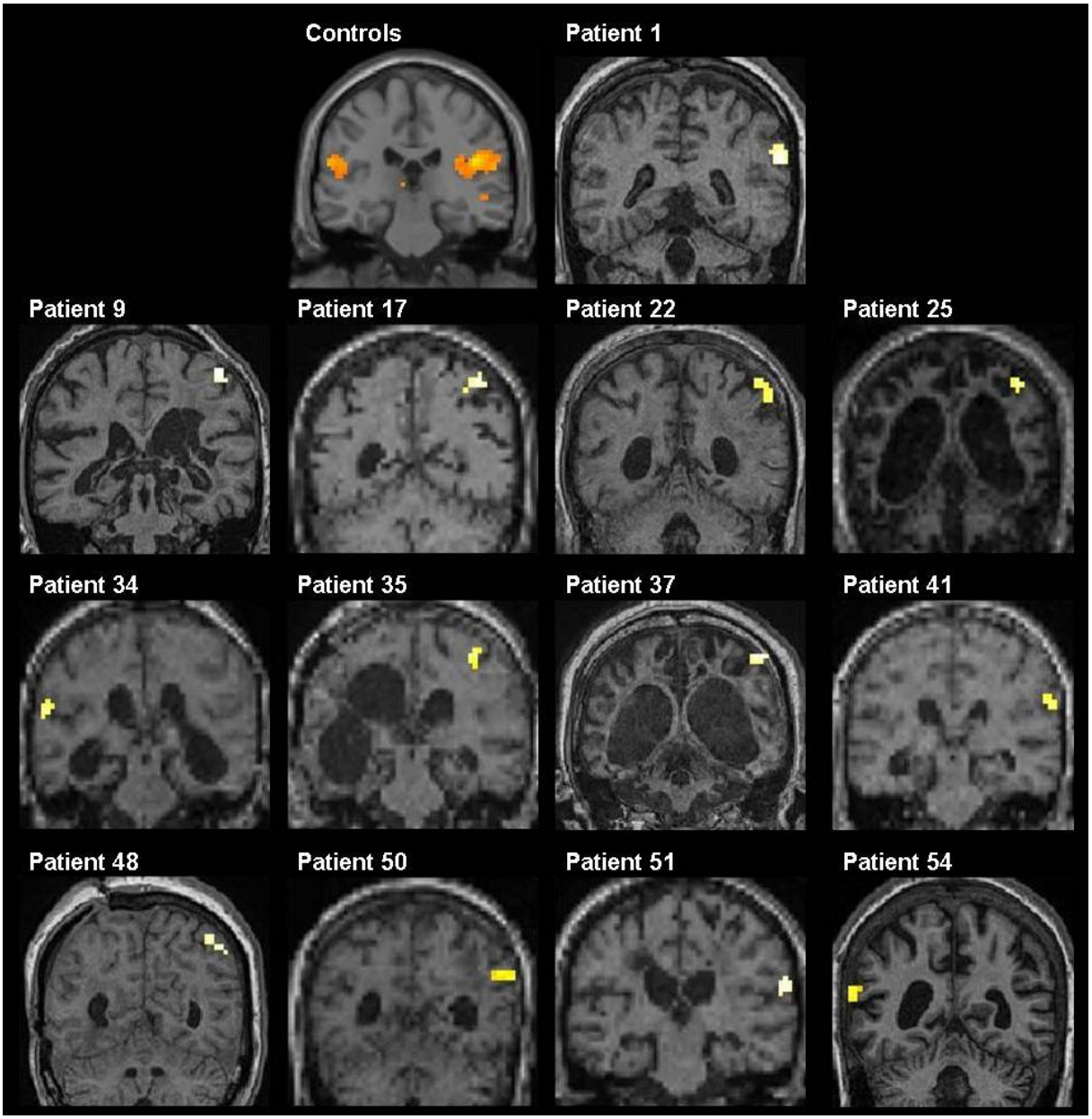

Figure 4. Brain responses in the secondary somatosensory cortex in pain experiment. These scans were obtained from a group of 15 healthy subjects and 13 patients with disorders of consciousness. The statistical threshold employed was an uncorrected p value of 0.001 for illustrative purposes.

Table 6 **Individual results of the pain experiment for each of the chosen ROIs in patients**

Patient number	ACC	AI	SII	SI	Thalamus	PI	Cerebellum
1	No	No	+	+	No	+	+
2	No	No	No	No	No	No	No
3	+	No	No	No	No	No	+
4	No	No	No	No	No	No	No
5	+	No	No	No	No	No	No
6	No	No	No	No	No	No	No
7	+	No	No	No	No	No	+
8	+	+	No	No	No	No	+
9	No	No	+	No	No	+	No
10	No	+	No	No	No	No	+
11	No	+	No	No	+	No	+
12	No	No	No	No	No	No	+
13	No	No	No	No	No	No	No
14	No	No	No	No	No	No	No
15	No	No	No	No	No	No	+
16	No	No	No	+	No	No	No
17	No	+	+	+	No	No	+
18	No	No	No	No	No	No	No
19	No	No	No	No	No	No	No
20	No	No	No	No	No	No	No
21	No	No	No	No	No	No	No
22	+	No	+	No	No	+	No
23	No	No	No	No	No	No	No
24	No	No	No	No	No	No	+
25	No	+	+	No	No	No	No
26	No	No	No	No	No	No	No
27	No	No	No	No	No	No	No
28	No	No	No	No	No	No	+
29	No	No	No	No	No	No	No
30	No	No	No	No	No	No	No

Table 6 (Continued.)

Patient number	ACC	AI	SII	SI	Thalamus	PI	Cerebellum
31	No	No	No	No	No	No	No
32	No	No	No	No	No	No	+
33	No	No	No	No	No	No	No
34	No	+	+	No	+	No	+
35	No	No	+	No	No	No	+
36	No	No	No	No	No	No	No
37	No	+	+	+	No	No	No
38	+	+	No	No	+	No	+
39	+	+	No	+	No	No	+
40	+	No	No	No	No	No	No
41	No	No	+	No	No	No	No
42	No	No	No	No	No	No	No
43	No	No	No	No	No	No	No
44	No	No	No	No	No	No	No
45	No	No	No	No	No	No	+
46	No	No	No	No	No	No	+
47	+	+	No	No	No	No	+
48	No	No	+	+	No	No	No
49	No	No	No	No	No	No	No
50	No	No	+	+	No	No	+
51	No	No	+	+	No	+	No
52	No	No	No	+	No	No	No
53	No	No	No	No	No	No	No
54	+	No	+	+	+	No	+

Results thresholded at small volume correction corrected $p < 0.05$. ACC, anterior cingulate cortex; AI, anterior insula; SII, secondary somatosensory cortex; SI, primary somatosensory cortex; PI, posterior insula; +, significantly positive BOLD signal in the pain stimulation condition compared with baseline condition; No, no significant response.

4.4 Discussion

Earlier research has shown that noxious stimulation elicits robust activation in the PM (Jones et al., 1991; Peyron et al., 2000; Iannetti and Mouraux, 2010). In line with these earlier findings, signals in those regions that are associated with pain experience were clearly indentified in 15 age-matched healthy subjects. Moreover, the individual analysis showed that the electrical painful stimuli produce very robust sensory activation in 100% of the participants and affective activation in 60% of them, suggesting that the current experimental paradigm is suited to identify signs of pain processing in non-communicative patients. The reason that 6 healthy controls did not show significant neural responses in the affective subsystem of the PM could be the relative mild intensity of the pain stimulation (5 mA)[1]. The 6 subjects identified their experience as pain, but showed no emotional and behavioral signs typically associated with the unpleasant aspect of pain, supposing that they might be feeling a pain that does not hurt. This assumption was confirmed by the behavioral data that the average rating of the pain stimulation was only 3.93 (SD = 1.28) on a visual analogue scale (VAS, from 0 = non pain at all to 10 = worst pain imaginable).

Interestingly, a statistically significant relative age effect on pain processing was found among the 15 healthy subjects. The age-related changes in the structure and function of the nociceptive system have been extensively studied over the years. Whereas most clinical studies have documented an age-related increase in clinical pain, experimental data are quite contradictory, indicating that pain sensitivity increases, decreases or remains unchanged over the individual's life span (Gibson and Farrell, 2004; Lautenbacher et al., 2005). The effects vary substantially depending on the methodological differences between studies and the

[1] Although ethical aspects of DOC are actually outside the scope of the present study, it should be said at this point that applying pain stimuli in patients unable to give an informed consent is a severe problem that we largely discussed with both patient's families and ethical experts before starting the experiments. Being unable to obtain subjective pain thresholds in patients, we selected very mild pain stimuli.

different dimensions of pain sensitivity under investigation (Kunz et al., 2009). In the present study, the neural responses in the PM were much more extensive in the younger subjects than in the older subjects (Figure 3). Based on these data, negative results in the older DOC patients should be interpreted with caution as even a fully conscious old subject may activate very few regions of the PM in response to the pain stimulation.

One important part of the definition of UWS is the inability to experience pain (or any other subjective feeling). This assumption may have far-going consequences, from a small surgery performed without anesthesia up to serious ethical and legal decisions, even the end-of-life decisions in such patients. Despite this definition, not only patients' relatives but also many health professionals believe that UWS patients can perceive pain. Demertzi et al. (2009) provided results from a European survey that the opinions of 1166 medical professionals are divided as to whether patients in UWS can feel pain or not (56% answered "yes"). The present results support and extend these findings by demonstrating the involvement of sensory subsystem of the PM in 50% of UWS and 62.5% of MCS patients. More importantly, neural responses in the affective part of the PM were found to be activated in about 30% DOC patients. Of course, an increase of brain metabolism in some brain areas cannot strongly prove the presence of subjective experience. However, given pain-related changes in higher-order brain structures such as anterior insula and ACC, which are considered to be related to affective awareness and autonomic regulation of pain (Vogt, 2005; Panksepp, 2005), it will be risky to still argue that the patients are unable to feel pain. Note that among these were also chronic patients (several years after the incident) with very severe hypoxic brain injury.

The SII showing specific involvement in pain processing in healthy subjects served as functional localizer in DOC patients. Thirteen patients (5 UWS and 8 MCS) showed some significant signal changes, which varied somewhat across individuals, in this higher-level area

during pain stimulation (Figure 4). Previous studies by Laureys et al. (2002) and Boly et al. (2005) showed that pain-related activations in UWS patients were observed exclusively in the primary sensory part of the pain matrix, isolated and disconnected from the higher-level cortices. In contrast, the present data provide strong evidence that not only MCS but also UWS patients may retain preserved pain processing function in the higher-order brain regions including SII, insula and ACC. This is in accordance with a previous PET study by Kassubek et al. (2003), who found activations of the sensory and affective parts of the PM even in long-term UWS patients.

For both objective and ethical reasons, rather weak pain stimuli were applied in the present experiment. We cannot rule out that they were weaker than in the comparable PET studies, which would partially explain the differences in results. Moreover, it has been shown that MRI scanner noise significantly reduces unpleasantness ratings of pain stimulation (Boyle et al., 2006). The difference in the physical environment should, therefore, also be considered when discussing neuroimaging studies on human pain perception. Importantly, these limitations may be supposed to decrease, rather than enhance, the pain responsiveness of the patients. They may also have resulted in rather weak responses of the MCS patients, in which nearly normal activations could be expected. The fact that the present experiment was strongly biased toward false negative findings underscores the importance of positive ones. If stimuli of such low intensity induced neural responses in the PM in 32/54 DOC patients, so how more pronounced, and perhaps in a greater fraction of the sample, might be pain-related brain activation in response to a real pain event such as toothache? From practical point of view, therefore, a conclusion from the present data may be drawn that the medical staff should carefully examine both UWS and MCS patients for any clinical sign or potential source of pain and treat them appropriately, assuming in the case of doubt that pain is subjectively experienced unless strong evidence for the opposite is obtained.

5. Emotional experiment

5.1 Theoretical and empirical background

Pain and emotion are inherently subjective experiences that can be verbally or non-verbally (i.e., changes in facial gestures) communicated to others. Like pain experience, it is very difficult to infer emotional states by observing overt behavioural responses to external stimuli (Demertzi et al., 2012). Recent efforts using fMRI to address the question whether DOC patients can perceive emotions have provided initial evidence of the residual functional substrates with respect to increased cortical activity in response to emotional stimuli, such as images of close family members (Zhu et al., 2009). In some ways, sounds are even stranger than visual images. Auditory stimuli with emotional content, such as infant cries or meaningful stories told by a relative, elicit more extensive brain activation than sounds without meaning and resulted in higher-level brain processing in MCS patients (Laureys et al. 2004b; Schiff et al. 2005). Preserved cognitive processing was also indentified in a case study in which the mother's voice elicited a strong activation of the amygdala and insula in a 17 years old MCS patient (Bekinschtein et al., 2004). Similarly, Di et al. (2007) investigated brain activation in response to presentation of the patient's own name spoken by a familiar voice in a group of 7 UWS and 4 MCS patients. Activations in the primary auditory cortices were observed in 3 US patients, but none of these patients recovered. In contrast, two other UWS patients who exhibited widespread activation in higher-level associative temporal cortex subsequently improved to the MCS in the following 3 months (Di et al. 2007).

However, the results from theses previous studies are inconsistent mainly due to the large variability of stimuli. Another way in the assessment of residual emotional awareness may consist in addressing the functions of pain matrix (PM) in response to a strong negative

emotional stimulation. As already mentioned in Section 4.1., the PM entails two large systems: a lateral sensory system including the primary and secondary somatosensory cortices (SI and SII, respectively), the thalamus, the cerebellum and the posterior insula (PI); and the medial affective system including the amygdala, the anterior insula (AI) and the anterior cingular cortex (ACC). A serious of studies have indicated that the PM responds not only to direct nociceptive (extero- or interoceptive) stimulation but also to complex stimuli describing another person's pain and suffer, such as picture of cutting skin with a knife (Singer et al., 2004; Jackson et al., 2006; Lamm et al., 2007; Benuzzi et al., 2008).

Because such pictures cannot be used in UWS patients most of whom are unable to control their gaze, Lang et al. (2011) introduced a new paradigm using pain cries from the international Affective Digitized Sound System (IADS; Stevenson and James, 2008). Other stimuli of human voice, which do not express pain or suffer, served as control. Pain cries, compared with control stimuli, elicited consistent responses in both sensory and affective components of the PM. Interestingly, the ACC demonstrated a significantly negative BOLD response (possibly indicating deactivation) in most healthy participants, whereas in the other PM areas consistent positive responses (i.e., activations) were observed. Pain-related stimuli also strongly activated the primary and secondary auditory cortices. Moreover, the responses in nine regions including the AI and the auditory cortex significantly correlated with Interpersonal Reactivity Index scores (IRI; Davis, 1983, 1996), which might give rise to the speculation that these responses underlie the experience of affective empathy.

However, the reliability of the results obtained in individual subjects rather than across a group is critical for any clinical application where interpretation will be made on a single subject basis. For this reason, the present study should first validate the experimental

paradigm developed by Lang et al. (2010) in a group of age-matched healthy controls and then investigate neural activity changes during auditory stimulation in DOC patients.

5.2 Methods

5.2.1 Stimuli and experimental design

Twenty auditory stimuli (10 pain-related; 10 control) used in this study were selected from International Affective Digitized Sounds (IADS), which is a database of 111 standardized emotional sounds characterized along the affective dimensions of valence, arousal, and dominance (Stevenson & James, 2008). Affective reactions to these sounds were assessed using the Self-Assessment Manikin (SAM; Bradley & Lang, 1994). The ten pain-related sound samples (No. 261, 276, 277, 278, 279, 285, 286, 290, 292, and 423) were chosen on the basis that healthy individuals scored them high in pain and empathy. Other ten stimuli (No. 110, 206, 220, 221, 226, 230, 252, 262, 270, and 802) with low scores containing snoring, singing and yawning were selected as control stimuli. Pain-related and control stimuli were matched in their basic physical features [81 versus 78 dB for pain-related and control condition, $t(18) = 0.27$; basic formant 653 versus 509 Hz, $t(18) = 1.09$]. In a previous work (Lang et al., 2011), pain-related stimuli were rated as significantly more arousing and more unpleasant than the control stimuli (arousal, $t = 4.31$, $p = .001$; valence, $t = 9.85$, $p < .001$).

The stimuli were presented to the patients in a block design, with five pain-related blocks, five control blocks and five baseline blocks. Each block lasted for 30 seconds and included the presentation of four different sound samples in one condition, whereas in baseline block only the attenuated machine noise could be heard. Each sound sample had duration of 6 s and the interstimulus interval was 1.5 s.

Subjects heard the auditory stimuli via MRI-compatible headphones with efficient gradient noise suppression (up to 45dB) and a filter system with more than 90 dB RF-suppression (MR

confon System, Magdeburg, Germany). The task sequence was controlled by a PC running "Presentations" software (Neurobehavioral Systems, Albany, CA, USA). The order of the blocks was pseudo-randomized to make sure that no more than two blocks from the same condition were presented consecutively.

5.2.2 Data analyses

Data were analyzed as previously described in detail (section 3.2.4 and 4.2.2). A voxel-level threshold of $p < 0.001$ uncorrected was used to identify significant differential activations between conditions. Only clusters of at least 10 adjacent voxels are reported to avoid false positives. While in 15 healthy controls group analysis was performed, single subject analyses in 54 patients checked the reliability of individual brain activation for the contrast pain cries>conrol. A region of interest (ROI) analysis was additionally performed to test the a priori hypotheses. Given the knowledge that the PM is of particular importance for pain related empathy, and also based on the previous findings from the healthy controls using the same experimental paradigm (Lang et al., 2011), ROIs of the anterior cingulate cortex (ACC), insula, primary and secondary somatosensory cortices (SI, SII), thalamus, cerebellum, superior temporal gyrus (STG) and middle temporal gyrus (MTG) were obtained for emotional paradigm. The ROI threshold was set at $p<0.05$ with family-wise error (FWE) corrected for multiple comparisons.

5.3 Results

5.3.1 Signal changes for the pain cries in healthy subjects

Hearing the pain cries expressed by other people resulted in activation of a widely distributed network of brain areas, reflecting the sensory and affective processing of the stimuli. Contrast between pain-related and control stimuli revealed clusters comprising the bilateral secondary somatosensory cortex, the bilateral middle/superior temporal gyri and the bilateral posterior insula cortex. Note that these two clusters surpassed a Family-Wise-Error corrected p value of <0.05 on cluster level (the left cluster with 362 voxels and right cluster with 436 voxels). Further differential responses were detected in the left anterior insula and in the left thalamus. Bilateral activations in the cerebellum and the putamen were also observed.

Contrasting the control condition with pain-related condition revealed stronger responses in the right anterior cingulate cortex. The areas are listed in Table 7 according to anatomical regions, Brodmann areas (BA), MNI coordinates, cluster sizes, and Z-score. Figure 2 shows the average differential activations across all 15 healthy participants, projected on a standard anatomical template.

These results suggest that the PM and the middle/superior temporal gyri show the most specific signal changes during pain cries condition. Thus these regions were defined as ROIs for subsequent analyses.

Individual results are listed in Table 8. Almost all the healthy subjects (fourteen of the 15, 93.3%) significantly responded to pain cries (i.e, responses were observed in at least one affective PM region or at least two other areas: primary and secondary somatosensory cortices,

posterior insula, thalamus, cerebellum and superior/middle temporal gyrus). Pain-related stimuli activated the sensory subsystem of PM in 13/15 (86.7%) volunteers. Affective activation was observed in 11/15 (73.3%) volunteers. 66.7% of subjects showed brain activities in both sensory and affective components of PM (Table 9).

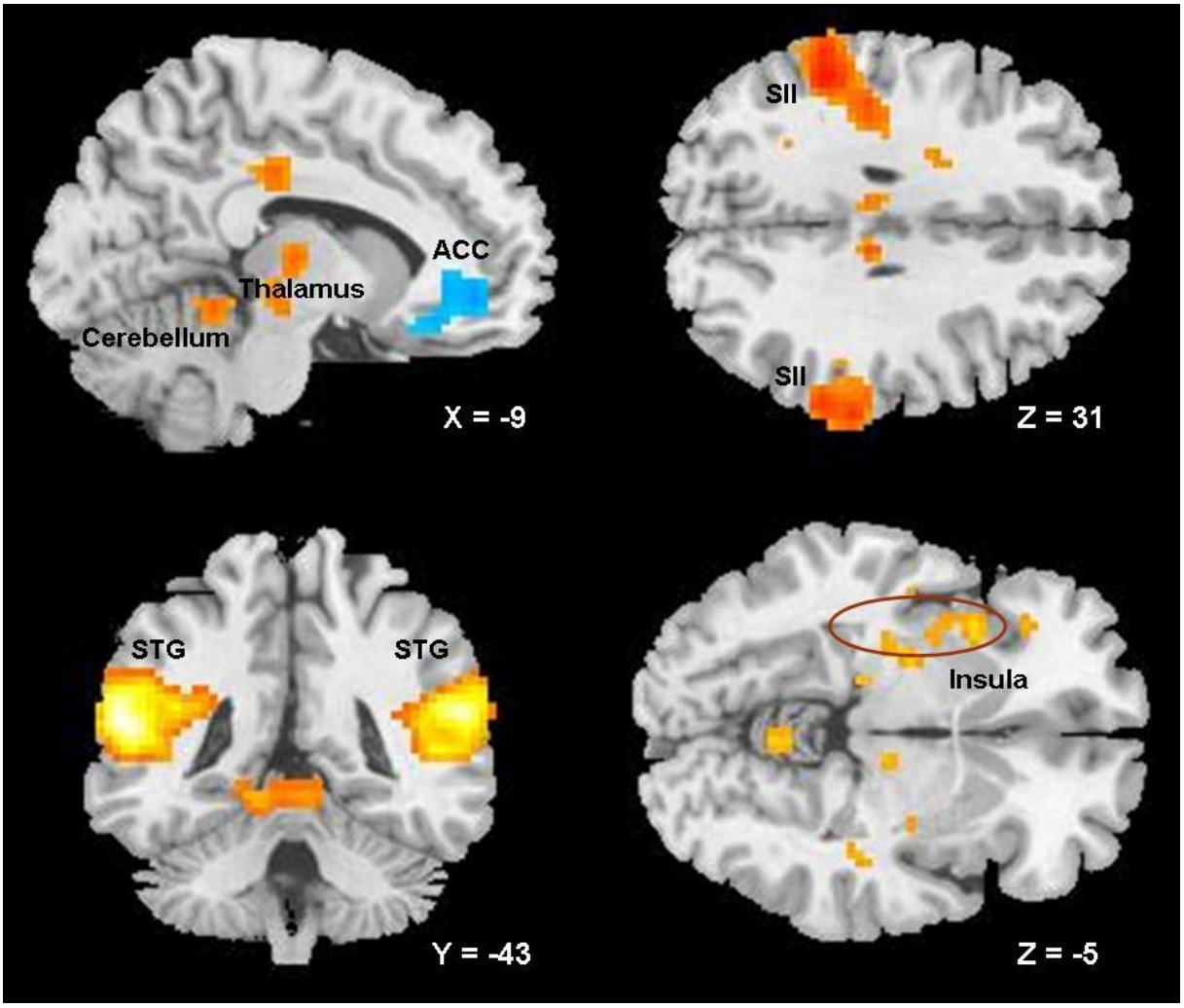

Figure 5. Significant clusters from the random-effects contrast of pain-related stimuli versus control stimuli. Brain responses are color-coded with red/yellow indicating areas with a positive BOLD signal and blue/green referring to a negative BOLD signal. The statistical threshold employed was an uncorrected p value of 0.001 for illustrative purposes.

Table 7 **Clusters of significant signal change (activation and deactivation) in emotional experiment**

Region	L/R	BA	Cluster size (voxels)	Peak in MNI			z score
				x	y	z	
Activation							
inferior parietal lobule*	R	40	436	54	-40	22	5.04
middle/superior temporal gyri*	R	22/41/42		51	-49	19	4.43
posterior insula*	R	13					
middle/superior temporal gyri*	L	22/41/42	362	-57	-43	15	5.03
inferior parietal lobule*	L	40		-51	-40	28	4.33
posterior insula*	L	13					
anterior insula	L	13	72	-36	11	-5	3.66
thalamus	L		22	-12	-16	7	3.44
cerebellum	L+R		33	3	-49	-11	3.31
superior temporal gyri	L	22	29	-51	-13	1	4.29
putamen	R		21	27	5	10	3.77
putamen	L		22	-27	-13	-5	3.48
Deactivation							
anterior cingulate cortex	R	10	18	9	29	-14	3.76

*Statistical maps were thresholded at p<0.001, uncorrected at the voxel level, with minimum cluster size of 10 voxels. *, Clusters surpassing a Family-Wise-Error corrected p value of <0.05 on cluster level.*

Table 8 **Individual results of the emotional experiment for each of the chosen ROIs in healthy controls**

Subject number	Sex	Age	ACC	AI	SII	SI	Thalamus	PI	Cerebellum	STG/ MTG
1	F	52	+	No	+	No	+	No	+	+
2	F	29	-	No	No	No	No	+	+	+
3	M	46	+	+	+	+	+	+	+	+
4	M	29	No	No	+	No	+	No	No	+
5	F	31	-	No	+	+	No	No	+	+
6	F	35	-	+	+	No	+	No	+	+
7	M	32	-	+	+	+	No	+	+	+
8	M	62	No	No	No	No	No	No	+	+
9	F	47	-	No	No	No	No	No	+	+
10	M	52	No	No	+	+	No	No	No	+
11	F	58	-	+	+	+	No	+	+	+
12	M	48	-	No	+	No	+	No	No	No
13	F	28	-	+	No	No	No	No	+	No
14	M	33	-	+	+	+	+	+	+	+
15	M	54	No	No	No	No	No	No	No	No

Results thresholded at small volume correction corrected $p < 0.05$. Sex (F, female; M, male); ACC, anterior cingulate cortex; AI, anterior insula; SII, secondary somatosensory cortex; SI, primary somatosensory cortex; PI, posterior insula; STG/MTG, superior/middle temporal gyrus; +, significantly positive BOLD signal in the experimental condition compared to control condition; -, significant negative BOLD response; No, no significant response.

Table 9	**Frequencies of significant activations in emotional experiment (in %)**			
	Sensory Responders	Affective Responders	Sensory AND Affective Responders	Non-responders
UWS N = 30	23.3	26.7	3.3	53.3
MCS N = 24	33.3	12.5	8.3	54.2
Controls N = 15	86.7	73.3	66.7	6.7

Patients were regarded as "affective responders" if at least one of the two ROIs strongly involved in the affective stimulus processing (ACC and anterior insula) was significantly activated. Since there are several other ROIs in the empathy experiment, we scored a patient as a "sensory responder", if at least two of these "non-affective" ROIs (primary and secondary somatosensory cortices, posterior insula, thalamus, cerebellum, and the auditory cortex) were activated. Of course, a higher responder rate would be obtained if the activation of a single region is counted, but a more conservative criterion is preferred.

5.3.2 Signal changes for the pain cries in DOC patients

As shown in Table 9, 46.7% (14/30) of the UWS and 45.8% (11/24) of the MCS patients demonstrated neural responses to pain cries of other people in at least one affective PM region or at least two other sensory areas. Sensory areas were activated in 7/30 (23.3%) UWS and 8/24 (33.3%) MCS patients. Activities in affective areas were found in 8/30 (26.7%) UWS and 3/24 (12.5%) MCS patients. One (3.3%) UWS and two (8.3%) MCS patients demonstrated activations in both sensory and affective PM subsystems. Considering that the negative BOLD response in the rostral ACC to pain cries compared with neutral stimuli was

robustly observed in the healthy controls (see also Lang et al., 2010), five patients (4 UWS and 1 MCS) were identified whose results closely match this activation pattern (Figure 6). Table 10 shows the individual results. There was no significant difference between the UWS and MCS groups in all aspects of activation patterns.

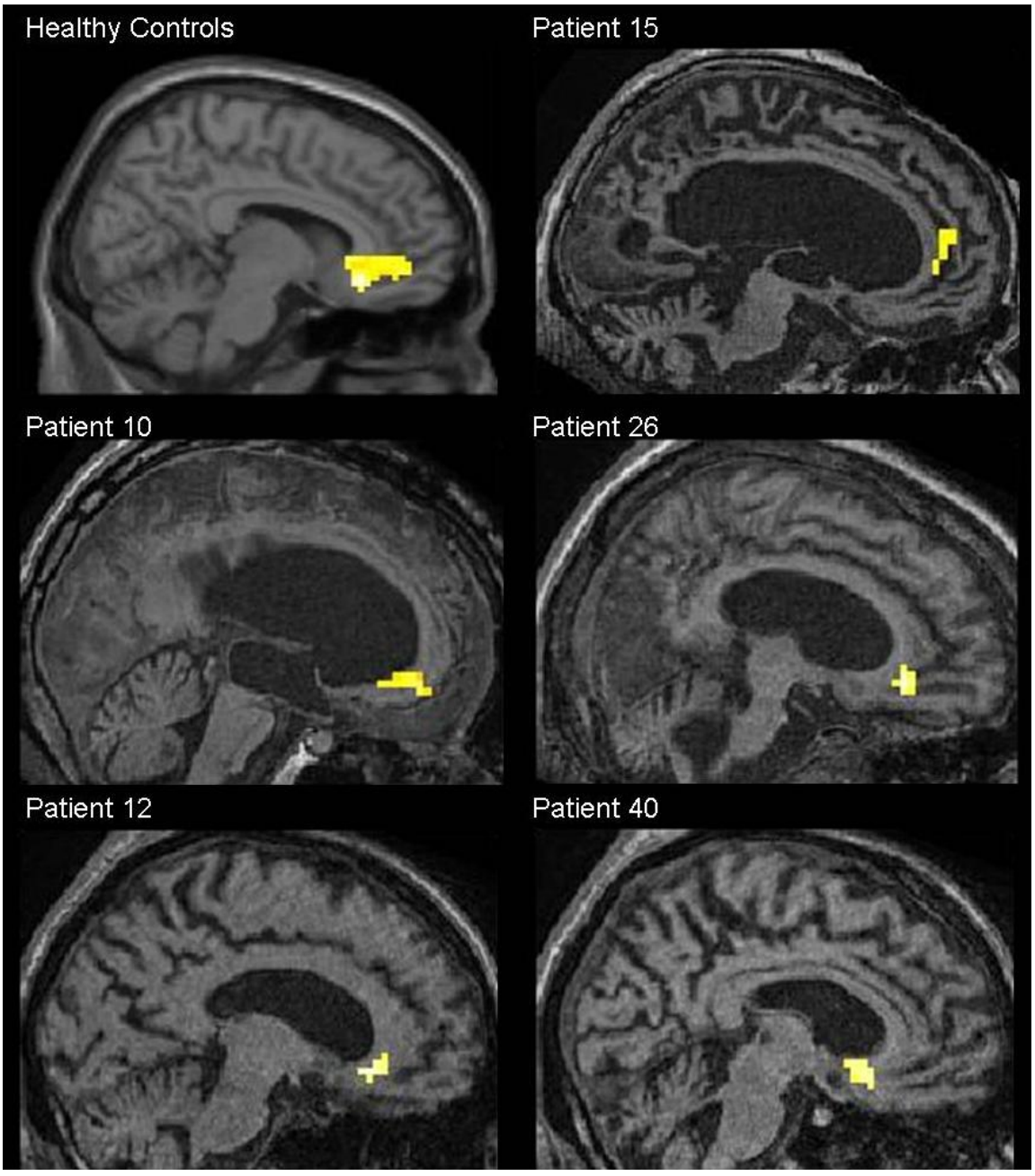

Figure 6. Negative BOLD response in the rostral ACC to pain cries compared with control stimuli (activation of contrast control>pain cries). These scans were obtained from a group of 15 healthy subjects and five DOC patients. The statistical threshold employed was an uncorrected p value of 0.001 for illustrative purposes.

Table 10 **Individual results of the emotional experiment for each of the chosen ROIs in patients**

Patient number	ACC	AI	SII	SI	Thalamus	PI	Cerebellum	STG/MTG
1	No	No	+	+	No	No	+	No
2	No	No	No	No	No	No	No	No
3	+	+	No	No	No	No	No	No
4	No	No	No	No	No	+	+	+
5	No	No	No	+	No	No	+	+
6	No	No	No	No	No	No	+	No
7	No	No	No	No	No	No	No	+
8	+	+	No	No	No	No	No	No
9	No	No	+	No	No	No	No	No
10	-	+	No	No	No	No	+	No
11	No	No	No	No	No	No	No	No
12	-	No	No	No	No	No	No	No
13	No	No	No	No	No	No	No	No
14	No	No	No	No	No	No	No	No
15	-	No	No	No	No	No	+	No
16	No	No	No	No	No	No	No	No
17	No	No	No	No	No	No	+	+
18	No	No	No	No	No	No	No	No
19	No	No	No	No	No	No	No	No
20	No	No	No	No	No	No	No	No
21	No	No	No	No	No	No	No	No
22	+	+	No	No	+	+	+	+
23	No	No	+	No	No	No	+	No
24	No	No	No	No	No	+	No	+
25	No	No	No	No	No	No	No	No
26	-	No	No	No	No	No	No	No
27	No	No	No	No	No	No	No	No
28	+	+	No	No	No	No	No	+
29	No	No	No	No	No	No	No	No
30	No	No	No	No	No	No	No	+

Table 10 (Continued.)

Patient number	ACC	AI	SII	SI	Thalamus	PI	Cerebellum	STG/MTG
31	No	No	No	No	No	No	No	+
32	No	No	No	No	No	No	No	+
33	No	No	No	No	No	No	No	+
34	No	No	No	No	No	No	+	+
35	No	No	No	No	No	No	No	No
36	+	+	+	No	+	+	+	+
37	No	No	+	No	No	No	No	No
38	No	No	No	No	No	No	No	+
39	No	No	No	No	No	No	No	+
40	-	No	No	No	No	No	No	No
41	No	No	+	+	No	No	+	No
42	No	No	No	No	No	No	No	No
43	+	+	+	+	No	+	+	+
44	No	No	No	No	No	No	No	No
45	No	No	+	+	No	+	+	+
46	No	No	No	No	No	No	No	+
47	No	No	No	No	No	No	No	No
48	No	No	No	No	No	No	No	No
49	No	No	No	No	No	+	No	+
50	No	No	No	No	No	No	No	No
51	No	No	No	+	No	No	No	+
52	No	No	No	No	No	No	No	No
53	No	No	No	No	No	No	+	No
54	No	No	No	No	+	No	+	No

Results thresholded at small volume correction corrected $p < 0.05$. ACC, anterior cingulate cortex; AI, anterior insula; SII, secondary somatosensory cortex; SI, primary somatosensory cortex; PI, posterior insula; STG/MTG, superior/middle temporal gyrus; +, significantly positive BOLD signal in the experimental condition compared to control condition; -, significant negative BOLD response; No, no significant response.

5.4 Discussion

Healthy subjects showed activation in various brain regions of the PM during the presentation of pain cries expressed by other people when compared to the presentation of control stimuli. This result is in good agreement with previous study by Lang et al. (2010) using the same experimental paradigm in a group of 22 healthy controls. However, it should be stressed that a statistical voxel-level threshold of $p < 0.001$ uncorrected with a cluster extent of 10 voxels was applied in the present study for the whole-brain analysis, whereas Lang et al. (2010) used a more conservative corrected threshold of $p < 0.05$ to identify significant differential activations between conditions. The lower threshold was chosen for three reasons: first, the subjects in the present study were older (mean age = 43.60) than in Lang et al. (2010; mean age = 26.24). Empirical studies examining emotion and aging have shown that older adults may have less neural activity in response to negative emotional stimuli when compared to young adults (Iidaka et al., 2002; Gunning-Dixon et al., 2003; Fischer et al., 2005; Tessitore et al., 2005); Second, the scanner used in the present study has a lower magnetic field (1.5T versus 3T); Third, the aim of the present study was to validate the neural responses at the individual level instead on a group level, therefore, an additional ROI analysis was applied to test the a priori hypotheses in each of the subjects.

Having positively validated this paradigm in normal subjects, with significant neural responses of the predefined ROIs in 14/15 subjects (sensory activation in 86.7% and affective activation in 73.3% of them), it is striking to see the wide variance in measured responses from the DOC patients. By definition, UWS patients are unable to have any subjective experience, including the experience of pain (Jennett, 2002; Laureys, 2010). This fact contradicts to the widely spread opinion of the patients' caregivers, most of whom believe that their patients do feel pain (Demertzi et al., 2009). Brain imaging data to this issue remain

equivocal. All positron emission tomography studies to date have shown significant activation in response to pain stimuli in UWS patients. However, some showed that these activations are restricted to the primary sensory regions (e.g., Laureys et al., 2002, Boly et al., 2005). Kassubek et al. (2003) found significant responses in the affective-cognitive structures of the PM in each UWS patient, which might be interpreted as an affective response to pain. It should be noted that all these studies used rather small samples of UWS patients (eight to 15).

But, if UWS patients might experience their own pain, can some of them also experience pain of others? In the present study 30 UWS and 24 MCS patients listened to sounds of human voice expressing pain and suffer. 46.3% of them demonstrated significant brain activations partially corresponding to those found in healthy individuals (Lang et al., 2010), and in one UWS and two MCS patients the activations were virtually identical to those of healthy subjects. Of course, partial or even full activations of the PM of the brain do not strictly prove the subjective perception and emotional experience of the stimuli. Even less want we to discuss whether the patients with significant PM responses experienced empathy with persons whose cries they heard, because the answer will largely depend on the definition of "empathy" (Batson, 2009). However, it is possible to argue that given such activations, one who denies subjective experience in UWS patients, now ought to find proofs supporting his/her skepticism.

The brain structures with the strongest activity in DOC patients were the cerebellum and the superior temporal gyrus (STG). In healthy population the cerebellum actively responds to stimuli indicating pain and suffer of others (Singer et al., 2004; Jackson et al., 2005; Moriguchi et al., 2007), even though the exact meaning of these responses remains unclear (Moulton et al., 2010). Interestingly, cerebellar responses to nociceptive stimuli appear to be

independent of the subjective experience of pain; however, this conclusion cannot be simply transferred to the stimuli signalizing others' pain.

Although the STG does not belong to the PM, its activation in the present condition is not surprising. Both STG and MTG have been shown to respond to emotional features of auditory stimuli (e.g., Wildgruber et al., 2005; Phillips et al., 1998; Sander and Scheich, 2001, 2005). Healthy individuals evaluated pain sounds used in the present as higher in arousal and valence than control sounds (see also Lang et al., 2010).

Eleven patients (20.4%) showed responses in the ACC, in six of which BOLD responses were significantly positive, and in the other five, significantly negative (Figure 6). In healthy subjects a negative BOLD response in the ACC to pain cries compared with control stimuli was obtained robustly. The ACC is probably the highest brain region in the hierarchy of affective responses closely related to the limbic system (e.g., Vogt, 2005; Fan et al., 2011). The two subgroups of patients with positive and negative BOLD responses are too small to find a reliable difference between them, although a trend can be seen in Table 1 & 10 for patients with positive BOLD responses to have a more severe brain lesion than in patients with negative responses. It might putatively be hypothesized that in highly complex networks of emotional regulation (e.g., in healthy persons) negative correlations between different control levels can emerge, resulting in a deactivation at the highest levels (i.e., ACC). The simpler the network, the less probable is the development of such complex negative loops. This hypothesis, however, remains highly speculative given that we do not even know whether a negative BOLD response is, indeed, related to neural deactivation (Shmuel et al., 2006) or not (Yuan et al., 2011).

6 Mental imagery experiment

6.1 Theoretical and empirical background

As described in the above sections, a series of fMRI studies has demonstrated that some DOC patients retain islands of residual sensory, emotional and cognitive functions. However, it is important to stress that in the absence of a full understanding of the neural correlates of consciousness, a normal or close to normal activation in response to a passive stimulation does not confirm the absolute presence of awareness because studies of learning during anaesthesia have shown that many aspects of human cognition can occur without consciousness (Laureys et al., 2006; Boly et al., 2007). This concern can be elegantly addressed using an active stimulation paradigm, the so called mental imagery.

There is often a rich phenomenology associated with visual or auditory images conjured up by one's imagination, though not nearly as detailed as those derived from direct perception. This internally generated movement, toward experiences that are not associated with particular objects in the environment or the body, is defined as mental imagery (Chalmers, 1996). Kotchoubey et al. (2003) performed a movement intention task and demonstrated in a completely paralyzed patient in the end stage of amyotrophic lateral sclerosis that conscious awareness can be attained in the absence of voluntary motor responses. The patient was instructed to imagine a fast right hand movement after hearing a high-pitch tone and a left hand movement after a low-pitch tone. A distinct negative ERP deflection with a peak latency about 250 ms was recorded to high-pitch tones at the C3 area, and to low-pitch toned at the C4 area, showing that the patient was really preparing his left or right motor cortex according to the instruction even though no task-related muscle activity could be obtained (see also Kotchoubey and Lang, 2001d).

Similarly, Owen et al. (2006) asked a UWS patient to imagine playing tennis and visiting all the rooms in her house. Comparison of each type of imagery yielded activations that are indistinguishable from those obtained from a group of healthy volunteers performing the same task (Boly et al., 2007). This study provided clear evidence of awareness and command-following in the absence of any overt behavioural response. Interestingly, the patient showed several months later inconsistent visual tracking and evolved into MCS. Probably, she was in a stage of transition from vegetative to recovery of consciousness at the time of assessment (Gosseries et al., 2011a). Recently, Monti et al. (2010) applied the same paradigm in 54 DOC patients and found that 4 UWS and 1 MCS patients retain the capacity for a volitional response to instructions.

In light of the previous studies, the mental imagery task was performed to determine what proportion of this group of subjects could also reliably modulate their brain activity, indicating nearly intact consciousness.

6.2 Methods

6.2.1 Stimuli and experimental design

The mental imagery experiment was an exact replication of previous work done by Owen et al. (2006) and Boly et al. (2007). Using a standardized auditory instruction procedure, patients were asked to perform two types of imagery task for 30-seconds-long epochs in alternation with rest periods cued with the word "relax". In the motor imagery task, the subjects were instructed to imagine playing tennis, swing an arm to hit the ball back hard, as on a tennis court during a competition. In the spatial imagery tasks, the subjects were asked to moving around the rooms in their house, concentrating on visualizing all the details in the room, rather than on walking. Each instruction was repeated six times and presented to the patients in a single session block design.

The auditory instructions were presented to the subjects via MRI-compatible headphones with efficient gradient noise suppression (up to 45dB) and a filter system with more than 90 dB RF-suppression (MR confon System, Magdeburg, Germany). The order of the blocks was pseudo-randomized and the task sequence was controlled by a PC running "Presentations" software (Neurobehavioral Systems, Albany, CA, USA).

6.2.2 Data analyses

The ROI analysis was carried out within the supplementary motor area (SMA) for motor imagery and the parahippocampal gyrus (PPA) for spatial imagery based on previous literature (Monti et al., 2010). Small volume correction across anatomical ROIs was applied and thresholded at $p<0.05$ with family-wise error (FWE) corrected for multiple comparisons.

6.3 Results

6.3.1 Signal changes for the motor and spatial imagery in healthy subjects

Motor imagery (imagine playing tennis) resulted in bilateral hemodynamic changes in the supplementary motor area, the middle/superior temporal gyri and in the inferior parietal lobule. Spatial imagery (imagine walking from room to room) specifically activated bilaterally the parahippocampal gyrus, the posterior parietal cortex, the posterior cingulate cortex and the left premotor cortex (Table 11 and Figure7).

As can be seen in Table 12 and summarized in Table 13, 86.7% (13/15) of subjects showed brain responses to the instructions. SMA activation during the motor imagery task was observed in 60% (9/15) of subjects. PPA was activated in 80% (12/15) of subjects during the spatial imagery task. 53.3% (8/15) subjects demonstrated successful modulation of their brain activity in both mental imagery tasks.

Table 11 Clusters of significant signal change in mental imagery experiment

Region	L/R	p value	Cluster size (voxels)	Peak in MNI x	y	z	z score
Navigation > Tennis							
parahippocampal gyrus	R	0.014	33	30	-37	-8	4.25
parahippocampal gyrus	L	0.011	37	-18	-37	-11	3.70
premotor cortex	L	<0.001	108	-24	8	52	5.08
posterior parietal cortex	L	<0.001	913	-6	-67	52	4.76
posterior parietal cortex	R			27	-64	52	4.66
posterior cingulate cortex	R	<0.001	199	9	-52	13	4.48
posterior cingulate cortex	L	<0.001	172	-9	-55	10	4.38
Tennis > Navigation							
supplementary motor area	L+R	0.007	67	-6	-1	64	3.18
middle/superior temporal gyri	R	<0.001	412	60	-37	4	4.80
inferior parietal lobule	R			54	-31	28	4.50
middle/superior temporal gyri	L	<0.001	642	-54	-43	7	4.56
inferior parietal lobule	L			-54	-45	28	4.39

Clusters identified with a threshold of p < .05 Family-Wise-Error corrected for multiple comparisons

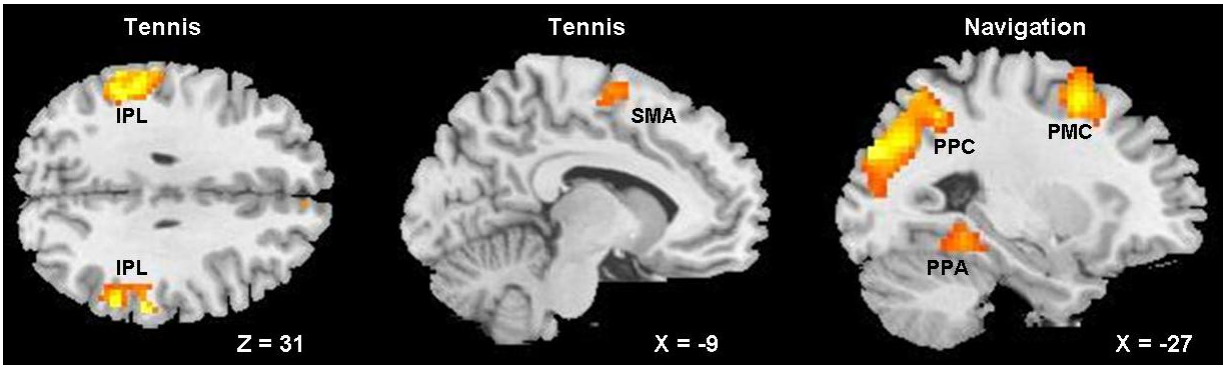

Figure 7. Significant clusters from the random-effects contrasts of tennis>navigation and navigation>tennis . The statistical threshold employed was an uncorrected p value of 0.001 for illustrative purposes.

Table 12 **Individual results of the mental imagery experiment for each of the chosen ROIs in healthy controls**

Subject number	Sex	Age	PPA	PPC	PMC	SMA	IPL
1	F	52	+	+	No	+	+
2	F	29	+	+	No	No	+
3	M	46	+	+	+	+	+
4	M	29	+	+	+	+	+
5	F	31	+	+	+	+	+
6	F	35	+	+	+	No	No
7	M	32	+	+	+	+	+
8	M	62	+	+	+	No	No
9	F	47	No	+	No	No	No
10	M	52	No	No	No	No	+
11	F	58	+	+	+	+	+
12	M	48	No	+	No	+	+
13	F	28	+	+	+	No	No
14	M	33	+	+	+	+	+
15	M	54	+	+	+	+	+

Results thresholded at small volume correction corrected $p < 0.05$. Sex (F, female; M, male); PPA, parahippocampal place area (spatial imagery task); PPC, posterior parietal cortex (spatial imagery task); PMC, premotor cortex (spatial imagery task); SMA, supplementary motor area (motor imagery task); IPL, inferior parietal lobule (motor imagery task); +, significantly positive BOLD response; No, no significant response.

Table 13 Frequencies of significant activations in mental imagery experiment (in %)

	Motor Imagery Responders	Spatial Imagery Responders	Motor AND Spatial Responders	Non-responders
UWS N = 30	6.7	6.7	3.3	90
MCS N = 24	8.3	8.3	0	83.3
Controls N = 15	60	80	53.3	13.3

Patients were regarded as motor imagery responders if supplementary motor area was significantly activated in the contrast tennis>navigation; Spatial imagery responder, significant activation in parahippocampal place area in the contrast navigation >tennis.

6.3.2 Signal changes for the motor and spatial imagery in DOC patients

As shown in Table 13 and Figure 8, 3/30 (10%) UWS and 4/24 (16.7%) MCS patients demonstrated significant brain response in mental imagery tasks. Motor imagery (imagine playing tennis) elicited SMA activation in 2/30 (6.7%) UWS and in 2/24 (8.3%) MCS patients. Spatial imagery (imagine walking around the rooms) activated the PPA in 2/30 UWS and 2/30 MCS patients. Only one UWS patient (3.3%) could modulate his brain activity in both motor imagery and spatial imagery task. The two patient groups (UWS/MCS) did not significantly differ from each other in terms of frequencies of activations. The individual results are presented in Table 14.

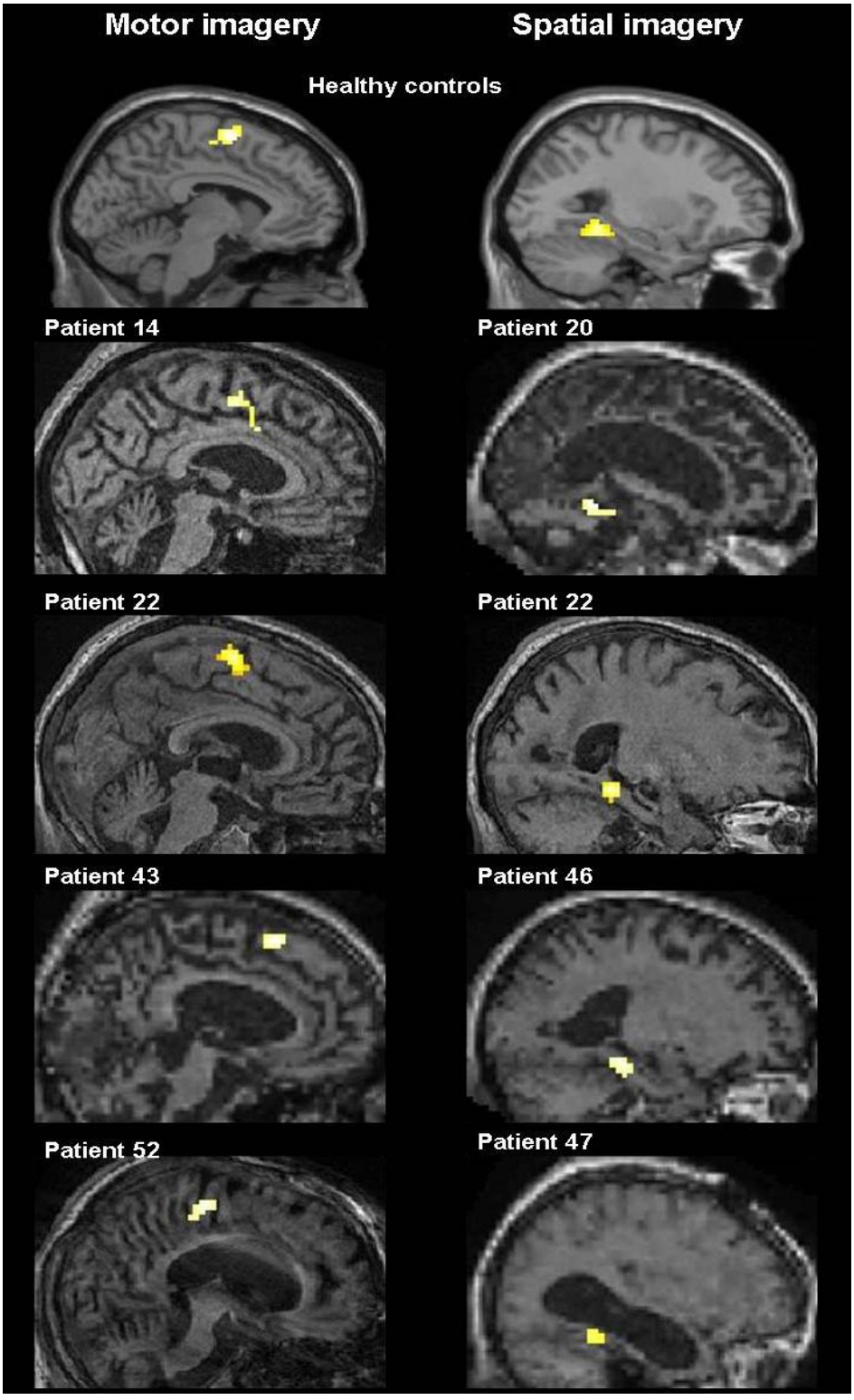

Figure 8. Brain response in the supplementary motor area in motor imagery task (contrast tennis>navigation) and in the parahippocampal place area in spatial imagery task (contrast navigation>tennis). These scans were obtained from a group of 15 healthy subjects and seven patients with disorders of consciousness. The statistical threshold employed was an uncorrected p value of 0.001 for illustrative purposes.

Table 14 **Individual results of the mental imagery experiment for each of the chosen ROIs in patients**

Patient number	PPA	PPC	PMC	SMA	IPL
1	No	No	No	No	+
2	No	No	No	No	No
3	No	No	No	No	No
4	No	No	No	No	No
5	No	No	No	No	No
6	No	No	No	No	No
7	No	No	No	No	No
8	No	+	No	No	No
9	No	No	No	No	No
10	No	No	No	No	No
11	No	No	No	No	No
12	No	No	No	No	No
13	No	No	No	No	No
14	No	No	No	+	+
15	No	No	No	No	No
16	No	No	No	No	+
17	No	No	No	No	No
18	No	No	No	No	No
19	No	No	No	No	No
20	+	No	No	No	No
21	No	No	No	No	No
22	+	+	No	+	No
23	No	+	No	No	No
24	No	No	No	No	No
25	No	No	No	No	No
26	No	+	No	No	No
27	No	No	No	No	No
28	No	No	No	No	No
29	No	No	No	No	No
30	No	No	No	No	No

Table 14 (Continued.)

Patient number	PPA	PPC	PMC	SMA	IPL
31	No	No	No	No	No
32	No	No	No	No	No
33	No	No	No	No	No
34	No	No	No	No	+
35	No	No	No	No	No
36	No	No	No	No	No
37	No	No	No	No	+
38	No	No	No	No	No
39	No	No	No	No	+
40	No	+	No	No	No
41	No	+	No	No	No
42	No	No	No	No	No
43	No	No	No	+	+
44	No	No	No	No	No
45	No	No	No	No	No
46	+	No	+	No	No
47	+	No	No	No	No
48	No	No	No	No	No
49	No	No	No	No	No
50	No	No	No	No	No
51	No	+	No	No	No
52	No	No	No	+	+
53	No	No	No	No	No
54	No	+	No	No	No

Results thresholded at small volume correction corrected $p < 0.05$. Sex (F, female; M, male); PPA, parahippocampal place area (spatial imagery task); PPC, posterior parietal cortex (spatial imagery task); PMC, premotor cortex (spatial imagery task); SMA, supplementary motor area (motor imagery task); IPL, inferior parietal lobule (motor imagery task); +, significantly positive BOLD response; No, no significant response.

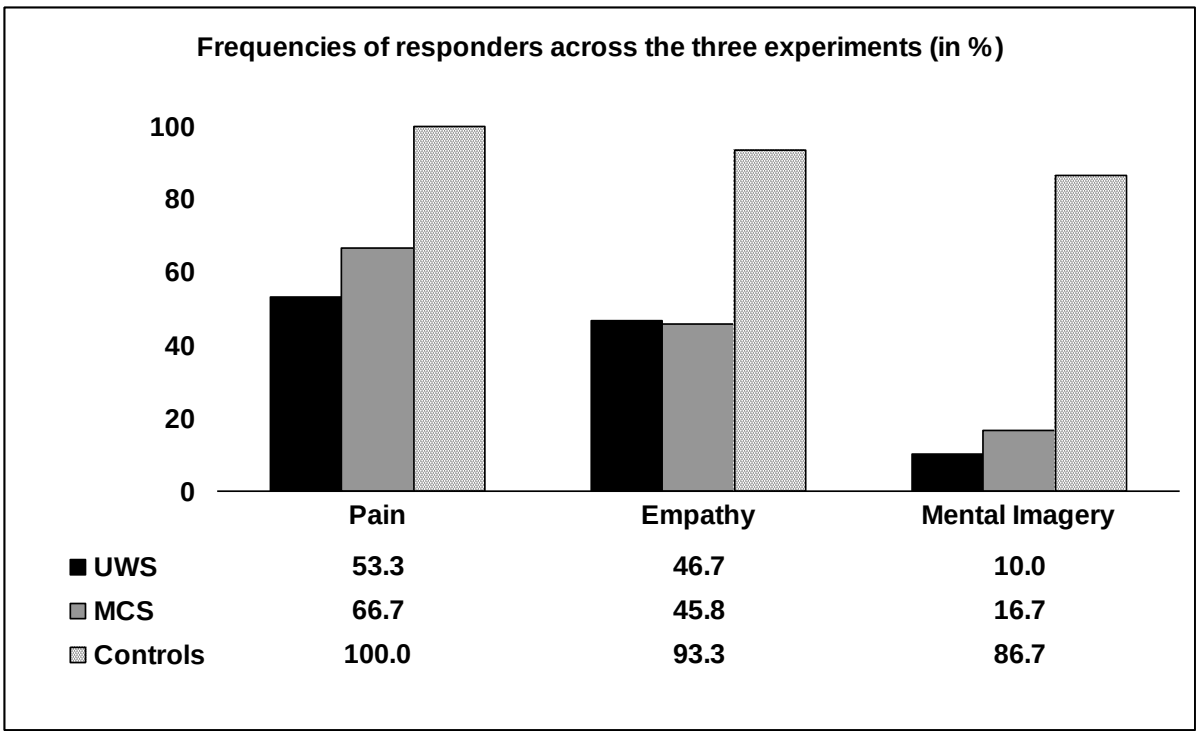

Figure 9. Frequencies of responders across the three experiments. In pain and emotional experiments, patients were regarded as responders if activations were observed in at least one of the two subsystems of PM (sensory and affective). In mental imagery experiment, patients were regarded as responders if they could modulate their brain response in at least one of the two imagery tasks.

6.3.3 Differences between the three experimental paradigms

In the pain experiment, thirty-two (59.3%) DOC patients (16 UWS and 16 MCS) with significant activations in the PM were regarded as responders. In the emotional paradigm, twenty-five (46.3%) DOC patients (14 UWS and 11 MCS) significantly responded to pain cries according to a rather conservative criterion (i.e., activation in at least one affective PM region or at least two other ROIs). In the mental imagery experiment, seven (13%) DOC patients (3 UWS and 4 MCS) who demonstrated successful modulation of their brain responses in at least one of the two imagery tasks were identified as responders. The data are

essentially different if the three experimental paradigms are compared with regard to the frequency of responders (Figure 9). Significantly more DOC patients responded to the electrical pain stimulation or pain cries of other people than to the auditory imagination instructions: $\chi 2(2) = 25.78$, $p<0.001$. While the number of non-responders differed significantly between paradigms in UWS [$\chi 2(2) = 14.07$, $p = 0.001$] and MCS [$\chi 2(2) = 12.35$, $p = 0.002$] patient group, no significant difference was found in the control group [$\chi 2(2) = 2.143$, $p = 0.343$].

One UWS patient (No.22) who demonstrated sensory and affective activations that were very similar to the healthy controls in both pain and emotional experiments also showed volitional responses during motor and spatial imagery tasks. Figure 10 presents an overview of the results.

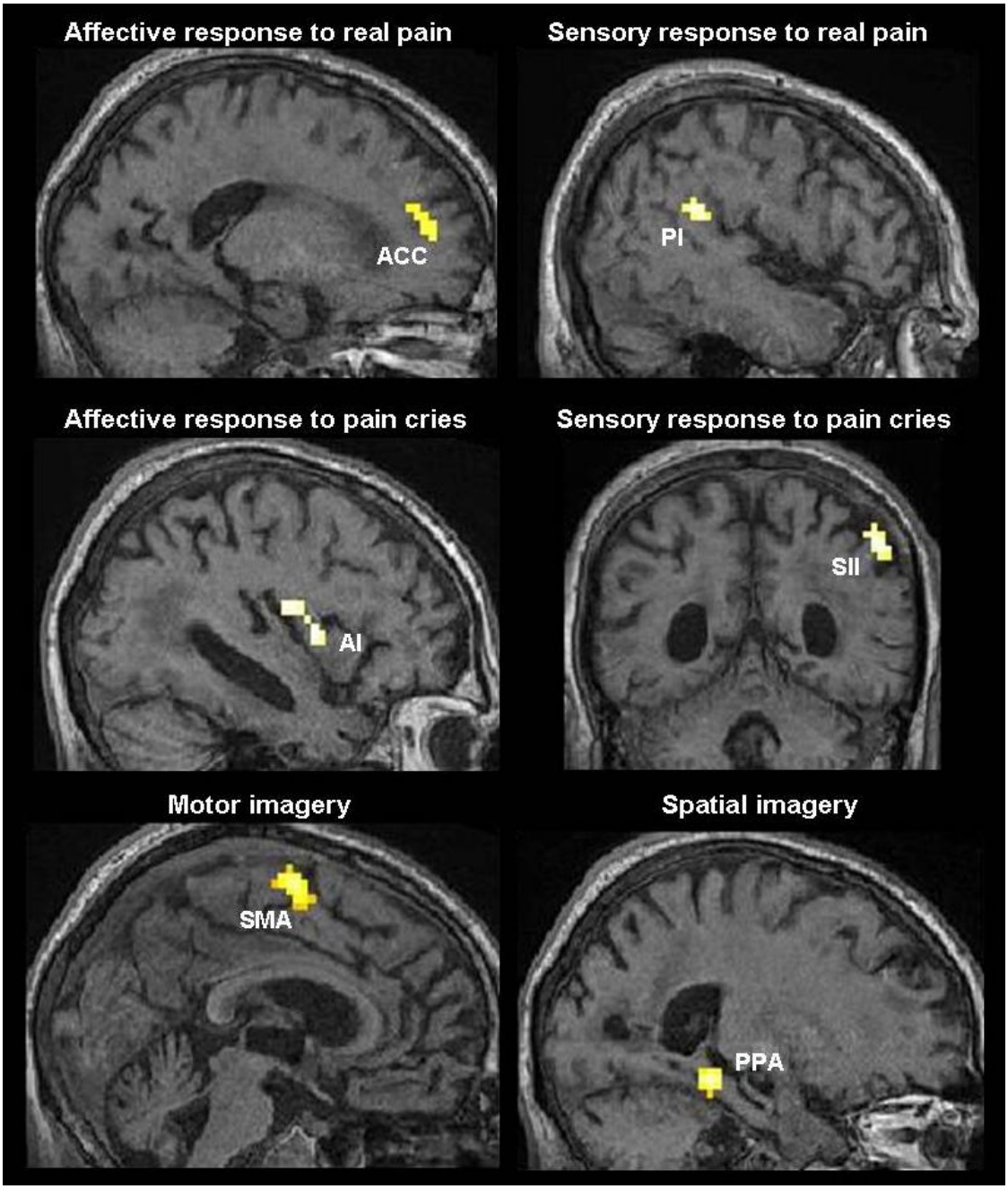

Figure 10. Patient 22 who had been diagnosed as being in UWS showed normal responses in all the three experiments. ACC, anterior cingulate cortex; PI, posterior insula; AI, anterior insula; SII, secondary somatosensory cortex; SMA, supplementary motor area; PPA, parahippocampal place area.

6.4 Discussion

Boly et al. (2007) demonstrated that command-following with spatial and motor imagery produces task-specific differential brain activation in healthy subjects. The activation pattern obtained from the 15 volunteers in the current experiment agrees with the earlier findings. However, at the single-subject level, only thirteen of the fifteen healthy subjects successfully performed the mental imagery task. The two participants who were unable to modulate their brain responses confirmed their attempts to follow the instructions during the experiment. This fact gives rise to the concerns regarding the interpretation of the negative findings. The reason for the negative results in healthy controls remains poorly understood. It is not possible to determine whether this failure is due to the low sensitivity of the method (e.g., low power to detect differences), to the loss of motivation to perform the task, to the misunderstanding of the instructions, or to some other unknown reasons (see also Bardin et al., 2011). Therefore, the absence of significant brain activations in response to the mental imagery instructions should never be taken as proof for a lack of consciousness, as demonstrated by the two healthy volunteers who failed to generate voluntary and reliable BOLD responses in predefined neuroanatomical regions.

On the contrary, positive findings do confirm that the patient has understood and followed the command and hence must be conscious. Of the fifty-four DOC patients, three UWS and four MCS patients showed volitional brain activity when asked to perform imagery tasks (Figure 8). Among these seven patients, one patient fulfilling the behavioural criteria for UWS demonstrated activations even in both motor and spatial imagery task. The activations obtained can only represent the clear act of the patients' intentions because the only difference between the conditions that induced task-dependent responses was in the instructions given at the beginning of each block. For this reason, it is impossible to explain these positive findings

without accepting that the patients retained preserved higher cognitive functions, such as language comprehension, working memory and attention maintenance, and executive control (Owen et al., 2006; Laureys et al., 2006).

Indeed, fMRI based command following with mental imagery demands higher cognitive functions than required for pain and emotion processing. In a middle-size sample of DOC patients reliable brain responses in a strongly emotional task (hearing highly unpleasant cries of pain and suffer) were obtained in 46.3% patients; in the pain experiment, 59.3% of patients were identified as responder. In contrast, only seven patients (i.e., 13%) showed reliable brain responses in the mental imagery task. This percentage agrees with that already reported in the literature, in which Monti and colleagues (2010) conducted a multicentric fMRI study enrolling 54 DOC patients and found five of them were able to wilfully modulate their brain activity. Therefore, our findings are in line with the suggestion (Panksepp 2005) that brain mechanisms underlying affective consciousness can survive even very severe lesions that make impossible higher conscious functions such as attention, working memory or language comprehension.

7 General discussion

Undoubtedly, the gray zones between the different clinical entities in the spectrum of DOC after coma are beginning to be better understood, especially thanks to the increasingly powerful neuroimaging technique. A number of previous studies using neuroimaging methods have demonstrated preserved cognitive processing in DOC patients (Laureys et al., 2002; Boly et al., 2005; Owen et al., 2006; Coleman et al., 2007; Kotchoubey et al., 2011a). The challenge now is to move from single case reports and small patient samples to studies comprising large series of patients further addressing the sensitivity and specificity of the functional neuroimaging assessment. To this end, paradigms capable of accurately detecting the residual cognitive functions were developed and applied in a group of 54 non-communicative DOC patients to determine how prevalent such retained functions are. The results revealed that 59.3% of patients were able to process painful stimuli, 46.3% of them could even process pain cries of other persons, only 13% of patients showed responses to the imagination instructions. This significant difference with respect to successful responders between the three experimental paradigms was not found in healthy controls. Thus the first hypothesis was confirmed: neural correlates of affective awareness are indeed more robust than correlates of cognitive awareness and can survive even a very severe brain damage.

Furthermore, the data of affective awareness provide evidence that the two functionally specialized networks of pain (sensory and affective) can be dissociated. For example, in the pain experiment, the sensory subsystem was activated in all healthy subjects and in 55.6% of patients, whereas the affective component was activated in only 60% of healthy volunteers and in 29.6% of patients, suggesting they might be feeling a pain that does not hurt. In the emotional experiment, the frequency of observed sensory and affective activations did not differ significantly (27.8% of patients showed sensory responses; 20.4% demonstrated

affective responses). From a neurological standpoint, when one experiences strong pain as a result of some bodily injury, then both the sensory and the affective subsystem of the PM should be active. But if one experiences vicarious pain, then the two components can be dissociated (Vignemont and Jacob, 2010). The result from the present study is in line with Vignemont and Jacob (2010) that the experience of vicarious pain can be primarily generated by the selective activation of only one of the two components of the PM.

It is important to remark that the 54 patients enrolled in the current study were all from non-traumatic aetiologies (most commonly, hypoxic-ischemic neuronal injury). Patients with non-traumatic brain damage are generally considered as the most severe subgroup of all DOC patients in terms of the extent of brain injury and outcome (Multi-Society Task Force on PVS, 1994; Kotchoubey, 2005a). Several studies have shown that the traumatic patients are superior to patients with non-traumatic aetiologies with respect to the residual brain functions (Kotchoubey 2005a, Monti et al., 2010; Bardin et al., 2011). Moreover, Owen and Coleman (2007) suggested that in non-traumatic cases, standard clinical assessment may be sufficient to rule out any potential of cognitive functions, without the need for fMRI.

Due to the considerable neuromorphological and neurophysiological differences between traumatic and non-traumatic aetiologies, only non-traumatic patients were included in the present study. Despite the fact that they belong to the most severe subgroup of DOC, many of them demonstrated reliable brain activity in response to external stimuli. More importantly, the obtained brain response did not correlate with the clinical assessment (CRS-scores). This is again in accordance with numerous previous data indicating that clinical assessment based on behavioural tests does not necessarily match the neuroimaging or neurophysiological data (Kotchoubey et al., 2005b; Staffen et al., 2006; Coleman et al., 2007; Wijnen et al., 2007; Fischer et al., 2010).

7.1 Differences between MCS und UWS

The most interesting aspect of the data obtained is that both UWS and MCS patients showed similar neural responses across all the three experiments. Currently, the differential diagnosis is made following prolonged and extensive behavioural assessment based on the very subtle distinction between reflexive reactions in UWS and weak, inconsistent but reproducible intentional actions in MCS. Indeed, the clinical data showed significant differences for CRS-scores and degree of atrophy between MCS and UWS patients. However, there was no significant difference with respect to fMRI responses between the two groups, even though the neuroimaging results were slightly better in MCS than in UWS patients.

As regards pain responses, these data do not agree with those from the Coma Science group who found a clear difference in brain activation between MCS and UWS (Boly et al., 2004, 2008). It should be stressed, however, that Boly et al. investigated only one MCS patient in 2004, and only five MCS patients in 2008, whereas the sample in present study comprised twenty-four patients in the most severe non-traumatic conditions within the MCS range. Thus the contradiction between the previous data and those of the current study may rather be apparent, because also in the present study a subgroup of MCS patients could be identified whose results are as good as the results of the PET studies by Boly and colleagues. MCS patients are as different as UWS patients, and vary from those who display only rare and slightest signs of conscious behaviours (MCS minus) to those who even try to communicate with their doctors and caregivers, but no reliable communication code can be established (MCS plus) (Bruno et al., 2011). Therefore, one should not be surprised that within a moderate-size, unselected MCS sample a broad range of responses could be observed.

This lack of differences between MCS and UWS may also reflect the high rate of misdiagnosis (Childs et al., 1993; Andrews et al., 1996), which implies that a large number of patients diagnosed as UWS are actually not in this condition. One may consequently assume that if behavioural assessment is considered to be rather insufficient in reliably differentiating MCS from UWS, it can not be taken as a strong foundation for the evaluation of neuroimaging technique but can only be used as a rough categorization of patients (Kotchoubey, 2007). However, this assumption could be misleading, as brain imaging methods would be more important in the diagnostics of DOC than clinical assessment and thus would be adopted to inform clinical decision making.

Using event-related brain responses to stimuli of different complexity levels, Kotchoubey et al (2005b) investigated 98 DOC patients and found no difference in cortical information processing between MCS and UWS patients. Similarly, Wijnen et al. (2007) found that the division line between DOC patients with better and worse ERP responses did not coincide with the clinical distinction between UWS and MCS. Also Coleman et al. (2007), who applied a hierarchical fMRI auditory processing paradigm to test the retained language processing in 14 DOC patients, failed to show any difference in brain responses between the two clinical groups. The findings in present study complement these observations by showing that a subset of UWS patients retained cognitive functions in processing of pain stimulation, pain cries of others and even command following, that were virtually indistinguishable from those obtained from a group of MCS patients performing the same task. This not only gives some insight into the nature of DOC, but may also be clinically relevant in providing additional information to current behavioural assessments.

7.2 Interpretation of positive results

Although fMRI technique provides a new method for evaluating consciousness when standard clinical assessment is unable to supply that information, its interpretation is complex. The coupling of neuronal activity and local hemodynamic in severely brain damaged patients may be different from that in healthy subjects, making interpretation very difficult. To answer the question whether the presence of normal brain activation in patients reflects a level of consciousness, additional requirements need to be satisfied. First, the stimuli are carefully matched, ideally identical. Second, differential activation across the two conditions is observed. Third, the observed activation is in the appropriate neuroanatomical location obtained from a group of fully conscious control subjects.

Many studies suffered from the problem described above. For example, Di et al. (2007) used fMRI to measure brain activation in response to the patient's own name spoken by a familiar voice. However, cerebral hemodynamic responses to the own name were compared only with the responses to the scanner noise. The two conditions consisted of stimuli differed in all possible aspects. Therefore, the activation observed might have reflected a low-level orienting response to speech in general, an emotional response to the speaker or any one of a number of possible cognitive processes relating to the unmatched auditory stimuli (Owen and Coleman, 2008). A recent study by Qin et al. (2010) did not meet the requirements either. They conducted an fMRI experiment in healthy subjects to identify brain regions specifically associated with self perception through the use of different auditory stimuli that had different grades of self-relatedness. Then they applied these regions as functional localizers to examine the relationship between self and consciousness level in DOC patients, however, in a different experimental design. The activation pattern obtained in healthy controls could be very different in another design and thus no specific evaluation could be made for the patients.

Of course, it is extremely difficult to create a control stimulus that matches the characteristics of the experimental stimulus in all aspects, and so a compromise is necessary. In the present emotional study, sound samples containing singing and yawning were selected as control stimuli, matching with the pain-related stimuli in the basic physical features. They served the purpose of revealing perceptual processes that are crucial for affective awareness. Therefore, the cognitive specificity is guaranteed. It would be very difficult to explain the positive results without accepting that the patients retained the ability to perceive at least some difference in the emotional valence between the two sets of auditory stimuli. By extension, although empathy is not necessarily proven by the activation of PM, the positive results do confirm that most of the preceding stages of empathy are intact. In order for a difference between sounds containing painful and neutral human voices to be detected it is necessary (1) that these sounds are perceived, (2) that the human voices of other persons are recognized and (3) that the emotional meaning of these voices is comprehended. All of these stages are essential for empathy process, and go well beyond those processes in previous studies (e.g., Laureys et al., 2004b; Di et al., 2007) which contrasted emotional auditory stimuli (infant cries and the patient's own name) merely with meaningless noise.

The choice of the experiment is also critical for the interpretation of fMRI data. The experiment should be complex enough to ensure that the cognitive processes of interest will be studied, preferably beyond the simple stimulus perception, but not so complex that the tasks could easily overstrain the cognitive functions of a weak or inattentive patient (Owen and Coleman, 2007). For example, the very simple stimulation in the current pain experiment might elicit relatively automatic responses from the brain, which means the response comes in the absence of consciousness, thus it is difficult to argue that the patient really experiences pain. A series of studies of learning and memory during deep sleep and general anaesthesia in healthy subjects have shown that some aspects of human cognition can occur without

conscious awareness (Davis et al., 2007; Badgaiyan, 2012). In contrast, positive results in the more complex mental imagery task provide unequivocal evidence that a patient is conscious because he has to retain such higher-level cognitive functions as language comprehension, working memory and attention maintenance etc. Although it is unclear whether an activation in the pain experiment implies the patients' real experience of pain, the presence of appropriate activation would suggest that some pain processing is preserved. Moreover, based on the existing data of pain studies in DOC patients that the lower-order pain-related activations (e.g., SI activation) in UWS patients were isolated and functionally disconnected from higher-order associative cortices (Laureys et al., 2002; Boly et al., 2005), and also on the theoretical arguments for a more robust affective awareness (Panksepp, 2005), we might consider that the activation in SII, ACC or insula is of greater significance than the activity in SI or thalamus, which can in some conditions be induced by sensory stimuli that fail to reach a level of consciousness.

7.3 Interpretation of negative results

Not only positive fMRI results encounter limitation in the interpretation, negative results are also ambiguous as they can represent true negative results, a drop in signal intensity, habituation, fluctuations in arousal, lack of attention, etc. Two types of negative findings should be distinguished:

The first type of negative findings is that the patients produce significant activations; however, the pattern of these activations is completely different than those obtained from a group of healthy controls performing the same task. Several factors could account for these abnormal response patterns. DOC patients are a very heterogeneous group, classified mainly by clinical criteria and resulting from a wide range of brain lesions. The differences between the patients

with most severe brain damage and the healthy subjects are apparently great and, in addition to the state of consciousness, may involve education, quality of life, opportunities, motivation and emotion, habitual reinforcements, and plenty of other factors (Kotchoubey and Lang, 2011d). Therefore, rather than completely healthy individuals, other patients with similar brain lesions but differing from the main group in terms of some critical variable (e.g., fully conscious), can be considered as a control for DOC patients (Kotchoubey and Lang, 2011d). Furthermore, due to the nature of statistical assessment, the selection of ROIs is arbitrary, not to mention that the method used to create ROI is strongly affected by the severe distortion of the brain regions compared with the healthy control brain. In addition, both neuromorphology and functional neurophysiology may be severely altered and have encountered some amount of cortical remapping in these patients. These issues may further increase the probability of obtaining abnormal activations, especially when using healthy controls as a benchmark. Thus, although the functional meaning of the irregular responses remains unclear, it is a miscarriage to ignore them and regard them just as non-responses.

The second type of negative findings is the lack of significant activation. In fact, false negative findings are common in fMRI investigations especially when analysis is performed at single subject level. Negative results are largely determined by the statistical power of the test. In the present study, a statistical threshold of $p<0.05$ corrected for multiple comparisons using the FWE procedure was applied to provide rigorous control for false positives, which means, at the same time, less sensitivity of detecting activation patterns. Indeed, when the statistical threshold was substantially reduced to $p<0.01$ uncorrected, appropriate activation could be found in several patients who did not reach significance at corrected level. This indicates that these patients may retain some relevant processing, but the neural activities are either too weak or too variable to be statistically reliable. Negative results are also determined by other factors, such as fluctuation in arousal/alertness, habituation or deficit of attention.

This is of particular relevance to DOC patients, especially for MCS patients in which fluctuations are even a part of definition of their diagnosis. It is also important to stress that some patients enrolled in the present study exhibited large head movement (up to 20mm translation and 20 degree rotation) during the fMRI measurement. Such spontaneous movement can produce strong task-irrelevant noise into fMRI time series, reducing the power of statistical analysis (Wilke, 2012).

To sum up, the absence of a positive finding should not be used as evidence for the lack of consciousness. The results presented in the current study suffer from the tendency to underestimate the cognitive capacities of the DOC patients. It has been recommended to regard the positive data as the lower limit of the patients' real processing abilities (Kotchoubey, 2005a).

7.4 Benefits for DOC patients

Although the findings are encouraging, it is still a matter of debate whether DOC patients could benefit from the potential of functional neuroimaging technique. The existing clinical assessments can evaluate the volitional motor responses and confirm the presence of consciousness reliably, as long as the patient is able to produce a voluntary motor output. Therefore, those patients with preserved motor behaviour would only benefit to a limited extent. In the present study, some MCS patients who showed inconsistent but reproducible behavioural response to command in the clinical examination demonstrated no cerebral response in the fMRI experiments, probably due to the false negative problem discussed above. In this patient group, brain imaging methods really have some theoretical and practical difficulties. This is also a reason of why neuroimaging assessment cannot replace clinical tests based on motor behaviours which are unequivocal and reproducible. However, if bedside

clinical examinations fail to detect any signs of consciousness, then fMRI technique may provide valuable information about the residual cognitive processing without demanding the patients to show any motor response.

One patient (No.22) fulfilling the clinical diagnostic criteria for UWS showed not only pain and emotion perception responses that were very similar to that of the control subjects for both tasks but also voluntary responses when asked to perform mental imagery tasks. These findings, especially the latter one, are strongly suggestive of a higher level of cognitive function beyond UWS and even beyond MCS. Although the positive findings are not intended to change the clinical diagnosis, they raise considerable concerns about tracking the further recovery of consciousness for this patient as well as planning and monitoring an appropriate treatment. More importantly, the additional information provided by functional neuroimaging may lead up to a dramatic change in motivation towards the patient from his care teams, changing their previously negative views.

The 54 patients in the present study are a group of clinically non-communicative patients with most severe non-traumatic brain damage. This group is no longer homogenous if we consider their cognitive function state as evaluated using functional neuroimaging technique. The hierarchical fMRI assessment employed here has opened a door into a world of possibilities to give the patients a great opportunity to respond on the basis of neural signs of consciousness that are inaccessible to clinical behavioural tests. Although there are several important limitations that need to be overcome, the strict methodology using ROI analysis and corrected statistical threshold provide a valuable tool to detect residual cognitive function or even volition in DOC patients. In some circumstances, this may be the only way to differentiate the conscious patient from the unconscious one.

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9 Abbreviations

ACC	Anterior cingulate cortex
AI	Anterior insula
BIS	Bispectral index
BOLD	Blood oxygen level dependent
BA	Brodmann area
CRS-R	Coma recovery scale revised
DOC	Disorders of consciousness
EEG	Electroencephalogram
ERP	Event-related potential
FDG	Fluorodeoxyglucose
fMRI	Functional magnetic resonance imaging
FWE	Family wise error
GCS	Glasgow coma scale
IADS	International affective digitized sounds
IPL	Inferior parietal lobule
IRI	Interpersonal reactivity index
ISI	Interstimulus interval
MCS	Minimally conscious state
MEG	Magnetoencephalography
MNI	Montreal neurological institute
MRI	Magnetic resonance imaging
MTG	Middle temporal gyrus
PET	Positron emission tomography
PI	Posterior insula

PM	Pain matrix
PMC	Premotor cortex
PPA	Parahippocampal place area
PPC	Posterior parietal cortex
PVS	Persistent vegetative state
ROI	Region of interest
SAM	Self-assessment manikin
SD	Standard deviation
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
SMA	Supplementary motor area
SMART	Sensory modality assessment and rehabilitation technique
SPECT	Single-photon emission computerized tomography
STG	Superior temporal gyrus
UWS	Unresponsive wakefulness syndrome
VAS	Visual analog scale
VS	Vegetative state
WHIM	Wessex head injury matrix