Brain connectivity and sensory stimulation in patients with disorders of consciousness

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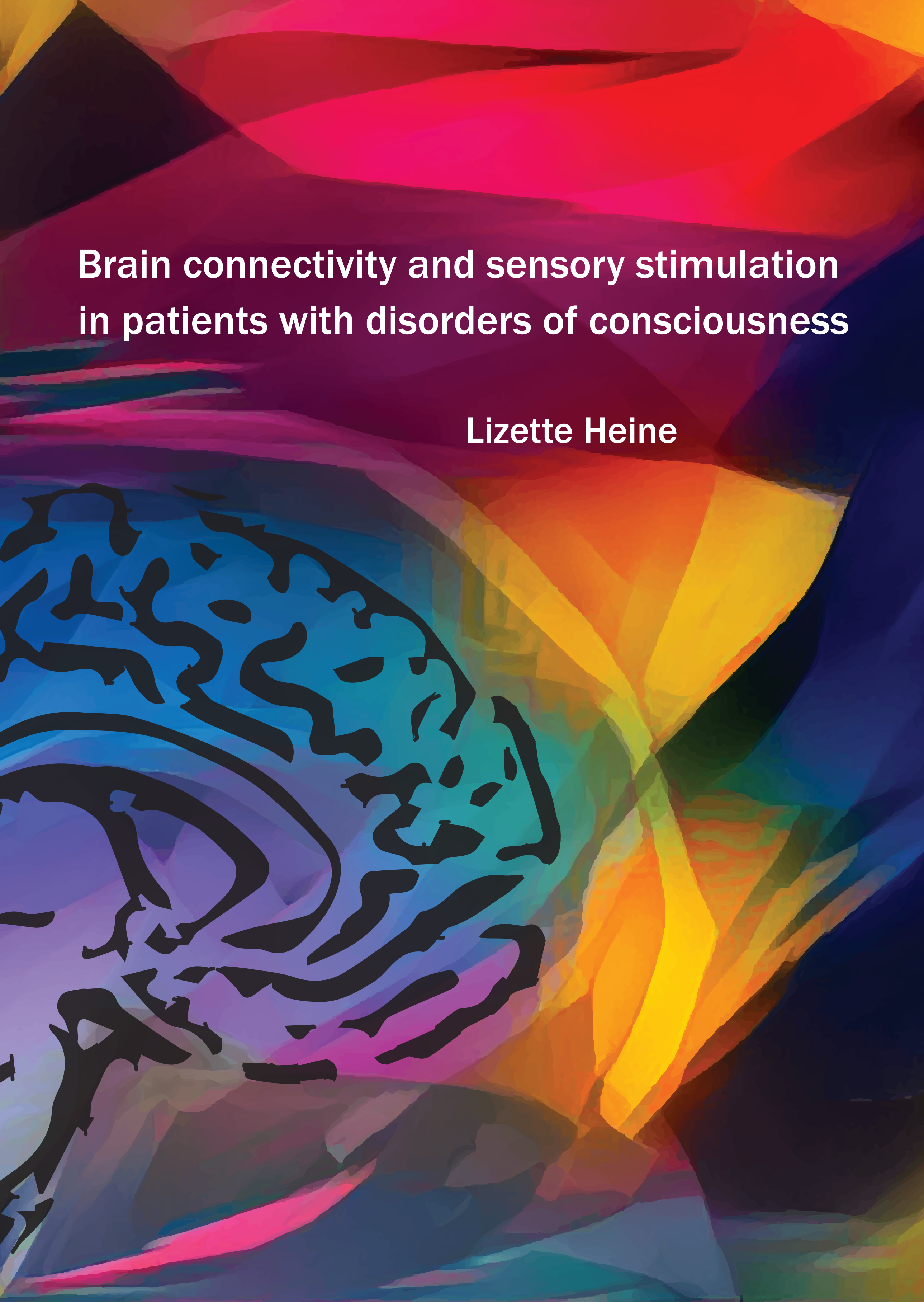

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List of Publications

This thesis is based on the following publications:

Articles

I. **Function-structure uncoupling in patients with severe brain injury as measured by MRI-DWI and FDG-PET**
Annen J*, **Heine L***, Ziegler E, Frasso G, Bahri M, Di Perri C, Stender J, Martial C, Wannez S, D'Ostilio K, Amico E, Antonopoulos G, Bernard C, Tshibanda F, Hustinx R, Laureys S.
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II. **Effects of preference and sensory modality on behavioral reactions in patients with disorders of consciousness**
Heine L, Tillmann B, Hauet M, Juliat A, Dubois A, Laureys S, Kandel M, Plailly J, Luauté J, Perrin F.
under review

III. **Exploration of functional connectivity during preferred music stimulation in patients with disorders of consciousness**
Heine L*, Castro M*, Martial C, Tillmann B, Laureys S, Perrin F.
Frontiers in psychology, 2015, 6:1704, doi: 10.3389/fpsyg.2015.01704

IV. **Functional connectivity in visual, somatosensory, and language areas in congenital blindness**
Heine L, Bahri A M, Cavaliere C, Soddu A, Reislev N, Laureys S, Ptito M, Kupers R.
Frontiers in Neuroanatomy, 2015, 138(pt9):2619-31,
doi: 10.3389/fnana.2015.00106

V. **Intrinsic functional connectivity differentiates minimally conscious from unresponsive patients**
Demertzi A*, Antonopoulos G*, **Heine L**, Voss H, Crone S,Kronbichler M,
Trinka E, Angeles C, Bahri M, Phillips C, Di-Perri C, Gomez F,

Tshibanda L, Soddu A, Vanhaudenhuyse A, Charland-Verville V, Schiff N, Whitfield-Gabrieli S*, Laureys S*.
Brain, 2015, 6:1704, doi: 10.3389/fpsyg.2015.01704

VI. **Technology-based assessment in patients with disorders of consciousness**
Di Perri C, **Heine L**, Amico E, Soddu A, Laureys S, Demertzi A.
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VII. **Resting state networks and consciousness: alterations of multiple resting state network connectivity in physiological, pharmacological, and pathological consciousness States**
Heine L, Soddu A, Gomez F, Vanhaudenhuyse A, Tshibanda L, Thonnard M, Charland-Verville V, Kirsch M, Laureys S, Demertzi A.
Frontiers in Psychology, 2012, 3:295, doi: 10.1093/brain/awv169

Bookchapters:

I. **Behavioral Responsiveness in Patients with Disorders of Consciousness**
Heine L, Laureys S, Schnakers C.
in: Brain Function and Responsiveness in Disorders of Consciousness, *Springer International Publishing*, 2016

II. **Imaging correlations in non-communicating patients.**
Heine L, Di Perri C, Soddu A, Gomez F, Laureys S, Demertzi A.
in: Clinical neurophysiology in disorders of consciousness: Brain function monitoring in the ICU and beyond, *Springer International Publishing*, 2015

III. **Consciousness and disorders of consciousness**
Heine L, Demertzi A, Laureys S, Gosseries O.
in: Brain mapping: An encyclopedic reference, *Elsevier*, 2015

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Other publications:

I. **Repeated behavioral assessments with the Coma Recovery Scale-Revised in patients with disorders of consciousness**
Wannez S, **Heine L**,Thonnard M, Annen J, Charland-Verville V, Martens G, Aubinet C, Martial C, Bodart O, Vanhaudenhuyse A, Chatelle C, Thibaut A, Schnakers C, Demertzi A, Gosseries O*, Laureys S*.
under review

II. **A method for Independent Component Graph Analysis of resting-state fMRI**
Paula D, Ziegler E, Abeyasinghe P, Tushar D, Cavaliere C, Aiello M, **Heine L**, Di Perri C, Demertzi A, Noirhomme C, Charland-Verville V, Vanhaudenhuyse A, Stender J, Gomez F, Tshibanda L, Laureys S, Owen A, Soddu A.
under review

III. **Mapping the functional connectome traits of levels of consciousness**
Amico E, Marinazzo D, Di Perri C, **Heine L**, Annen J, Martial C, Dzemidzic M, Laureys S*, Goni J*.
under review

IV. **Structural constraints to information flow within cortical circuits : a TMS / EEG-dMRI study**
Amico E, Bodart, O, Gosseries O, **Heine L**, Rosanova M, Van Mierlo P, Martial C, Massimini M, Marinazzo D*, Laureys S*.
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V. **The neural correlates of consciousness in patients who have emerged from minimally conscious state: a multimodal imaging study**
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VI. **Clinical response to tDCS depends on residual brain metabolism and grey matter integrity in patients with minimally conscious state**
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Brain Stimulation, 2015, 8(6):1116-23,doi: 10.1016/j.brs.2015.07.024

VII. **Beyond the gaze: communicating in chronic locked-in syndrome**
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VIII. **Correlation between resting state fMRI total neuronal activity and PET metabolism in healthy controls and patients with disorders of consciousness**
Soddu A, Gomez F, **Heine L**, Voss HU, Bruno MA, Vanhaudenhuyse A, Phillips C, Demertzi A, Chatelle C, Schrouff J, Thonnard M, Thibaut A, Truong J, Charland-Verville V, Noirhomme Q, Tshibanda JF, Salmon E, Schiff ND, Laureys S
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IX. **Diagnostic precision of multimodal neuroimaging methods in disorders of consciousness - a clinical validation study**
Stender J, Gosseries O, Bruno MA, Charland-Verville V, Vanhaudenhuyse A, Demerzti A, Chatelle C, Thonnard M, Thibaut A, **Heine L**, Soddu A, Boly M, Schnakers C, Gjedde A, Laureys S.
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X. **Assessment of visual fixation in vegetative and minimally conscious states**
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BMC Neurology, 2014, 16:14-147,doi:10.1186/1471-2377-14-147

XI. **Detection of visual pursuit in minimally conscious patients: a matter of visual plane?**
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XII. **Measuring consciousness in coma and related states**
Di Perri C, Thibaut A, **Heine L**, Soddu A, Demertzi A, Laureys S.
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XIII. **Looking for the self in pathological unconsciousness**
Demertzi A, Vanhaudenhuyse A, Brédart S, **Heine L**, Di Perri C, Laureys S.
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XIV. **Mindsight: Diagnositcs in disorders of consciousness**

Guldenmund P, Stender J, **Heine L**, Laureys S.

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Glossary

Active paradigm Experimental condition which requires the subject to perform a specific task on request.

CRS-R Coma Recovery Scale-Revised.

DMN Default mode network. Resting state network of distinct, remote, and cooperating brain areas. DMN activity has been linked to self-related and internal processes.

DOC Disorders of consciousness. This term refers to altered states of consciousness as a result of severe acquired brain injuries and describes patients in coma, vegetative state/unresponsive wakefulness syndrome, and minimally conscious states.

DTI Diffusion-tensor imaging. MRI technique that measures water molecule diffusion revealing the structural integrity of axon tracts in the brain.

EMCS Emergence from minimally conscious state. No clinical disorder of consciousness. These patients show reliable functional communication and/or functional object use.

FA A measure of directionality of water diffusion assumed to be related to myelination of white matter.

fMRI Functional magnetic resonance imaging. Non-invasive neuroimaging technique that measures neuronal activation based on blood-oxygen-level dependent (BOLD) changes.

Functional connectivity The temporal correlation of time courses of spatially distant, functionally related brain regions or voxels of the brain.

Gustatory stimuli Stimuli using the sense of taste.

LIS Locked-in syndrome. A clinical condition wherein patients are awake and aware, but with severe motor impairments sometimes so severe that they cannot move any part of their body. The primary means of communication is through eye movements.

MCS Minimally conscious state. A clinical disorder of consciousness wherein patients are awake but show fluctuating signs of awareness without being able to functionally communicate with their surroundings.

NCC Neural correlates of consciousness. These NCC are defined as the neural mechanisms jointly sufficient for any one specific conscious experience.

Olfactory stimuli Stimuli using the sense of smell.

Passive paradigm Experimental condition during which there is the administration of external stimulations such as auditory, tactile or visual stimuli while the subject is not asked to do anything in particular.

PET Positron emission tomography. Invasive neuroimaging technique that measures brain metabolism energy turnover.

SUV The ratio of the imaged radioactivity concentration (using PET), and the injected concentration in the whole body. Values are generally lower in patients compared to conscious control subjects.

Tactile stimuli Stimuli using the sense of touch.

UWS Unresponsive wakefulness syndrome. A clinical disorder of consciousness wherein patients are awake but not aware of themselves and their surroundings.

Abstract

This thesis assesses brain connectivity and sensory stimulation in patients with disorders of consciousness (DOC). These are serious conditions where massive brain damage can lead to a dissociation between arousal and awareness, leaving patients after a comaperiod in an unresponsive wakefulness syndrome (UWS), or minimally conscious state (MCS). As an introduction, Chapter 1 focuses on the behavioral profile of the various clinical conditions of diminished consciousness, as well as available methods and paradigms for diagnostic assessment. The work described in the rest of this thesis explores these methods to gain a better understanding of consciousness.

Part I of this thesis is dedicated to the resting paradigm. This paradigm assesses spontaneous brain activity and thus does not rely on patient cooperation. It is utilized here with structural magnetic resonance imaging (MRI), positron emission tomography (PET), and functional MRI to explore brain structure and function in severely brain damaged patients. In Chapter 2 we demonstrate that function and structure are linked by showing a positive relationship between glucose metabolism and white matter integrity. This link is present in the default mode network (i.e., network of cooperating brain areas related to internal thought), but not in the whole brain. Furthermore, we found a stronger relationship between structural integrity with the thalamus in patients who have emerged from MCS as compared to DOC patients. In Chapter 3 we review literature describing a decrease in resting state functional connectivity (i.e., the correlation between spatially distant, functionally related brain regions) in diminished levels of consciousness due to pathology, physiology and pharmacology. Chapter 4 extends this by showing significant differences between functional connectivity in MCS and UWS in most resting state networks. Using the functional connectivity between auditory and visual areas, single subject diagnostic classification is shown, pointing towards the importance of multisensory integration in these patients.

Part II of this thesis explores the passive paradigm to assess behavior and brain-function in patients. Passive paradigms try to indicate covert cognitive processing through assessment of brain function after external sensory stimulation. Chapter 5 shows that sensory stimulation without personal relevance, as used in several assessment scales does not seem to increase the presence of oriented responses, a sign of consciousness. As instead preferred stimuli might improve responsiveness, we then analyzed functional connectivity during preferred

music in Chapter 6. This preliminary study showed that functional connectivity is stronger during preferred music compared to the noise condition in brain regions of the auditory network that might be linked to autobiographical memory. Chapter 7 explored this effect as a testing context and found that auditory stimuli triggered higher responsiveness compared to olfactory stimuli. Furthermore, an effect of preference can be seen with better scores for preferred stimuli compared to neutral ones.

Part III of this thesis concerns the question of wether assessment of brain function in blind, conscious people could eventually teach us something on the presence or absence of vision in brain damaged patients. Our data reveal increased functional connectivity within both the ventral and the dorsal visual streams in congenitally blind participants as compared to healthy control participants. However, connectivity between the two visual streams was reduced in blind subjects. Our results underscore the extent of cross-modal reorganization and the supra-modal function of the occipital cortex in congenitally blind individuals.

Two conclusions can be drawn from this thesis. First, brain connectivity, as explored in part I, is linked to consciousness. The brain's function and structure are intimately related to each other, and the decrease in brain function can be used to distinguish between the clinically indicated states of consciousness.

Second, sensory stimulations as described in part II have the power to improve responsiveness. Preferred stimuli might momentarily enhance brain function and behavioral responses. The use of preferred stimuli, such as music, as a testing context might optimize the diagnostic assessments of the fluctuating pattern of minimally conscious patients. The use of preferred stimuli might thus be advised as a testing context when diagnostic doubts exist.

Résumé

Cette thèse évalue la connectivité cérébrale et la stimulation sensorielle chez les patients en état de conscience altérée (ECA). Ces troubles de la conscience apparaissent lorsquune lésion cérébrale sévère mène à une dissociation entre léveil et la conscience après une période de coma, caractérisant ainsi un syndrome déveil non répondant (ENR) ou un état de conscience minimale (ECM). À titre dintroduction, le chapitre 1 traite du profil comportemental des différentes entités cliniques liées à la diminution de la conscience ainsi que des méthodes et paradigmes disponibles pour lévaluation diagnostique. Le travail qui est décrit par la suite explore ces méthodes et vise une meilleure compréhension de la conscience.

La partie I de cette thèse est dédiée au paradigme de repos. Ce paradigme mesure lactivité cérébrale spontanée et ne dépend donc pas de la coopération du patient. Il est utilisé avec limagerie par résonance magnétique (IRM) structurelle, la tomographie par émission de positons (TEP) et lIRM fonctionnelle afin dexplorer les fonctions et structures cérébrales chez ces patients sévèrement cérébrolésés. Dans le chapitre 2, nous démontrons que fonctions et structures sont liées en montrant une relation positive entre le métabolisme glucidique et lintégrité de la matière blanche. Ce lien est présent dans le réseau du mode par défaut (cest-à-dire le réseau des aires cérébrales liées aux réflexions internes), mais ne concerne pas le cerveau dans son ensemble. En outre, nous avons trouvé une plus forte relation entre lintégrité structurelle du thalamus et sa fonction chez des patients ayant émergé dun ECM, en comparaison avec des patients en ECA. Dans le chapitre 3, nous avons passé en revue la littérature décrivant une diminution de la connectivité fonctionnelle au repos (cest-à-dire la corrélation entre les régions cérébrales spatialement éloignées mais fonctionnellement reliées) chez des patients montrant des niveaux de conscience réduits pour des raisons pathologiques, mais aussi physiologiques ou pharmacologiques. Le chapitre 4 élargit ce sujet en montrant des différences significatives entre la connectivité fonctionnelle chez les patients en ECM et chez les patients en ENR dans la plupart des réseaux du repos. Nous avons développé, via la connectivité fonctionnelle entre les aires auditives et visuelles, une classification diagnostique individuelle qui souligne limportance de lintégration multi-sensorielle chez ces patients.

La partie II de cette thèse explore le paradigme passif afin dévaluer le fonctionnement comportemental et cérébral des patients en ECA. Les paradigmes passifs tentent de dévoiler les processus cognitifs latents en évaluant la fonction cérébrale après une stimulation

sensorielle externe. Le chapitre 5 montre que les stimulations sensorielles dépourvues de signification personnelle, telles quelles sont utilisées dans plusieurs échelles dévaluation, ne semblent pas favoriser lapparition de réponses orientées (signes de conscience). Puisque lutilisation des stimuli préférés des patients pourrait toutefois améliorer leur réactivité, le chapitre 6 se consacre à lanalyse de la connectivité fonctionnelle lors de lécoute dune musique favorite. Cette étude préliminaire montre que la connectivité fonctionnelle est plus importante lors dune musique favorite par rapport au bruit ambiant dans des régions cérébrales du réseau auditif qui pourraient être liées à la mémoire autobiographique. Le chapitre 7 explore cet effet en situation dévaluation et démontre que le stimulus auditif déclenche une réactivité plus élevée en comparaison au stimulus olfactif. De plus, un effet de préférence peut être observé par de meilleurs scores pour des stimuli préférés par rapport aux neutres.

La partie III de cette thèse concerne lévaluation du fonctionnement cérébral chez des personnes aveugles et conscientes, sintéressant à ce quil peut nous enseigner sur la cécité chez des patients cérébro-lésés. En effet, nous avons mesuré la connectivité fonctionnelle chez des personnes aveugles pour cause congénitale. Nos données montrent une connectivité fonctionnelle plus élevée au sein des voies visuelles ventrale et dorsale chez ces participants. Cependant, la connectivité entre ces deux voies visuelles était réduite chez ces sujets aveugles. Nos résultats soulignent létendue de la réorganisation intermodale et des fonctions supra-modales du cortex occipital chez des individus aveugles congénitaux.

Deux conclusions ressortent de cette thèse. Premièrement, la connectivité cérébrale, telle quexplorée dans la partie I, est liée à la conscience. Les fonctions et structures cérébrales sont intimement connectées les unes aux autres, et la réduction des fonctions cérébrales peut être employée pour distinguer les différents tableaux cliniques détat de conscience.

Deuxièmement, les stimulations sensorielles telles quelles sont décrites dans la partie II peuvent améliorer la réactivité des patients. Les stimuli préférés pourraient momentanément améliorer les fonctions cérébrales ainsi que les réponses comportementales. Lutilisation de stimuli préférentiels, tels que la musique, dans une situation dévaluation, pourrait également optimiser les examens diagnostiques des patients en ECM qui présentent généralement une fluctuation de leur état de conscience. Lutilisation des stimuli préférentiels pourrait donc être recommandée en situation dévaluation lorsque certains doutes persistent quant au diagnostic.

Samenvatting

In dit proefschrift is hersenconnectiviteit en sensorische stimulatie in patiënten met bewustzijnsstoornissen (disorder of consciousness; DOC) onderzocht. Dit zijn ernstige aandoeningen waarbij grote schade aan de hersenen kan leiden tot een dissociatie tussen waakzaamheid en besef, waardoor patiënten na een periode van coma in een niet responsief waaksyndroom (unresponsive wakefulness syndrome (UWS)) of een minimaal bewuste staat (minimally conscious state (MCS)) terecht kunnen komen. Als inleiding richt hoofdstuk 1 zich op het gedragsmatige profiel van de verschillende klinische entiteiten van bewustzijnsstoornissen, alsmede de beschikbare methoden en paradigmas voor diagnostiek. Het werk beschreven in in de rest van dit proefschrift verkent deze methodses om een beter begrip te krijgen van bewustzijn.

Deel I van dit proefschrift is gewijd aan het rust-paradigma. Dit paradigma bestudeerd spontane hersenactiviteit en is daardor niet afhankelijke van samenwerking vanuit de patiënt. Het wordt hier gebruikt met structurele magnestic resonance imaging (MRI), position emission tomography (PET), en functionele MRI om de structuur en functie van de hersenen te bestuderen in deze patiënten met ernstig hersenletsel. In hoofdstuk 2 demonstreren we dat functie en structuur gelinkt zijn door het tonen van een positieve relatie tussen de glucosehuishouding en de integriteit van de witte stof. Deze link is aanwezig in het default mode network (d.w.z. het netwerk van samenwerkende hersengebieden gerelateerd aan interne gedachtes), maar niet in het gehele brein. Verder vonden we een sterkere relatie tussen de structurele integriteit met de thalamus in patiënten die uit een minimaal bewuste staat zijn gekomen vergeleken met DOC-patiënten. In hoofdstuk 3 geven we een overzicht van de literatuur die de afname van rust-staat functionele connectiviteit (d.w.z. de correlatie tussen van elkaar afgelegen, maar functioneel gerelateerde hersengebieden) beschrijft in patiënten met een verminderd bewustzijnsniveau door pathologische, physiologische, of parmacologische oorzaak. Hoofdstuk 4 breidt deze vinding uit door het tonen van significante verschillen in functionele connectiviteit tussen MCS en UWS in de meeste rust-staat netwerken. Met behulp van de functionele connectiviteit tussen auditieve en visuele gebieden was het tevens mogelijk om een diagnose te stellen op patiënt basis, wat wijst op het belang van multi-sensorische integratie in deze patiënten.

Deel II van dit proefschrift gebruikt het passieve paradigma om gedrag- en hersen-functionering te evalueren in patiënten. Passieve paradigmas proberen om verborgen cognitieve verwerking aan te duiden door evaluatie van de verwerking in de hersenen van externe sensorische stimulaties. Uit hoofdstuk 5 blijkt dat sensorische stimulaties zonder persoonlijke relevantie, zoals die gebruikt worden in verschillende beoordelingsschalen de aanwezigheid van georiënteerde reacties, een teken van bewustzijn, niet verhogen. Aangezien misschien niet gegeneraliseerde, maar favoriete stimulaties het reactievermogen verhogen, hebben we daaropvolgend functionele connectiviteit tijdens favoriete muziek geanalyseerd in hoofdstuk 6. Deze verkennende studie laat zien dat functionele connectiviteit sterker is tijdens favoriete muziek vergeleken met een geluid conditie in hersengebieden van het auditieve netwerk die mogelijk gelinkt kunnen worden aan autobiografisch geheugen. Hoofdstuk 7 verkent dit effect als een evaluatie-context en we bevonden dat auditieve stimuli een hoger reactievermogen verkrijgen in vergelijking met olfactieve (reuk) stimuli. Bovendien is er een effect van voorkeur, waarbij betere scores verkregen worden na favoriete stimuli ten opzichte van neutrale stimuli.

Deel III van dit proefschrift gaat over de vraag of analyse van de functionele connectiviteit in blinde en bewuste mensen ons uiteindelijk iets kan leren over de aan- of afwezigheid van zichtvermogen in patiënten. Onze data toont een toegenomen functionele connectiviteit in de ventrale en dorsale visuele stromen in de hersenen van blinde vergeleken met controle subjecten. Echter, de connectiviteit tussen deze twee visuele stromen was verlaagd in blinden. Onze data onderstrepen de omvang van de cross-modale reorganisatie en de supra-modale functie van de occipitale cortex in mensen met aangeboren blindheid.

Twee conclusies kunnen getrokken worden uit dit proefschrift: Ten eerste, de hersenconnectiviteit, zoals onderzocht in deel I, is gekoppeld aan bewustzijn. De functie en structuur van de hersenen zijn nauw gerelateerd, en de afname van hersenfunctie kan worden gebruikt om onderscheid te maken tussen de klinische bewustzijnstoestanden.

Ten tweede, sensoriële prikkels zoals beschreven in deel II hebben de kracht om het reactievermogen te verbeteren. Favoriete stimuli kunnen wellicht tijdelijk hersenfunctie en gedragsreacties vergroten. Het gebruik van favoriete stimuli, zoals muziek, als test-context zou de diagnostische beoordeling van het fluctuerende patroon van minimaal bewuste patiënten optimaliseren. Het gebruik van favoriete stimuli zou dus geadviseerd kunnen worden als een test-context wanneer diagnostische twijfels bestaan.

Chapter 1

Introduction

Based on the following articles:

Consciousness and disorders of consciousness
Heine L, Demertzi A, Laureys S, Gosseries O.
in: Brain mapping: An encyclopedic reference, *Elsevier*, 2015

Imaging correlations in non-communicating patients
Heine L, Di Perri C, Soddu A, Gomez F, Laureys S, Demertzi A.
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Technology-based assessment in patients with disorders of consciousness
Di Perri C, **Heine L**, Amico E, Soddu A, Laureys S, Demertzi A.
Annali dell'instituto superiore di sanita, 2014, 6:1704,

1.1 Consciousness

Being conscious means you are aware and responsive to your surroundings. You are having a subjective, private, what is it like, experience related to, for example, auditory stimuli, visual stimuli, thoughts, or emotions. The physical origin of this subjective experience and its psychology is still being elucidated. Therefore, there is not yet a universal, all inclusive definition of consciousness. In a clinical setting, consciousness is reduced into two main components: wakefulness and awareness [1]. Wakefulness is related to arousal, or the level of vigilance. Awareness is related to subjective experiences and can be subdivided into awareness of the external world (e.g., sensory perception of the environment) and of the internal world (e.g., stimulus-independent thoughts, such as mental imagery and inner speech).

Sleep is an illustrative example to describe the relationship between wakefulness and awareness: the drowsier we become as we move towards deep sleep, the less aware we are of our surroundings and ourselves (figure1.1). A dissociation between wakefulness and awareness leads to states of diminished consciousness. Anesthesia, epilepsy, somnambulism (i.e., sleep walking) are examples of this.

Disorders of consciousness (DOC) are pathological states with such a dissociation between arousal and awareness. Arousal can be present, while awareness is absent or fluctuating. What differentiates DOC from other states of unconsciousness, such as those due to pharmacological anesthesia, sleep and epileptic seizures, is the prolonged impaired awareness followed by severe brain damage.

In this chapter we will first define the different states of consciousness following severe brain injury. We will describe the necessity of proper clinical evaluation and the most sensitive clinical scales to do so. We will then shortly present the neuronal characteristics of patients with DOC as measured by different neuroimaging techniques employing available paradigms for ancillary testing. Finally, we will briefly present the objectives of this thesis.

1.2 Disorders of consciousness

Coma

The main causes of coma are trauma, stroke, or anoxia (e.g., cardiac arrest). A coma is a transient state of unarousable unresponsiveness during which the patient lies with the eyes closed, and has no awareness of self and surrounding [1]. A coma must last at least one hour to be differentiated from fainting (i.e., syncope). Autonomic functions, such as breathing and thermoregulation, are reduced, which often requires respiratory assistance. In general, most patients recover from a coma within the first hours to weeks after injury. However, some

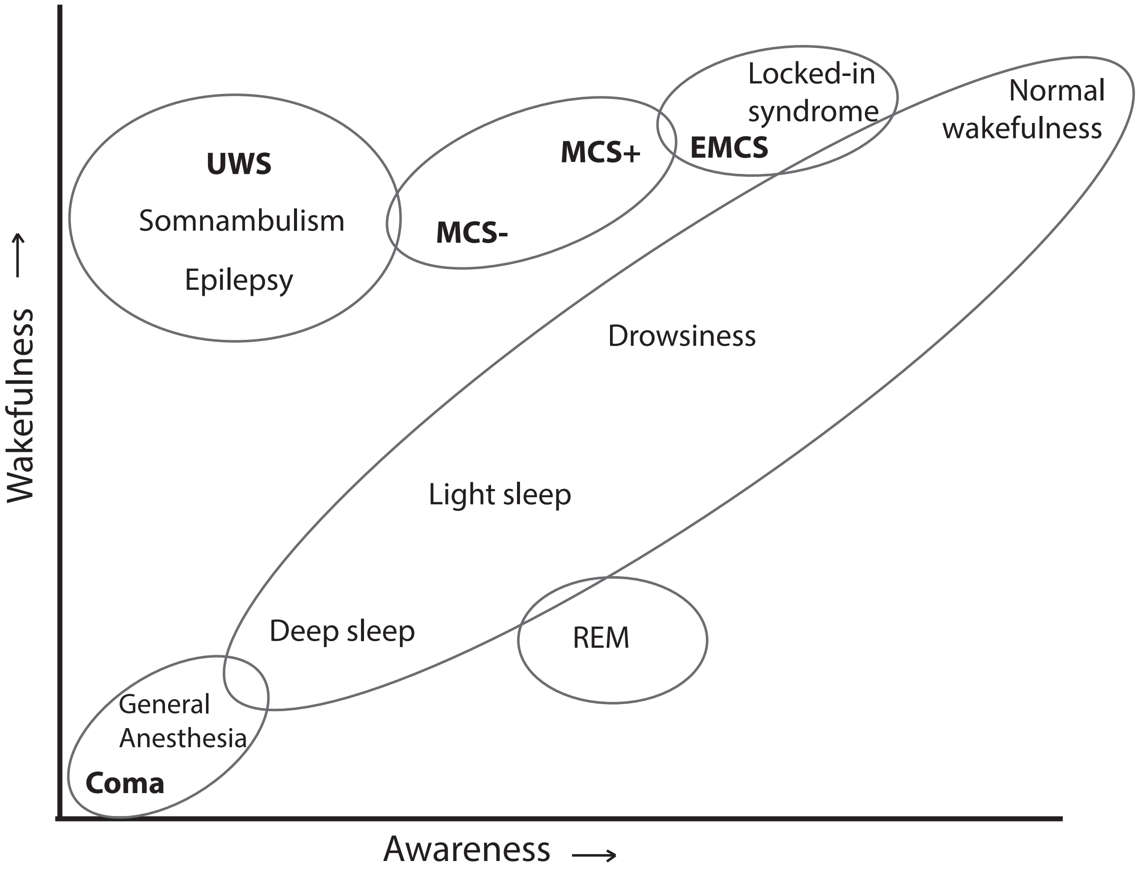

Figure 1.1 – A clinical definition of consciousness. Interaction between arousal and awareness in different states of (un)consciousness. REM: rapid eye movement, EMCS: emergence of the minimally conscious state, MCS+: minimally conscious state plus, MCS-: minimally conscious state minus, UWS: unresponsive wakefulness syndrome, LIS: locked-in syndrome. Adapted from [2], appendix A.

evolve into other disorders of consciousness (DOC) such as the unresponsive wakefulness syndrome (UWS) and minimally conscious states (MCS). Worth mentioning is brain death, another result of severe brain injury, defined by a permanent loss of all brain functions. Which means that the patient is persistently comatose without confounding factors (e.g., hypothermia, drugs), all brainstem reflexes are lost, there is no respiration, and neuroimaging shows an empty skull sign, or the absence of activity in the entirety of the brain [3] (table 1.1)

Unresponsive wakefulness syndrome

The unresponsive wakefulness syndrome (UWS) is the revised name for patients in a vegetative state (VS) [4]. These patients recover arousal, meaning that they show spontaneous or induced eye opening. Awareness however, is absent, and exhibited behaviors are unintentional or reflexive [5]. The patient is able to perform a variety of movements, such as grinding teeth, blinking and moving eyes, swallowing, yawning, crying, and smiling, but these are always reflexive/unintentional movements and unrelated to the context [6]. Adapted emotional responses cannot be elicited. Autonomic functions are generally preserved, and breathing occurs usually without assistance. This state can be persistent but also transient towards both decreases and increases in health and (conscious) state. They can improve to the minimally conscious state or further, or remain in the UWS. The UWS has been said to be permanent 12 months after traumatic brain injury, and 3 months after non-traumatic etiologies [6]. These patients may have in that case less than 5% chance of recovery, after which the difficult ethical and legal issues around withdrawal of hydration and nutrition may be discussed [6]. However small but possible [7], the chance of late recoveries highlights the need for a name avoid of vegetable-like connotations with a more neutral description of the behavioral profile [4]. Thus, for the remaining of this thesis the term Unresponsive wakefulness syndrome (UWS) will be used to indicate these patients (table 1.1).

Minimally conscious state

Conscious recovery consists of regaining fluctuating but reproducible non reflexive-oriented and/or voluntary behaviors. This state is called the minimally conscious state (MCS) [8]. For example, command following, visual pursuit as a direct response to moving or salient stimuli, localization of noxious stimulation, as well as contingent responses to emotional stimuli, are considered signs of consciousness. Furthermore, patients in MCS are more likely to experience pain and/or suffering [9]. The heterogeneity of this group of patients has led to the proposal of a stratification into MCS+ (plus) and MCS- (minus) based on the complexity of behavioral responses [10]. Patients in an MCS- show non reflexive-oriented responses such as visual pursuit or localization to noxious stimuli, while MCS+ refers to patients showing non reflexive voluntary responses such as command following, intelligible verbalization, and/or nonfunctional communication [11] (table 1.1).

When patients show reliable demonstration of functional communication (i.e., accurate yes-no responses to situational orientation questions) or functional object use (i.e., demonstration of the use of two different objects) on consecutive assessments, the patient is considered to have emerged from the MCS (EMCS) [12]. After emerging from MCS, these patients are no longer considered to suffer from a disorder of consciousness. However, they often remain confused, disoriented, sometimes agitated, and they might continue to need full-time care (table 1.1).

Locked-in syndrome

Although not a disorder of consciousness, the locked-in syndrome (LIS) is worth mentioning, as it can easily be misdiagnosed as a DOC. Classically, patients in LIS have fully intact cognitive abilities, while voluntary motor control is lost, with the exception of small eye movements. Ventral brainstem lesions damaging the corticospinal tract are the most common cause of a LIS. The primary mode of communication is via eye movements or blinking [13] (table 1.1).

1.3 Clinical assessment of consciousness

Clinically, behavioral assessment is based on the two clinical components of consciousness: arousal/wakefulness and awareness. Wakefulness is assessed by spontaneous or stimulus induced eye opening. Awareness can be devided into awareness of the external world and awareness of the internal world. The former is mainly assessed in consciousness through contingent behaviors (i.e., action or -emotional- reaction in response) towards specific environmental stimuli. Self-awareness is difficult to evaluate when only based on bedside observations (contrary to self-reports).

Correct diagnosis is highly important in DOC for prognostic, therapeutic and ethical reasons. The prognosis of patients in MCS is better than those in UWS [15]; in one study, twelve months after brain injury about half of the patients tracked in MCS had improved, compared to a very small percentage of patients in UWS [16]. In terms of therapeutic choices, the medical team may choose to apply pharmacological (e.g., with amantadine, zolpidem or palliative medication) and/or non-pharmacological interventions (e.g., deep brain stimulation, transcranial direct current stimulation) [17, 18], or make ethical decisions [19]. However, differentiating MCS from UWS can be challenging since voluntary and reflexive behaviors can be difficult to distinguish and subtle signs of consciousness may be missed. The behavioral assessment

Table 1.1 – Diagnostic criteria for patients with severe brain injuries

Clinical entities	DOC	Definition	Reference
Brain death	No	Irreversible coma Evidence for the cause of coma Irreversible loss of all functions of the brain, including brainstem reflexes Apnea Absence of confounding factors (e.g., drugs, hypothermia, electrolyte, and endocrine disturbances)	[14]
Coma	Yes	No wakefulness No awareness of self or environment Acute state (i.e., resolves in hours to maximum 4 weeks)	[1]
Unresponsive wakfulness syndrome	Yes	Wakefulness No awareness of self or environment No sustained, reproducible, purposeful, or voluntary behavioral responses to visual, auditory, tactile, or noxious stimuli No language comprehension or expression Relatively preserved hypothalamic and brainstem autonomic functions (e.g., respiration, digestion, thermoregulation) Bowel and bladder incontinence Variably preserved cranial-nerve and spinal reflexes Acute and/or chronic state	[4, 6]
Minimally conscious state (MCS)	Yes	Wakefulness **MINUS** Visual pursuit Contingent behavior Reaching for objects Orientation to noxious stimulation **PLUS** Following simple commands Intentional communication Intelligible verbalization	[10, 12]
Emergence from MCS	No	Functional communication Functional object use	[12]
Locked-in syndrome	No	Wakefulness Awareness Aphonia or hypophonia Quadriplegia or quadriparesis Presence of communication via the eyes Preserved cognitive abilities	[13]

of consciousness should be done through repeated examinations revealing reproducible, oriented or voluntary behavioral responses to various stimuli (the most common being auditory, verbal and motor stimuli) [20]. Further variance in diagnostic accuracy may result from biases induced through the environment, the patient and/or the examiner.

Concerning the environment, paralytic and sedative medications, movement restrictions through restraints and immobilization techniques, poor positioning and excessive ambient noise / heat / light can decrease or distort voluntary behavioral responses. Concerning the patient, fluctuations in arousal level, fatigue, subclinical seizure activity, underlying illness, pain, cortical sensory deficits (e.g., cortical blindness/deafness), motor impairment (e.g., generalized hypotonus, spasticity or paralysis) or cognitive deficits (e.g., aphasia, apraxia, agnosia) constitute a bias to the behavioral assessment and therefore decrease the probability to observe signs of consciousness. Moreover, while present in the official criteria for MCS [8], potentially meaningful affective behaviors (e.g., emotional behaviors such as crying during a specific song) are very difficult to assess objectively and are therefore not usually present in clinical routine. Lastly, examiner errors may arise when the range of behaviors sampled is too narrow, response-time windows are over or under-inclusive, criteria for judging purposeful responses are poorly-defined, or examinations are conducted too infrequently to capture the full range of behavioral fluctuation (Appendix G, [21, 22]).

The development of diagnostic criteria for MCS [8] (Giacino et al., 2002a) as mentioned before can help reduce the incidence of misdiagnosis [23, 24]. However, recent studies have found that around 40% of patients believed to be in UWS remain misdiagnosed [21, 25]. The use of standardized rating scales offers some protection from these errors, and behavioral assessment remains at present the gold standard for the assessment of consciousness.

The coma Recovery Scale Revised

The Coma Recovery Scale-Revised (CRS-R) [26] (table 1.2) is currently the most reliable and sensitive tool for the differential diagnosis of DOC [27]. It was developed to differentiate UWS from MCS and uses 23 hierarchically organized items in visual, motor, auditory, oromotor, communication and arousal subscales. The first five subscales give weighted scores to reflect presence of cognitively mediated responses (highest scores) towards low scores when no measurable responses, reflexive/non cooperative activity or brainstem reflexes are observed. The arousal subscale indicates the level of arousal ranging from attention through eye-opening to none. The use of self-referential stimuli such as ones own name and ones own face (using a mirror) should be used during CRS-R assessments to increase the patients responsiveness [28–30]. The CRS-R has excellent content validity and is the only scale currently available which includes all of the Aspen Workgroup criteria for good standardized administration and scoring [8]. It showed good inter-rater reliability, test-retest reliability, and internal consistency. Furthermore, confounding factors such as deafness, aphasia or blindness might be indicated when improbable scoring occurs, and thus could increase the accuracy of CRS-R scoring [31]. Resent research focused on the lack of evidence-based recommendations for repetition of assessments. Data of many years of standardized repeated assessment of patients at the Coma Science Group has shown that when using only one CRS-R assessment, a 36% chance of false negatives occurs. Rather, diagnostic assessment should be performed at least 5 times for accurate diagnosis [20].

Although the CRS-R is currently the gold standard for the behavioral examination of patients with disorders of consciousness, the environmental biases mentioned earlier can still play a role. To avoid misinterpretation, three out of four repetitions of each task are generally required for a positive result using the CRS-R, but the risk of false positives cannot be ruled out. Therefore, absence of adequate response to command does not necessarily prove a patient is unconscious. Finally, due to lack of a diagnostic ground truth, criterion validity and diagnostic value (i.e., the scales ability to establish an accurate diagnosis compared with the true diagnosis as measured by a reference standard) cannot be determined for any available scoring system [27].

Many other scales for the examination of consciousness exist, some of which will be briefly mentioned in chapter 5.

1.4 Ancillary testing of consciousness

Neuroimaging is in general viewed as an objective, unbiased tool for the assessment of consciousness in these patients, to be used as an aid next to clinical assessment [32]. The ultimate aim of neuroimaging in this context is probably to use these patients in the form of a lesion approach for the exploration of the neural correlates of consciousness (NCC). The NCC refers to the minimal neuronal mechanisms jointly sufficient for any one specific conscious experience [33]. This can be content specific, or a full NCC defined as the neural substrates supporting conscious experiences in their entirety, irrespective of their content [33]. The first step on the road to an understanding of the full NCC is to understand the level of residual consciousness on a patient-specific basis.

These neuroimaging studies use three paradigms: active, passive, and resting paradigms (figure 1.2). Several distinct methods are exploited for these paradigms. Positron emission tomography (PET), magnetic resonance imaging (MRI), and electroencephalography (EEG)

Table 1.2 – Coma Recovery Scale - Revised

	Auditory function
4	Consistent movement to command#
3	Reproducible movement to command#
2	Localization to sound
1	Auditory startle
0	None
	Visual function
5	Object recognition*
3	Pursuit eye movements*
2	Fixation^
1	Visual startle
0	None
	Motor function
6	Functional object use+
5	Automatic motor response*
4	Object manipulation*
3	Localization to noxious stimulation*
2	Flexion withdrawal
1	Abnormal posturing
0	None/flaccid
	Oromotor/verbal function
3	Intelligible verbalization#
2	Vocalization/oral movement
1	Oral reflexive movement
0	None
	Communication
2	Functional: accurate+
1	Nonfunctional: intentional#
0	None
	Arousal
3	Attention
2	Eye-opening w/o stimulation
1	Eye-opening with stimulation
0	Unarousable

* denotes MCS-; # denotes MCS+; + denotes emergence from MCS; ^denotes an MCS except for anoxic etiology.

are most often used to explore awareness and covert cognitive processes in healthy subjects and brain damaged patients [34, 35].

Active, passive, and resting paradigms

Active paradigms use wilfully modulated brain signals, for example by using mental imagery tasks, to detect command following similar to command response tests done at the bedside. Command following in patients with DOC is of major clinical importance because, according to standardized behavioural assessment, this behaviour differentiates patients in MCS from patients in UWS [10, 26]. The same rationale can be used when applying brain imaging. However, a criticism of using mental imagery tasks to unfold cognition and/or to communicate relies on patients limited short-term memory resources and restricted attention span. As a result, relatively long scanning intervals might be necessary to increase the signal-to-noise ratio, which in turn contributes to patients fatigue, and ultimately to lack of their vigilance [36]. Additionally, similar problems as those biasing bedside evaluations exist (e.g., sensory impairments, small or easily exhausted motor activity, pain, sedative medication, sleep disturbances and/or medical complications). In these cases, absence of responsiveness does not necessarily correspond to absence of awareness [37] (figure 1.2, top).

Passive paradigms measure brain responses to external sensory stimulation (e.g., auditory, somatosensory and visual) while the subject is not performing any mental task (figure 1.2, middle). The limitations of using this approach stem from patients pathologies and technical requirements. Indeed, patients have varying clinical presentation, such as visual problems, motor spasticity, somatosensory hypersensitivity and cortical auditory deafness, which can inhibit their detection of external stimuli. Furthermore, the technical setup of these examinations is not as simple as resting paradigms.

Alternatively, increasing attention is being paid to resting state paradigms [38]. This paradigm does not rely on tasks, or stimulation of some sort, instead spontaneous brain function is assessed when the subject receives no external stimulation. Usually subjects are asked to lay still, relax their mind (e.g., not to think of anything in particular), and close their eyes while not falling asleep or focus vision on a cross. Importantly for clinical studies, the resting state paradigm is particularly appealing because it does not require sophisticated experimental setup to administer external stimuli and surpasses the need for patients contribution (e.g., language comprehension and/or production of motor responses [38]). Hence, resting protocols are a suitable means to study clinical populations in which communication cannot be established at the bedside, such as patients with DOC. This means that this approach might thus bypass the limitations which are raised by the other experimental neuroimaging methods, as well as those affecting bedside behavioural assessment (figure 1.2, bottom).

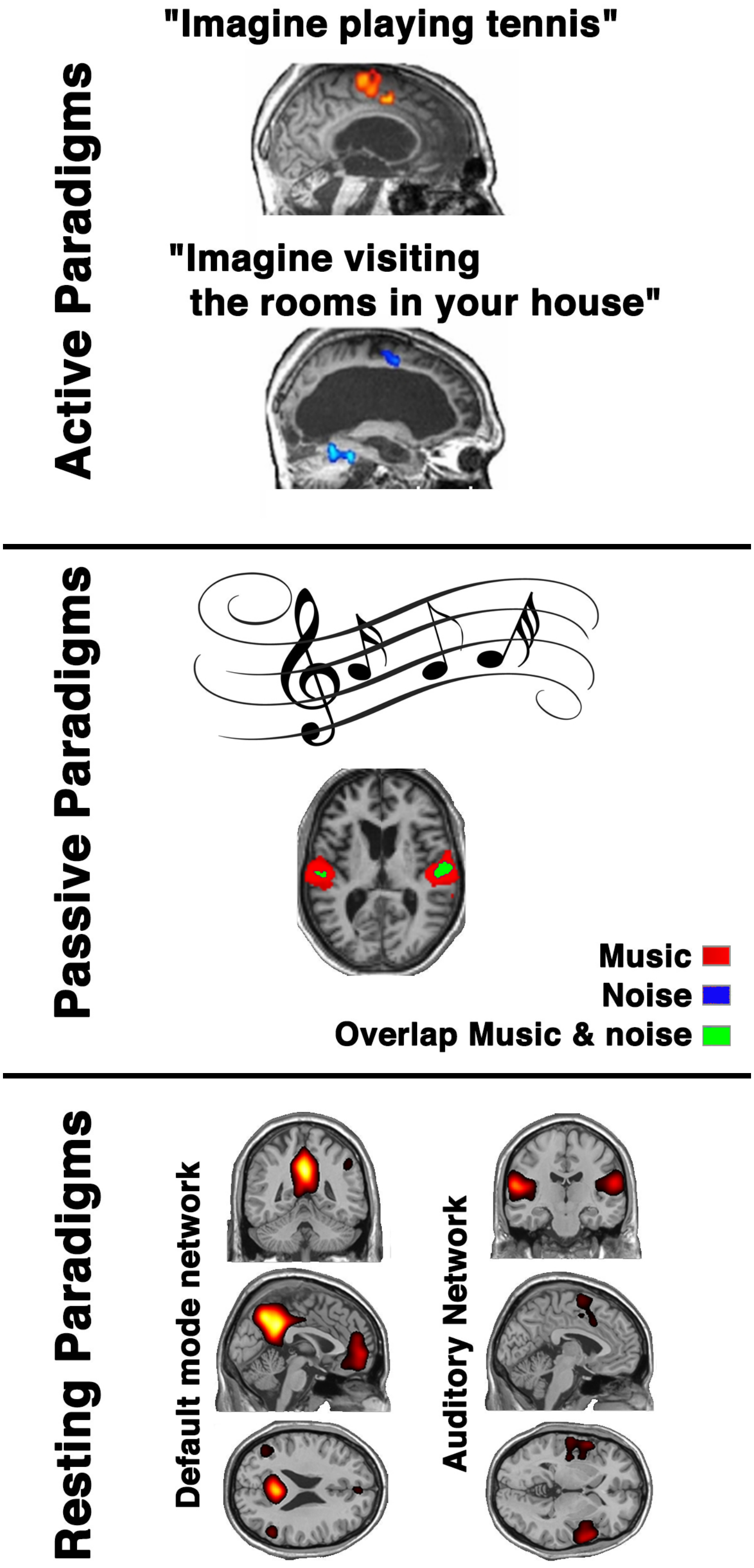

Figure 1.2 – Neuroimaging paradigms for the assessment of residual cognitive processes in DOC. Graphical representation of a possible neuro-imaging paradigms. Examples indicated here used MRI. Active paradigm where subjects are asked to perform a task [39]; passive paradigm where subjects are exposed to certain stimuli, for example music ([40], Appendix H); resting paradigms assess spontaneous activity without external stimulation ([41], Appendix E).

Positron emission tomography

PET gives an approximation of functional tissue integrity by measuring cerebral glucose consumption. 18-Fludeoxyglucose (FDG) is an analogue of glucose, and the resulting tracer concentrations imaged indicate the regional glucose uptake and hence indicate neural activity during resting paradigms. Using this method, clear differences can be seen between DOC and conscious subjects, and automatic classifiers are well equipped to differentiate between UWS and LIS [42].

In UWS patients FDG-PET has reliably shown a global massive decrease in metabolism of up to 40% of normal value [43–45]. However, the loss of consciousness is not related to a global dysfunction in cerebral metabolism, but rather to regional decreases (figure 1.3). Indeed, patients suffering from DOC show decreased metabolism in a widespread frontoparietal network, encompassing lateral prefrontal and posterior parietal areas as well as midline anterior cingulate/mesiofrontal and posterior cingulate/precuneal associative cortices [46]. This frontoparietal network can be functionally subdivided into two different networks: the extrinsic awareness network, and intrinsic awareness network.

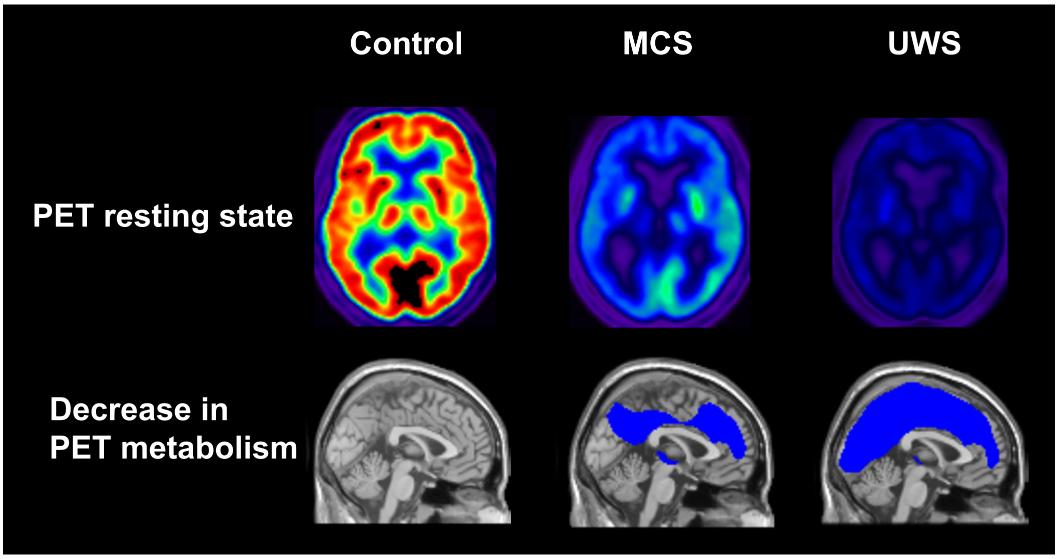

Figure 1.3 – PET metabolism in patients with DOC. Group-level studies utilizing PET in healthy and DOC (UWS and MCS patients). Higher images show cerebral metabolism as measured with PET in the three groups. Images are shown using the same color scale. Lower images show areas with significant metabolic impairments in UWS and MCS compared to healthy controls. Impairments is clear in the frontoparietal network. Statistical maps are thresholded at a family-wise error correction rate for multiple comparisons ($p<0.001$). Adapted from Appendix A.

The lateral areas of this network are considered to be implicated in external awareness (extrinsic awareness network, or executive control network), or awareness of the environment / sensory awareness. The intrinsic awareness network, more widely known as the default mode network (DMN; see page 21), is linked to the midline regions. These include mainly the medial prefrontal cortex, the precuneus and the bilateral posterior parietal cortex, and it is related to awareness of self and self-related processes, such as mind-wandering and autobiographical thinking [47]. In accordance with this, patients in MCS show higher glucose metabolism in the precuneus than patients in UWS [48]. The difference between the two states is most pronounced in the frontoparietal cortex [49], but thalamocortical metabolism is also impaired [50]. Interestingly, PET is especially sensitive in making a distinction between MCS and UWS as well as in prediction of long-term recovery in patients with an UWS [32].

Furthermore, PET studies using passive auditory and noxious stimulations have demonstrated a disconnection between primary sensory areas and large-scale associative fronto-parietal cortices in UWS patients. MCS patients do show activation patterns similar to healthy control subjects after noxious stimuli, possibly suggesting a potential for pain perception [9]. The proposal to subcategorize the MCS into MCS- and MCS+ was confirmed by resting PET analysis, where differences in language and sensorimotor areas are observed between patients in MCS- and MCS+ [11] .

Magnetic resonance imaging

Anatomical MRI helps to assess the extent of structural damage. Diffusion tensor imaging (DTI) is a measure of the directionality of water molecules that and can be used to map white matter tracts. Using this method it was shown that traumatic brain injury seems to be specifically affecting the corpus callosum [51]. Other studies on severe brain injury confirm this, and show decreases in fractional anisotropy (FA; a measure of directionality of water diffusion assumed to be related to myelination of white matter) in many large fibre tracts (including the corona radiata, corticospinal tracts, cingulum, external capsule, and corpus callosum), as well as negative correlations with cognitive and clinical outcomes [52, 53, 53–55]. Furthermore, white matter integrity dysfunction especially affects the tracts connecting the regions of the default mode network [56], in cortico-cortical and subcortico-cortical figures which again are related to cognitive function [57]. This shows the behavioral differences seen between these two groups of patients are also represented by differences in severity of brain-damage (figure 1.4, top)

Functional MRI (fMRI) can visualize brain function derived from blood-oxygen-level dependent (BOLD) changes, which is based on changes in the ratio of oxy- to deoxy-hemoglobin of the blood. fMRI has been largely used in patients with DOC in order to detect brain activity related to residual cognition, awareness and command following. Using fMRI, two mental imagery tasks have been shown to encompass reproducible cortical activations across healthy controls, namely thinking about playing tennis (encompassing primarily supplementary motor area) and imagining visiting the rooms of ones house (encompassing primarily parahippocampal cortex) [39] (figure 1.4 middle, and figure 1.2 top). Some UWS patients can show brain activity indistinguishable from healthy controls [58]. Since these patients were able to comprehend and execute the mental imagery commands in a sustainable manner, the behavioral diagnosis was challenged and the patient was no longer considered as in UWS [59].

Based on the command-following rationale, other mental tasks for evidencing response to command in patients with DOC have been employed. For example, with a silent picture naming task [60], hand moving tasks [61], and selective auditory attention tasks [62]. More sophisticated designs using mental imagery, and duration of the mental effort can be used in real-time to answer multiple choice questions [63], or spell words for real-time communication [64].

As active paradigms are subject to many of the same biases that affect behavioral examinations, efforts have also been put in the assessment of resting-state fMRI. This technique is used to investigate the spontaneous temporal coherence in BOLD fluctuations related to the amount of synchronized neural activity (i.e., functional connectivity) existing between distinct brain locations, even in the absence of input or output tasks [65]. During rest, the brain is organized in distinct functional networks [66]. In healthy subjects, these resting state networks, such as the default mode, visual, auditory, salience, sensorimotor and executive control networks can reliably be detected [67]. In patients, these functional connectivity patterns are disturbed [41, 68] (figure 3.1). Chapter 2 will go deeper into functional connectivity, resting state, and resting state networks. However, in short, functional connectivity has been shown to decrease as a function of the level of consciousness (Appendix E, [41]). Meta-analysis showed that this decrease is especially notable in the DMN, and most pronounced in UWS [69].

Electroencephalography

Resting state measures of electrical brain activity can also aid diagnosis with the advantage of being performed at the bedside [70]. For instance, recent studies have demonstrated an absence of electrophysiological characteristics of sleep [71, 72] in UWS. Studies using quantitative and connectivity EEG measures have demonstrated the ability of this technique to differentiate patients in MCS from those in UWS at the group level. EEG alpha activity is decreased in all DOC patients, whereas delta power is increased only in UWS [73]. Furthermore, different patterns decreases in information integration can be seen in the different DOC

[74]. Several studies using passive paradigms have assessed event-related potentials (ERP) in response to stimulations. The presence of an ERP response to stimuli and to odd stimuli within a sequence (mismatch negativity; MMN) serves as predictors of outcome. The P3 ERP response to unexpected stimuli also aids prognosis, and can be used as a response to a command paradigm by showing higher ERP when used in an active condition as compared to a passive situation [75]. As in fMRI active paradigms, some patients who are behaviorally diagnosed as UWS have been shown to be able to perform active mental imagery tasks [76].

EEG in combination with transcranial magnetic stimulation (TMS) is used to stimulate a brain region and assess cortical excitability (i.e., the amplitude of the initial response to TMS) and effective connectivity (i.e., causal interaction between the stimulated area and the subsequent activated cortical regions). This technique has been shown to successfully differentiate patients with UWS from MCS. Indeed, MCS patients demonstrate complex long-lasting widespread activation patterns, whereas patients in UWS show simple and local slow wave responses that indicate a breakdown of effective connectivity [77] (figure 1.4, bottom).

All these neuroimaging methods can aid diagnostic assessment, however they are not able to be used instead of bedside evaluation. None of the studies so far are yet able to accurately diagnose the single subject, and studied groups are not big enough to be applied on a clinical level.

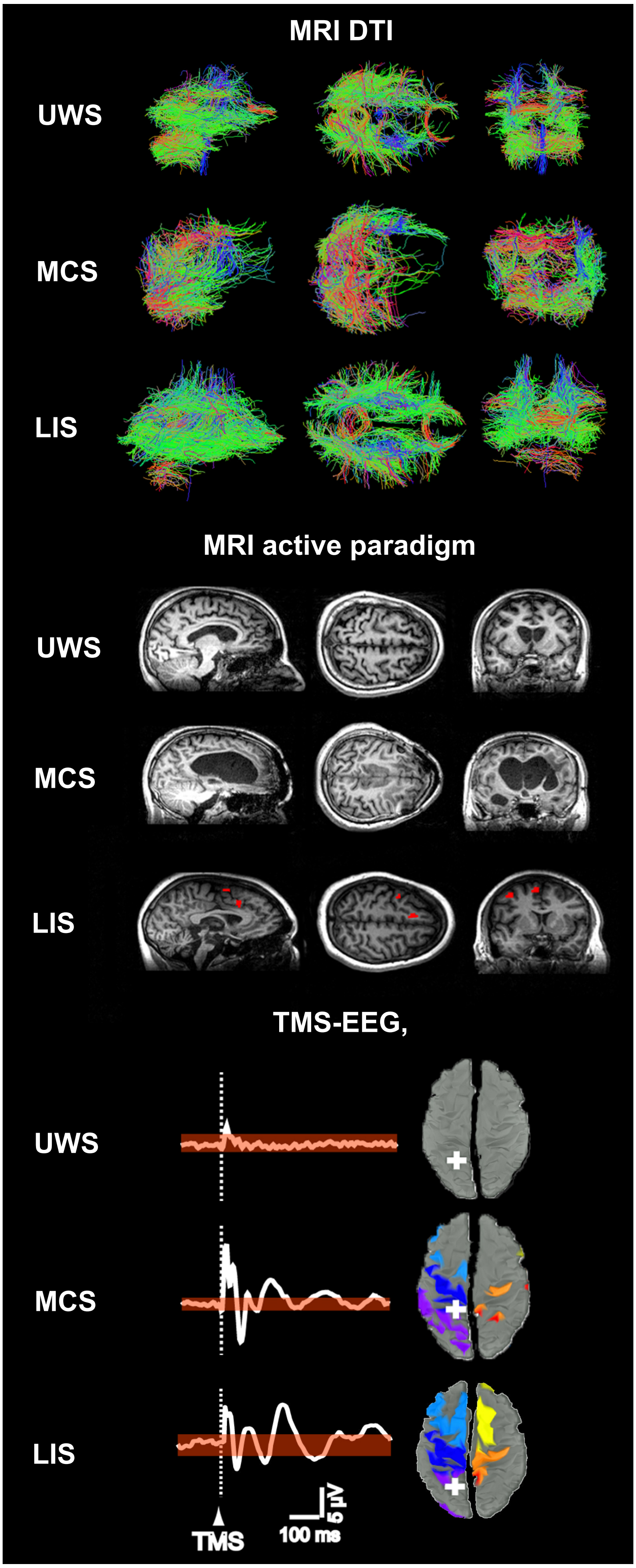

Figure 1.4 – Multimodal neuroimaging in three patients. Neuroimaging methods at the single-patient level: one patient is behaviorally diagnosed UWS, one as MCS, and one as LIS. top: MRI DTI indicates the amount of damage in structural connectivity (i.e., white matter tracts, color-coded by axis). middle: fMRI Active paradigms allow for motor-independent response to command (tennis imagery). bottom: TMS combined with EEG assesses cortical excitability and the effective connectivity. EEG evoked response under the stimulation area (left image) and the subsequent widespread of the activation (right, color-coded by brain area). When the result of multimodal neuroimaging assessment converges, greater confidence can be achieved in the assessment of the level of consciousness. Adapted from Appendix A.

1.5 Objectives of the thesis

The aims of this thesis are two-fold, concerning both brain connectivity and sensory stimulation.

Part I uses resting paradigms to assess brain connectivity. Using a lesion approach we hope to gain a better understanding of consciousness and its necessary neural underpinnings. Chapter 2 assesses brain function in the DMN using PET and structural MRI. Aim of this study is to explore the function-structure relationship, and how this is affected in severely brain injured patients. We then focus on one single method namely fMRI functional connectivity with the aim to review differences between brain function in conscious and unconscious states. Exploring the NCC cannot be done through assessment of brain damaged patients alone, so chapter 3 will review functional connectivity networks in physiological (sleep, hypnosis), pharmacological (sedation, anesthesia) and pathological (coma-related states) alteration of consciousness. Clinical care, prognosis, and ethical decisions are all dependent on diagnosis of DOC, making correct diagnostic assessments critical. Chapter 4 tries to answer the question if the observed differences in functional connectivity between UWS and MCS can be classified.

As mentioned, one of the challenges for clinical evaluation is the fluctuating pattern of responsiveness in the MCS. With the aim to further optimize diagnostic assessment we use sensory stimulation in the context of passive paradigms in part II. First aim here was to determine if sensory stimuli without personal relevance, as used in several behavioral scales, could provoke conscious behavioral responses (chapter 5). We hypothesized that contrary to stimuli without personal relevance, preferred stimuli might improve behavioral responsiveness. Chapter 6 assessed functional connectivity during patients' favorite music and classical resting state scans. To study if the indicated increased functional connectivity seen in the latter study during preferred music is due to the preferred characteristics or an effect of music, we continued in chapter 7 with a behavioral study using smell and audition.

Part III, and chapter 8 uses resting state fMRI to assess functional connectivity in congenitally blind subjects. The understanding of the brains' adaptation to sensory loss might someday aid diagnosis of these losses in non-communicating patients.

Part I

The resting paradigm: Brain function and structure in relation to consciousness

Introduction

This part will focus on the resting state, as mentioned this paradigm does not rely on tasks or external stimulations and thus is suitable in our severely brain injured population. Three chapters will utilize several different methodologies, namely structural MRI, functional MRI and PET. Chapter 2 will look at the changes in function and structure using PET and MRI respectively. We directly investigate, for the first time in severely brain injured patients, the relationship between functional brain activity and structural connectivity within the DMN in an objective and combined fashion. We will then return to single method analysis to explore the use of resting state functional connectivity analysis as measured using functional MRI. Chapter 3 will review changes in functional connectivity using fMRI under pathological, physiological and pharmaceutical unconsciousness. This is followed in chapter 4 by a study aiming at differentiating between MCS and UWS using resting state functional connectivity in these networks.

Default mode network

Using functional resting methods, the default mode network (DMN) has been studied extensively. This network of distinct, remote, and cooperating brain areas encompass precuneus/posterior cingulate cortex, mesiofrontal/anterior cingulate, and temporoparietal junction. It was initially identified in PET studies as regions less active when performance on cognitive tasks was compared to resting control condition [78–80] (see figure 1.2 bottom left; figure 1.5; red activations)

After this the DMN was also identified in functional MRI [81], and can now also be replicated in neurophysiological measures of synchronized phase-amplitude coupling of activity, such as with magnetoencephalography (MEG) [82].

While the DMN is usually defined in terms of functional connectivity, there are indications of clear structural underpinnings [83, 84]. The structural core of the network is centered in the posterior elements of the default mode network, and important for functional integration of the whole brain [85, 86].

FMRI resting state connectivity studies stress that the brain in a resting state is characterized by coherent fluctuations in the blood-oxygen-level-dependent (BOLD) signal. These BOLD fluctuations can be detected in the low frequency range (<0.1Hz) [87] and are distinct from respiratory and cardiovascular signal contribution [88]. This indicates that resting state functional MRI analysis is not only noise and unspecified neural activity, but correlated fluctuations in absence of tasks organize the brain in large-scale cerebral networks [65, 66].

The DMN can be explained in terms of cognitive function, its activity has been linked to self-related and internal processes, such as stimulus-independent thoughts [89], mind-wandering [90], social cognition [91], introspection [92], monitoring of the mental self [93], and integration of cognitive processes [81]. Anatomically, the ventral medial prefrontal cortex can be seen as a sensory-visceromotor link concerned with social behavior, mood, and motivation, while the dorsal medial prefrontal cortex is associated to self-referential judgment, and the posterior elements of the default mode network with the recollection of prior experiences [94].

Concerning patients, the DMN shows functional [47, 95, 96], metabolic [48], and structural [56, 97, 98] impairments [99]. Furthermore, disconnections with thalamocortical and cortico-cortical regions in the DMN in DOC correlate with clinical severity [47, 56, 57, 99]. Additionally, preservation of functional connectivity between frontal and parietal DMN regions is indicated to be a marker of recovery from coma after 3 months [100].

Anticorrelations

Since the early studies of resting state, it was suggested that the brains baseline activity can be organized in two brain networks showing anticorrelated activity to each other: an intrinsic and an extrinsic network [47, 101, 102]. The intrinsic network coincides with the DMN and is involved in the same cognitive processes as the DMN. The extrinsic system encompasses lateral frontoparietal areas resembling the brain activations during goal-directed behavior, and it has been linked to cognitive processes of external sensory input, such as somatosensory [103], visual [104] and auditory [105] stimuli. Previous studies showed that these two systems are of a competing character in the sense that they can disturb or even interrupt each other [106]. Such an anticorrelated pattern is also illustrated in activation studies on motor performance [107], perceptual discrimination [108], attentional lapses [109], feelings of dissociation during hypnosis [110], and somatosensory perception of stimuli close to somatosensory threshold [103]. These competing networks were furthermore behaviorally implicated in internal and external awareness, switching around every 20 seconds [47].

However, the fMRI anticorrelations have been subject of debate. It has been argued that the anticorrelated pattern could arise from the preprocessing procedure when the brains global signal is regressed out [111]. Nonetheless, anticorrelations have been found in studies which address the criticisms [112–114], and more importantly, anticorrelations have been found using EEG [113]. These anticorrelations have also been shown to reduce or disappear in decreased states of consciousness such as anesthesia [115], sleep [116], and in UWS patients [117].

More recently, it was shown that indeed, patients with an UWS and MCS show decreased positive correlations within the default mode network, but also the negative correlations between the external network and DMN disappear. These anticorrelations do appear again in EMCS patients [118]. Together, these studies show that anticorrelations have a physiological origin, and a reduction of these anticorrelations can be seen during unconscious states.

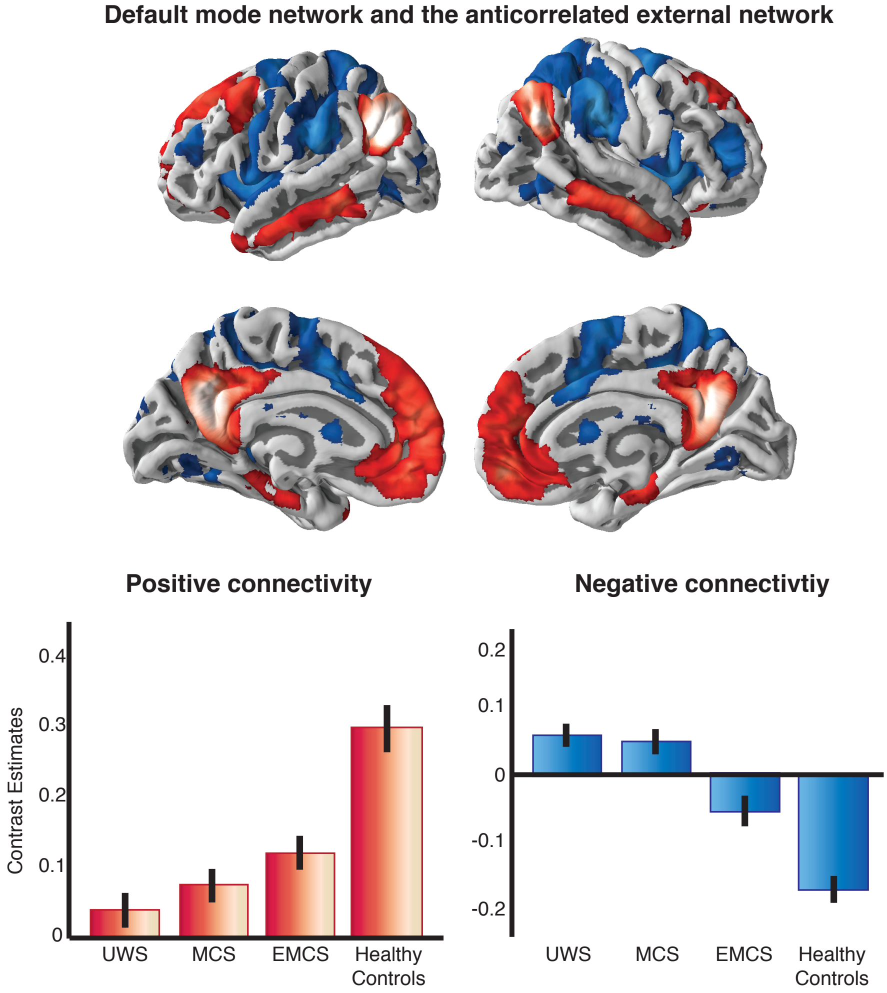

Figure 1.5 – Anticorrelated networks. Positive default mode network connectivity shown in red (i.e., within-network correlations) was decreased, albeit preserved, in UWS, MCS, and EMCS. Functional connectivity of healthy controls is significantly different from all patient groups, while no differences were identified between the groups of patients. Negative connectivity shown in blue (i.e., anticorrelations) are also diminished. Default mode network anticorrelations are only observed in EMCS and healthy controls. In UWS and MCS patients these anticorrelations become positive. Figure adapted from [118].

Chapter 2

Metabolic function, structural integrity, and function-structure connectivity

Based on:

Function-structure connectivity in patients with severe brain injury as measured by MRI-DWI and FDG-PET
Annen J*, **Heine L***, Ziegler E, Frasso G, Bahri M, Di Perri C, Stender J, Martial C, Wannez S, D'Ostilio K, Amico E, Antonopoulos G, Bernard C, Tshibanda F, Hustinx R, Laureys S.
Human Brain Mapping, June 2016

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2.1 Function and structure of the DMN

As described in the introduction, a substantial body of literature exists on grey matter metabolic (e.g., fluorodeoxyglucose PET; FDG-PET) and white matter structural (e.g., MRI-DWI; diffusion-weighted imaging) brain characteristics in DOC. Both these methods independently show severe impairments in DOC and EMCS patients (see introduction, or [35]). In short, at a global level, functional measures show that metabolism in DOC patients is decreased by up to 40-50 percent from their normal value [43, 44, 119–121], with specific dysfunction is in the DMN [46, 48]. More precisely, metabolic activity, functional connectivity, and structural integrity are reportedly more reduced in these regions than in the rest of the brain [99].

The cerebral metabolic reductions in DOC are proposed to result from widespread neuronal injury [48] or disruption of central excitatory drivers [122]. The latter mesocircuit hypothesis proposes that large-scale dysfunction is due to an important reduction of thalamic excitatory output to the cortex. The observations of impaired metabolism suggest that axonal deafferentiation may be a key driver. However, no study has directly investigated the structure (MRI-DWI)-function (PET metabolism) relationship and how it is affected in severely brain injured patients. We explored this DMN function-structure relationship in severely brain-damaged patients with varying levels of consciousness using measurements of metabolism (standardized uptake value; SUV to estimate glucose uptake) and white matter structural integrity (fractional anisotropy; FA, a measure of directionality of water diffusion assumed to be related to myelination of white matter) (figure 2.1).

We studied 25 chronic (>1month) severely brain injured patients and 25 healthy subjects using two neuroimaging modalities: diffusion-weighted MRI and 18F-FDG-PET imaging. The analysis focused on four regions per hemisphere (thalamus, frontal cortex, precuneus, inferior parietal cortex) comprising the default mode network.

Each subjects T1-weighted images were manually reoriented, and automatically labelled using the Desikan-Killiany atlas [123]. Labels were combined to produce the regions of interest. Grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) masks were produced by combining Freesurfer and FAST (part of FSL) segmentation methods. DWI were corrected for vibration artifacts, subject motion and eddy current-induced distortions, and when necessary volumes were removed when distorted by table-vibrations. Diffusion tensors were fit at each voxel using non-linear least squares fitting and fractional anisotropy (FA) was computed. Voxels with unidirectional diffusion were identified and used to estimate the diffusion-weighted signal response for a single fiber population. Next, non-negativity constrained spherical deconvolution was performed, fiber orientation distribution functions within

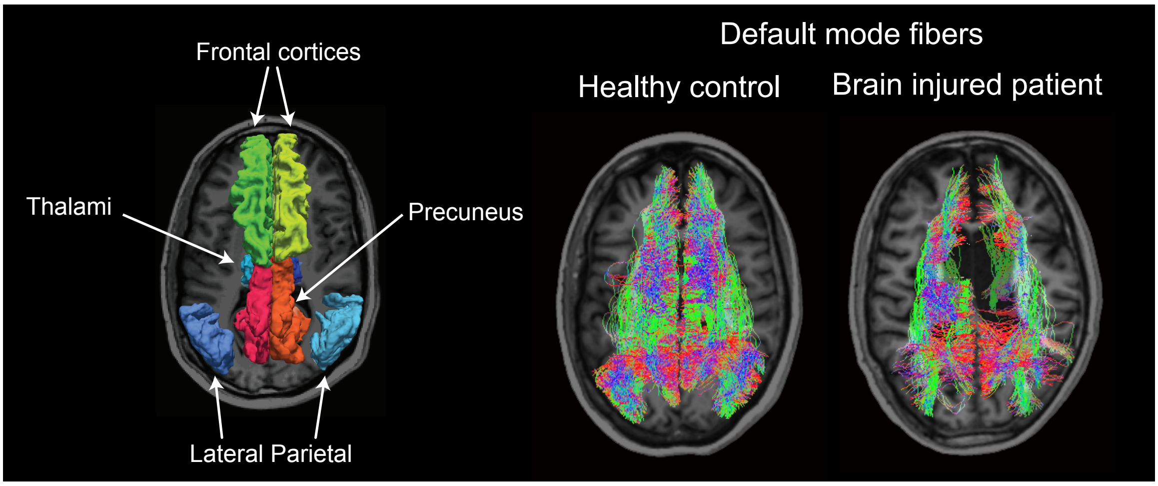

Figure 2.1 – Default mode network regions and the tracts between the regions in a healthy volunteer and brain injured patient. Left side shows ROI volumes with their respective naming. Right side of the figure shows tracts between the ROIs of the default mode network (represented on the left) for one control subject and one patient.

each voxel were estimated and probabilistic tractography was performed. A connectivity matrix for the eight-region connectome was computed using the streamline origin and termination points and the region-of-interest label mask. For each streamline the FA was averaged over all the voxels it passed through.

FDG-PET underwent partial volume correction using the Muller-Gartner-Rousset method [124]. Finally, following PVE correction the PET image was transformed into T1 space and the mean SUV value was extracted within each ROI (see figure 2.2 for the processing pipeline)

Using R (R Core Team (2014)) for statistical analysis we performed three main analyses. First we tested the difference between controls and brain injured patients in mean SUV and FA values using two-sample t-tests with Bonferroni correction. Subsequently, multivariate linear regression analysis with group and FA as regressor to model how group and structural integrity (FA) of DMN tracts relate to metabolism (SUV) in adjacent regions. Type II ANOVAs were used to assess significant main and interaction effects. We then focused on patients alone to better understand the variance within the brain injured group. Multiple linear regression models were used to investigate how demographic factors as diagnosis (DOC vs. EMCS), etiology (TBI or non-TBI), disease duration (sub chronic vs. chronic), gender, and age influence the function-structure relationship in the patient population. Type II ANOVAs were used to assess significant main and interaction effects.

Due to stringent exclusion criteria the final cohort consisted of 25 patients and 25 healthy controls (mean age of 36.3 years, 11 males for brain injured and 40.9 years and 13 males in the healthy control group). DOC patients had been clinically diagnosed as in an UWS (n = 7) or MCS (n = 12), and diagnosis was consistent during MRI and PET. Six subjects were diagnosed as EMCS (n = 6). The patient cohort consisted of sub acute (n = 10, >30 days to 3 months after onset) and chronic patients (n = 15, >3 months after onset) with a mean time since onset of 1.8 years (SD = 1.9 years). Patients suffered from traumatic brain injury (TBI, n = 12), anoxia (n = 11), both (n = 1), or infection (n = 1).

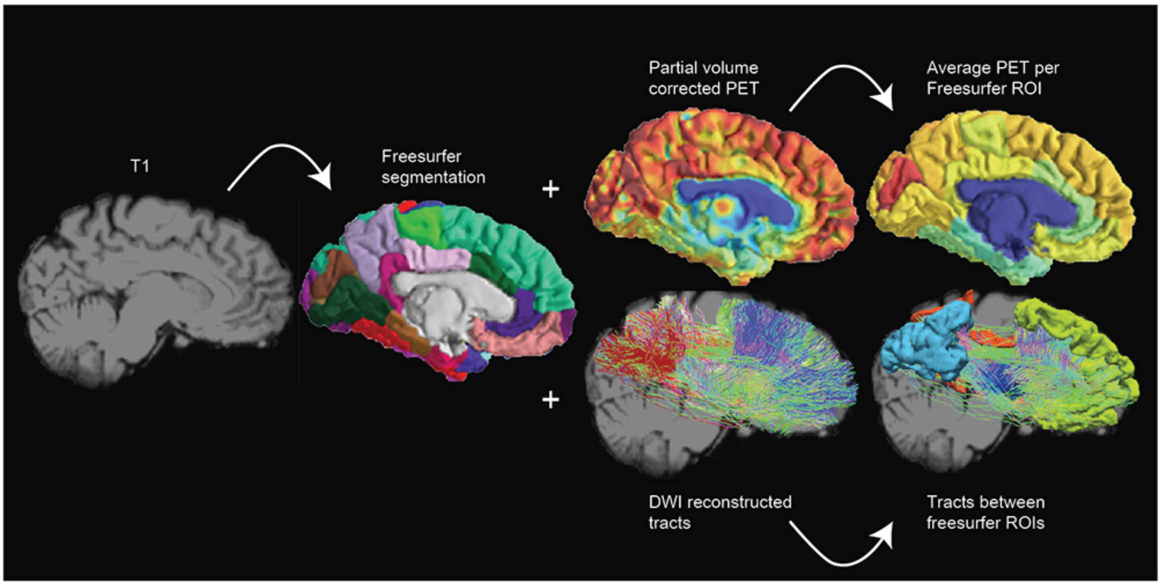

Figure 2.2 – Schematic of the processing pipeline. Data was assessed in subject space where the T1 MRI was segmented using Freesurfer. PET glucose metabolism was estimated by calculation of mean partial volume corrected standardized uptake values within the default mode network ROIs. Fractional anisotropy was extracted of the voxels that the DMN tract passed through.

2.2 Decreases in function and structure independently

We first assessed function (PET metabolism) and structure (DWI-FA) independently, to replicate previous studies focusing on either measure separately. Indeed, marked impairments in SUV and FA were observed in patients. Standardized uptake value in all DMN regions was lowered in brain injured patients compared to healthy controls, with a 39-42% reduction of metabolic rates in brain injured patients in the cortical DMN regions and thalamus (figure 2.3) This is in accordance with previous findings on a global brain scale [32, 43, 44, 120, 121] and within the DMN specifically [48, 50, 125]. Fractional anisotropy in all DMN tracts was diminished by about 13-23% in brain injured patients compared to healthy controls (figure 2.4), in line with previous reports [56, 97, 98]. Our results support recent findings of diminished structural integrity of corticocortical and subcortico-cortical DMN connections, which correlated with clinical severity in a group of 8 patients [57].

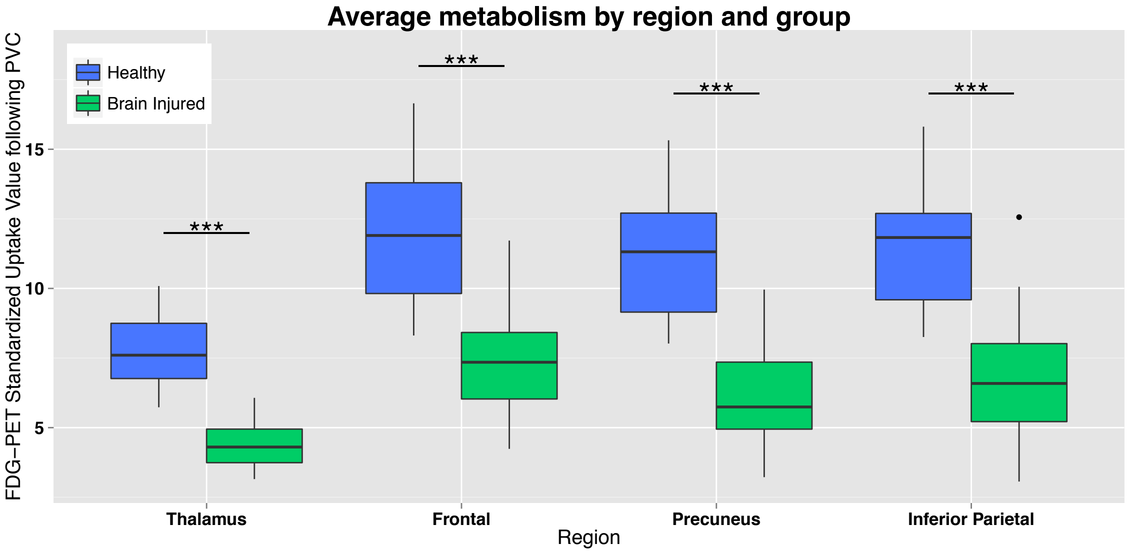

Figure 2.3 – PET functional activity in DMN. Standardized uptake value following partial volume correction (PVE) in the default mode network regions (average of standardized uptake values of left and right hemisphere) for healthy controls and brain injured patients. Brain injured patients show a decreased standardized uptake value compared to controls in all default mode network regions. *** = $p < 0.001$.

2.3 The structure-function relationship

The main aim of this study was to assess the function-structure relationship in the DMN and thalamus in healthy conscious subjects and coma survivors. First, as expected, we have replicated previous studies and shown that patients have significantly lower FA in all studied connections and SUV in all regions. Building on this, we showed that grey matter metabolic function can be partially explained by white matter anisotropy in several regions of the default mode network within the patient cohort. More specifically, metabolism of the frontal cortex, precuneus and inferior parietal cortex can be explained by fronto-inferioparietal, precuneal-inferiorparietal, and thalamo-inferioparietal as well as thalamo-frontal structural integrity (FA) (figure 2.5). These results are in line with the limited previous studies indicating there might be a link between structural integrity and glucose metabolism. For example, one study correlating metabolism with white matter bundles in the default mode network in healthy subjects

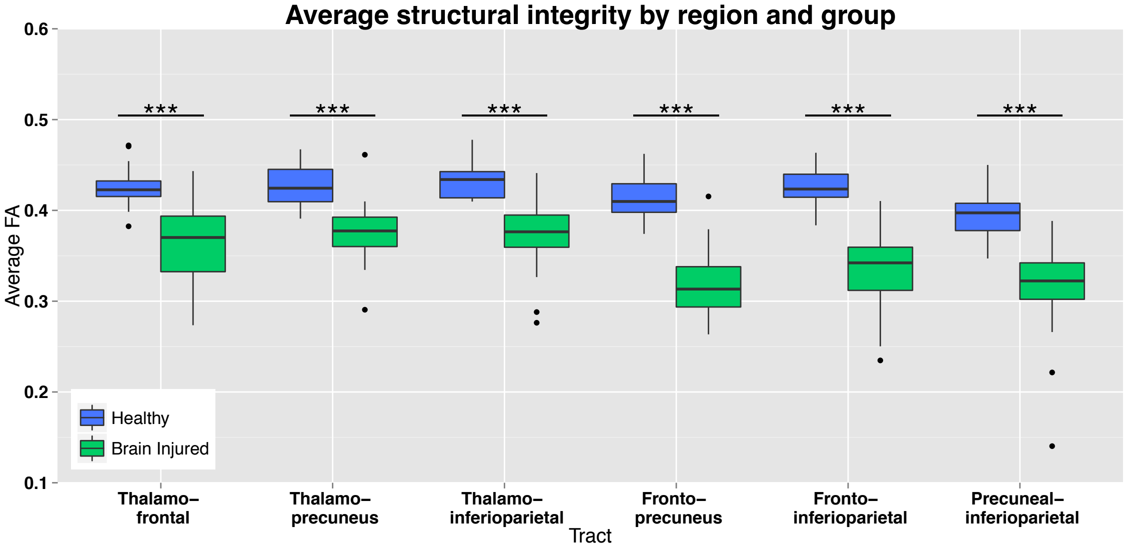

Figure 2.4 – MRI structural connectivity in DMN. Structural connectivity of tracts between the default mode network regions represented with fractional anisotropy (FA) of the voxels that the tracts pass through, in healthy controls and brain injured patients (average of left and right hemispheres). Brain injured patients show lower FA values compared to healthy controls subjects in all tracts. *** = $p < 0.001$.

found that working memory is related to a structure-function correlation in the cingulum [126]. Further studies have shown that diffusion measures have been correlated to glucose uptake in patients with Alzheimers disease and dementia [127–129], children with occipital lesions [130], normal aging [131] and epilepsy [132]. However, all of these studies use simple correlations instead of regressions measures, and thus do not take population-specific changes into account. This could result in false positive-correlations, driven by main effects of group on the (in) dependent variables. This could result in false positive-correlations, driven by main effects of group on the (in) dependent variables. We provide proof that metabolic function is indeed directly related to structural integrity, surpassing existing correlational results.

Interestingly, we did not find a structure-function relationship at the global brain level, suggesting that our results do not solely reflect general brain integrity. Instead, the function-structure relationship of the default mode network might be directly related to consciousness. This has been shown in single-modality studies, for example functional connectivity [47], white matter structural integrity [56, 97, 98], and metabolic function [48]. Here we show for the first time a direct function-structure relationship within this network.

As expected, healthy control subjects showed FA and SUV within normal range and therefore we are unable to make inferences about whether one drives the other. Next we investigated the function-structure relationship within our patient population, comparing EMCS with

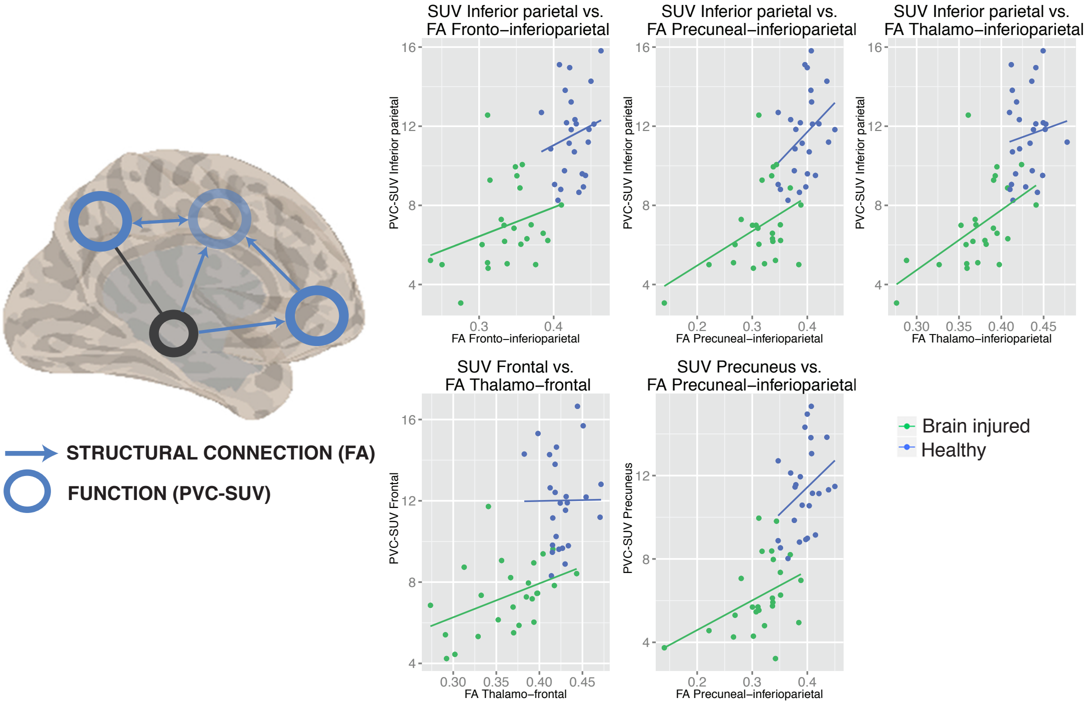

Figure 2.5 – Linear regression model of the function-structure relationship. Left side of the image shows a spatial representation of the function-structure relationships. Blue circles represent regions where standardized uptake value (SUV; as corrected for partial volume effects) depended on fractional anisotropy (FA), blue arrows for FA of tracts that drive SUV in adjacent regions. Grey versions represent non-significant analysis. Right side of image shows five scatterplots of the linear regression models for healthy controls (blue dots), and patients (green dots), and significant main effect of FA (lines). Abbreviations: FA: fractional anisotropy; SUV: Standardized uptake value.

DOC patients (MCS and UWS). EMCS patients are able to use objects and/or functionally communicate, and thus by definition conscious. Apart from one region-connection pair, all observed main effects of FA seen in the healthy vs. brain injured analysis were also observed in the analysis between the two patient populations, indicating that there is a positive linear relation between functional and structural integrity of the default mode network. Furthermore, in contrast to DOC patients, EMCS patients show a significantly stronger function-structure interaction between the function of the thalamus and the structural integrity of the thalamo-inferiorparietal tract. On the uni-modal level our results match previous research in post-comatose patients finding that structural cortico-thalamic connections are diminished [57] and thalamic metabolism is lowered (Fridman et al., 2014). These findings can be explained by the mesocircuit theory, which proposes that large-scale dysfunction is due to a global decrease of excitatory neurotransmission which in turn alters cerebral activity. More specifically, the globus pallidus is disinhibited and overactive, inhibiting the thalamic excitatory output to the frontal cortex (Schiff, 2010). By combining both functional metabolism and white matter structural information we here provide further evidence for the validity of this theory, supporting the hypothesis that thalamo-cortical connectivity plays an important role in emergence of consciousness [122]. We limited ourselves to the DMN because of the large body of literature on this brain-network relating to consciousness. Therefore, future research should extend these findings to more specific sub-cortical regions, such as the globus pallidus or specific thalamic regions.

We do not find any difference between patients based on etiology, even though several studies have shown that temporal dynamics of Wallerian degeneration vary given different etiologies [133, 134] and that traumatic brain injury, unlike anoxia, might selectively affect DMN white matter integrity [135, 136]. Multi-centric collaborations should provide sufficiently large data-sets to study these effects in the future.

We here assessed the function-structure relationship within healthy, conscious subjects and severely brain damaged patients with varying levels of consciousness through direct combined investigation of function (FDG-PET), and structure (MRI-DWI). Levels of structural integrity (FA) and metabolic function (SUV) are significantly diminished in patients compared to controls. Furthermore, a significant positive function-structure relationship can be observed within most regions of the default mode network. This relationship may be network-specific, as it does not appear at the whole-brain level. Finally, we show that EMCS compared to DOC show a significantly stronger thalamo-cortical function-structure relationship, which is in line with the mesocircuit hypothesis.

Other ways of assessing resting data exist. One of these is functional MRI, which can detect BOLD (blood-oxygen-level-dependent) changes in the brain. The next chapter will use this to assess spontaneous brain-activity during the resting paradigm, through functional connectivity.

Chapter 3

FMRI functional connectivity measures

Based on:

Resting state networks and consciousness: alterations of multiple resting state network connectivity in physiological, pharmacological, and pathological consciousness States
Heine L, Soddu A, Gomez F, Vanhaudenhuyse A, Tshibanda L, Thonnard M, Charland-Verville V, Kirsch M, Laureys S, Demertzi A.
Frontiers in Psychology, 2012, 3:295

3.1 Resting state functional connectivity networks during unconsciousness

Resting state functional MRI can teach us about spontaneous brain-functioning. Resting state networks, and the DMN are briefly introduced on page 14, and 21. Differences between these networks during consciousness and unconsciousness can teach us about the NCC, and thus we will describe resting state networks, and their respective changes during physiological (e.g., sleep and hypnosis), pharmacological (e.g., sedation and anesthesia), and pathological (e.g., coma and DOC) states of unconsciousness. Furthermore, the DMN is not the only resting state network important in consciousness, and the function of any brain region cannot be understood in isolation but only in terms of functional integration [137]. Therefore we will further focus this review on the bilateral frontoparietal, salience, sensorimotor, auditory, and visual networks [66, 67, 88, 107, 138] (figure 3.1).

As indicated before, resting state fMRI studies suggest that activity of the DMN is generally reduced as a function of the level of consciousness. Not only in patients [118], but also in other states of diminished consciousness. For example, it has been shown that with the advancement of sleep, connectivity between the frontal and posterior parts of the DMN decreases [139]. Decreases in functional connectivity were also observed in the posterior cingulate cortex (PCC) of the DMN under pharmacological unconsciousness with propofol [115, 140] and sevoflurane [141]. These studies suggest that DMN functional connectivity correlates, at least partially, with the level of ongoing conscious cognition. This is in agreement with functional connectivity studies on intermediate states of awareness. For example, hypnotic state show only small [110] or no connectivity decreases in the DMN [142]. Similarly, during light sleep [143, 144] there is no change, and during moderate sedation, little [145] or no changes [146] in DMN connectivity have been observed.

The frontoparietal network (figure 3.1) during normal wakefulness encompasses bilateral middle, inferior and superior frontal cortices, bilateral inferior parietal lobes, ACC/supplementary motor area (SMA) and bilateral insular cortices. Resting state independent component analysis identified this network in a lateralized manner. The left frontoparietal network is thought to be more involved in cognitive and language paradigms while the right frontoparietal network relates to perceptual, somesthetic and nociceptive processing [67, 147]. Activity in both these two networks is reduced during deep sleep [116] and anesthesia [115] whereas light sleep did not seem to mediate functional connectivity in these networks [144]. Taken together, these results highlight the involvement of the frontoparietal networks in the perception of the external world, in line with previous suggestion that activity of these areas is a necessary condition for conscious (i.e., reportable) visual perception [148].

The salience network (figure 3.1) encompasses fronto-insular and anterior cingulate cortices (ACC) with connections to subcortical and limbic structures. In normal conditions, this network is implicated in the orientation towards salient (e.g., homeostatic, cognitive, and emotional) stimuli [149], conflict monitoring, information integration, and response selection [150, 151] and it has been proposed that the salience network enables the switch between internal attention (the default mode) and task related states [152]. This network also showed modulations in connectivity under propofol anesthesia [153]. The salience network has also been linked to pain-related processes both during actual pain (Tracey and Mantyh, 2007), during resting state while anticipating pain (Ploner et al., 2010;Wiech et al., 2010), and after hypnotic suggestions for creating pain experiences in the absence of a noxious stimulus (Derbyshire et al., 2004). In altered states of consciousness, increased connectivity between the ACC and the insula under light sevoflurane sedation was observed (although connectivity between the insula and the secondary somatosensory cortex was reduced, [141]). Analysis of the salience network in comatose states could be beneficial for analysis of pain, as patients in MCS are more likely to feel noxious stimuli [9].

The (sensori-)motor network (figure 3.1) resembles the activations seen in motor tasks [65]. In normal wakefulness it encompasses the supplementary motor area (SMA), midcingulate cortex, bilateral primary motor cortex and bilateral middle frontal gyri [65, 154]. During light sedation the sensorimotor network shows increases in functional connectivity [141, 154].

The auditory network (figure 3.1), important in audition (e.g., tone/pitch discrimination, music, and speech) [147] in normal wakefulness, encompasses primary and secondary auditory cortices, including Heschls gyrus, bilateral superior temporal gyri, and posterior insular cortex. During normal wakefulness, resting state independent component analysis also identifies the visual network in three independent components. One network, the lateral visual network includes the middle temporal visual association area at the temporo-occipital junction and is most important in complex (emotional) stimuli [147]. The other networks include medial and occipital visual networks, important in simple visual (e.g., a flickering checkerboard) and higher-order visual stimuli (e.g., orthography), respectively [66, 138, 147, 155]. No difference in connectivity was identified between both these primary auditory and visual sensory networks and light sleep [144], or between awake and sedation [115, 141]. One study showed increased temporal synchrony in auditory and visual areas in light midazolam sedation [156]. The visual cortex (figure 3.1) has been shown to possess a higher amplitude of bold fluctuations when asleep [157]. This indicates that resting state activity continues in these areas during sleep, and thus is not dependent on consciousness. Finally, reliably indicated as possessing functional connectivity is the cerebellum. This network is associated with action and somesthesis [147], but not yet thoroughly studied in altered states of consciousness.

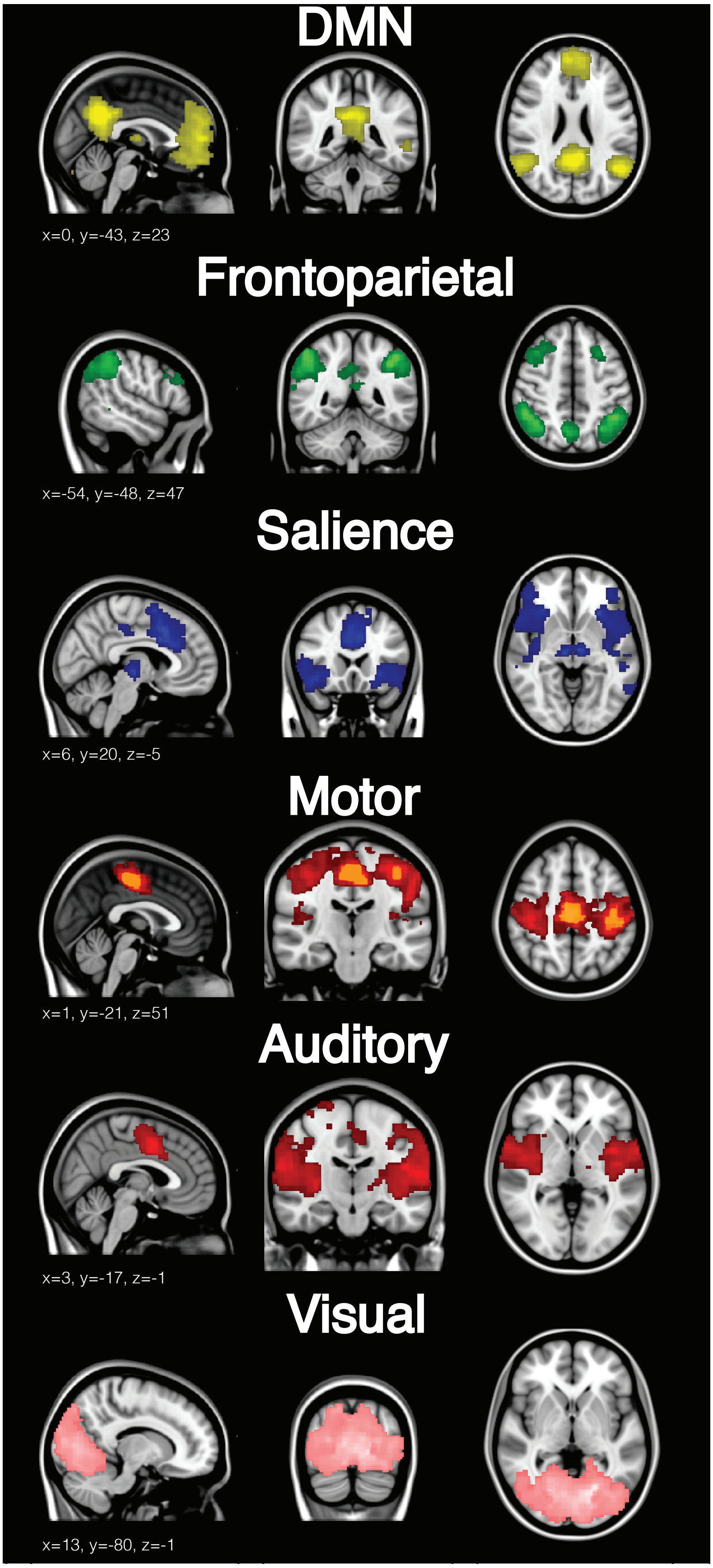

Figure 3.1 – Resting state networks. Functional connectivity maps of the six most studies resting state functional connectivity networks were produced in a group of 10 healthy conscious subjects using the CONN toolbox. For illustrative purposes, group-level spatial maps (z values) are rendered on a structural T1 magnetic resonance template and x, y, and z values indicate the MNI coordinates of the represented sections.

3.2 Analyzing resting state data from pathological brains: methodological issues

Functional connectivity refers to measures of similarity between the two time-courses, usually (and in the studies of this thesis) this is done through the Pearson correlations coefficient, measuring the linear dependence between the two signals. Depending on the adopted methodology, several issues need to be taken into account when analyzing resting state acquisitions from clinical populations. To date, two main approaches are employed; hypothesis-driven seed-voxel correlation analysis and data-driven independent component analysis. Each method has its own advantages, yet their methodological difficulties, especially in non-collaborative patients, must be acknowledged.

Hypothesis-driven method: seed based correlation analysis

The seed-voxel approach uses the extracted BOLD time course from a region of interest (ROI) and determines the temporal correlation between this signal (the seed) and the time course from all other brain voxels [101]. This creates a whole-brain voxel-wise functional connectivity map of covariance with the seed region. See figure 3.2 for an example of the main resting state networks, and representative seed regions within these networks. This is the most straightforward method to analyze functional connectivity between brain regions. The method gives a direct answer to specific hypotheses about functional connectivity of that region. It is attractive and elegant for many researchers as the data can be interpreted relatively easily when a well-defined seed area is used. When applying this approach to the study of resting state activity in patients with DOC, several controversial issues arise. A first general issue concerns the removal of artifactual influences on the signal. When the review on which this chapter is based was written in 2012, issues about regressing out the global activity from the BOLD signal [111, 158] already existed. In the years since, several steps have been taken to improve quality of the data. For example, other methods regress out signals found in white matter, ventricles or other noise components [159–161] before bandpass filtering [162]. This protects against confounding correlations as produced by other methods, like global signal regression [111, 112, 160, 161].

All resting state studies in this thesis use this method of confound-regression as implemented in open-source, SPM and MATLAB based CONN analysis toolbox [163]. More specifically, aCompCor models the influence of noise as a voxel-specific linear combination of multiple empirically estimated noise sources by deriving principal components from noise regions of interest (white matter, CSF) and by including them as nuisance parameters within the general linear models. Motion is also a severe confounder in resting state analysis, and proper methods of filtering are necessary [164]. Our analyses includes regression of the motion parameters (ART; http://www.nitrc.org/projects/artifact_detect), instead of deletion of motion contaminated images [114].

Next, patients with severe brain injuries may suffer from structural deformations resulting from traumatic brain injury and focal hemorrhages. Additionally, patients with severe chronic brain injuries usually develop atrophy and secondary hydrocephalus (i.e., ex-vacuo dilation of the ventricles). This implies that even if a statistical structural normalization procedure has been performed, the selection of a proper seed region can become difficult and will require visual inspection by an expert. This issue adds to the already intrinsic challenges of a priori selection of the seed region which can be damaging to network estimation when functionally inaccurate ROIs are chosen [165, 166]. Finally, as for all group-level analyses, one has to take into account intersubject variability, such as cortical folding or functional localization between individuals or groups [165] which can be extremely challenging in severely deformed brains.

Data-driven method: independent component analysis (ICA)

Data-driven methods are used to analyze whole brain connectivity patterns without the need for a priori seed regions. ICA is a widely used methodology with a high level of consistency in results within subjects [167]. It divides an entire dataset into different maximally statistical independent spatial components and thus is able to isolate cortical connectivity maps from non-neural signals [138]. Spontaneous activity is therefore automatically separated from noise, such as head motion or physiological confounds (e.g., cardiac pulsation, respiratory, and slow changes in the depth and rate of breathing) [168]. This method has the advantage that it can evaluate and compare the coherence of activity in multiple distributed voxels [165], and can divide multiple resting state networks into different components. However, ICA does not provide any classification or ordering of the independent components. It is therefore perceived as more difficult to understand due to the complex representation of the data. The most straightforward method for labeling the components is by visual inspection, but this lacks reproducibility and could be hard to perform in cases with a large component dimensionality. Alternatively, an automatic selection is preferable but the way to choose the right independent component remains a delicate issue. Merely performing a spatial similarity test with a predefined template has been shown not to be successful for choosing the right component [38, 96]. Some automatic approaches for component selection have been proposed, based on template matching using the goodness of fit as an outcome index. However, these methods have to be interpreted with care especially in cases of deformed brains as in patients with a traumatic brain injury or comatose state. It was recently proposed that when

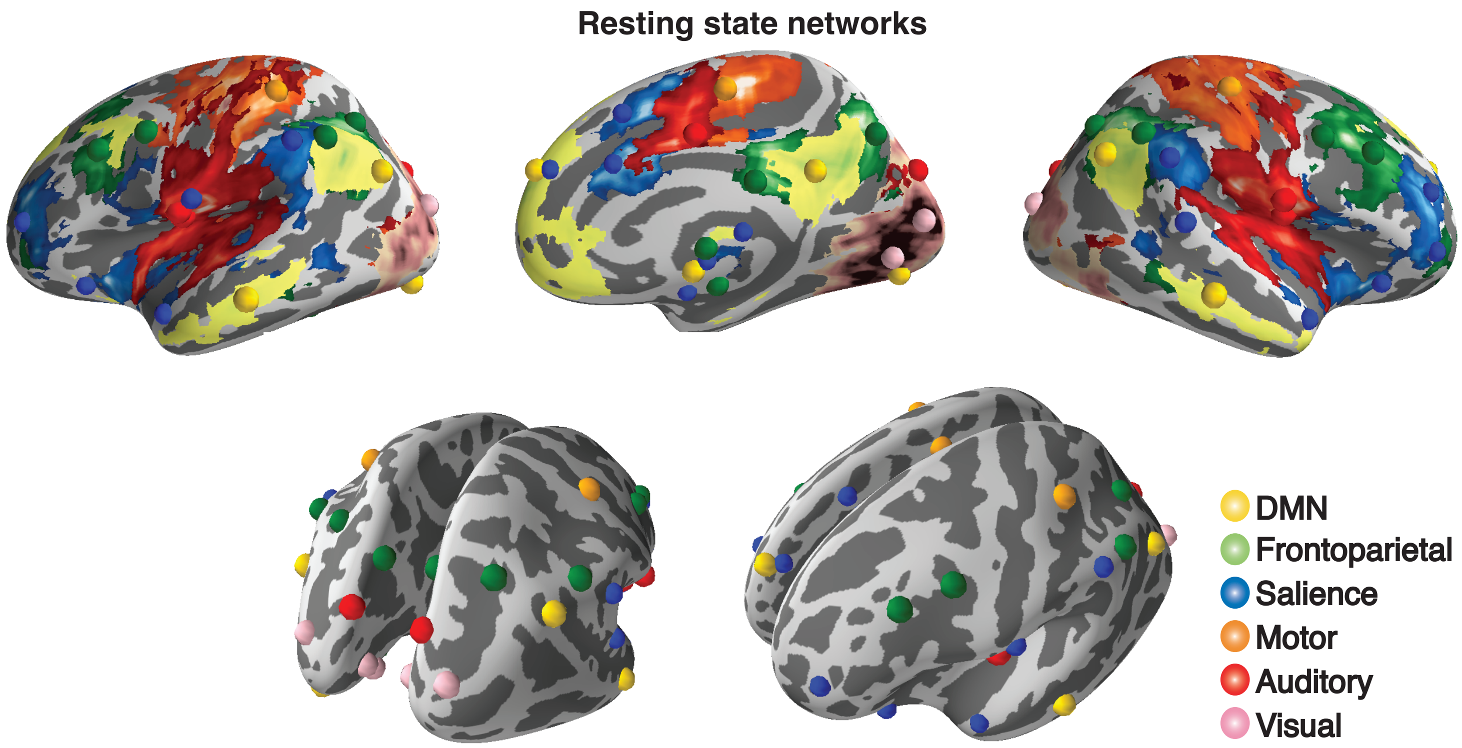

Figure 3.2 – Functional connectivity networks and representative seed regions. Functional connectivity maps of the six most studies resting state functional connectivity networks were produced in a group of 10 healthy conscious subjects using the CONN toolbox. Spheres show representative seeds / locations of peak activity (used in chapter 4).

selecting independent components in a patient population, spatial, temporal and a compromise between spatial and temporal properties of the network of interest need to be met [96]. For example, a component can be erroneously selected as the resting state network of interest if the selection is based on the spatial pattern ignoring the properties in the time-domain (figure 3.3). Additionally, the determination of the proper dimensionality (i.e., the right number of estimated components) remains unclear. Extracting many components can result in the spatial segregation of the network of interest into multiple sub-networks [67]. It was shown, for example, that the use of 75 components can reduce the DMN into four components and the sensorimotor network in six [155]. When applying ICA in pathological brains it is probably best not to select a large quantity of components, because high component dimensionality can further reduce the chances of identifying a network due to decrease in spatial pattern and spectral properties [169].

A more resent study indicated that seed based analysis might be more informative compared to ICA, but combination or multiple seed analysis is preferred when trying to correlate clinical scores of DOC patients to functional connectivity [99]. Other techniques to analyze resting state data exist such as methods that focus on the (fractional) amplitude of low frequency fluctuations ((f)ALFF; [171]), or on the small world characteristics using correlation and graph analysis [172, 173] or dynamic functional connectivity [174].

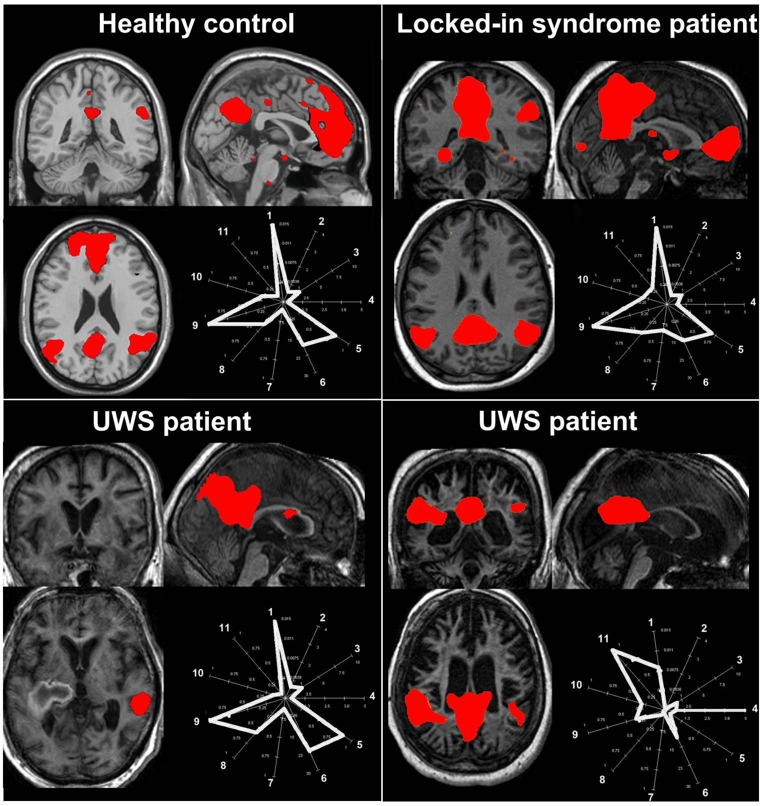

Figure 3.3 – The challenges of component selection. The figure illustrates the spatial pattern (brain maps, z values 0.810) and spatial-temporal properties [170] of the default mode network in healthy consciousness states. Upper and lower left figures show characteristic properties in both the spatial and the temporal domain (i.e., the fingerprints pick in the 0.02-0.05 Hz frequency band labeled with the number 9). Lower right figure shows the spatial pattern of the default mode network but the time course of this component is characterized by high frequency fluctuations, in the 0.1-0.25 Hz frequency band and high spatial entropy (labeled, respectively, with the number 11 and 4 in the fingerprint). Therefore, such activity cannot be considered of neuronal origin. A compromise in the selection of the appropriate network of interest in the space and time domain is needed. Fingerprint labels: (1) degree of clustering; (2) skewness; (3) kurtosis; (4) spatial entropy; (5) autocorrelation; (6) temporal entropy; power: (7) 0-0.008 Hz; (8) 0.008-0.02 Hz; (9) 0.02-0.05 Hz; (10) 0.05-0.1 Hz; (11) 0.1-0.25 Hz.

Chapter 4

Classification of resting state functional connectivity

Based on:

Intrinsic functional connectivity differentiates minimally conscious from unresponsive patients
Demertzi A*, Antonopoulos G*, **Heine L**, Voss H U, Crone SJ, Kronbichler M, Trinka E, Angeles C, Bahri M, Phillips C, Di-Perri C, Gomez F, Tshibanda L, Soddu A, Vanhaudenhuyse A, Charland-Verville V, Schiff N D, Whitfield-Gabrieli S*, Laureys S*.
Brain, 2015, 6:1704,

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Up to now, research on accurate single-patient categorization in MCS and UWS has been performed by means of transcranial magnetic stimulation in combination with EEG [77, 175] and by combining different EEG measures [176]. Patient separation by means of fMRI was not yet done. In this study we aimed at differentiating the MCS-UWS single-patient population by using functional connectivity. To this end, we studied systems-level resting state functional connectivity in severely brain injured patients in a comatose state, UWS, or MCS with the aim to (i) estimate the contribution of each network to the level of consciousness as determined by behavioral assessment; (ii) rank the capacity of each network to differentiate between patients in MCS and UWS; and (iii) automatically classify independently assessed patients.

Three clinical centers collected data from 73 patients in MCS, UWS, and coma. The main analysis was performed on the data set coming from one center (Liege) including 51 patients (26 MCS, 19 UWS, six coma; 15 females; mean age=49 years, SD=18 years; 16 traumatic, 32 non-traumatic of which 13 anoxic, three mixed; 35 patients assessed > 1-month post-insult) for whom the clinical diagnosis with the CRS-R was congruent with PET scanning. Group-level functional connectivity was investigated for the default mode, frontoparietal, salience, auditory, sensorimotor and visual networks using a multiple-seed correlation approach. Between-group inferential statistics and machine learning were used to identify each network's capacity to discriminate between patients in MCS and UWS. Data collected from 22 patients scanned in two other centers (Salzburg: 10 MCS, 5UWS; New York: 5 MCS, 1 UWS, 1 EMCS) were used to validate the classification with the selected features. Coma Recovery Scale-Revised total scores correlated with key regions of each network reflecting their involvement in consciousness-related processes.

4.1 Behavioral relation to functional connectivity networks

Resting state functional MRI connectivity of the default mode, frontoparietal, salience, auditory, sensorimotor and visual networks were first shown to correlate with behavioral CRS-R assessment scores (figure 4.1), highlighting their contribution to the level of consciousness. As seen in the previous chapter, results are in line with other studies in various forms of unconsciousness. Here, the positive correlation between CRS-R scores and the salience network anterior cingulate cortex could account for the preserved capacities of some patients to orient their attentional resources towards environmental salient stimuli, such as noxious stimulation, corroborating previous PET data [177]. The thalamus and cerebellar networks did not correlate to CRS-R total scores. The cerebellum has minimal implication in conscious-related processing [178, 179], but the thalamus is generally thought to be implicated in conscious processing [104, 178]. Taken together, the positive correlation between clinical scores and each network's functional connectivity highlight that the here studied networks are an appropriate means to study, at least to a certain degree, residual cognitive function in this patient cohort.

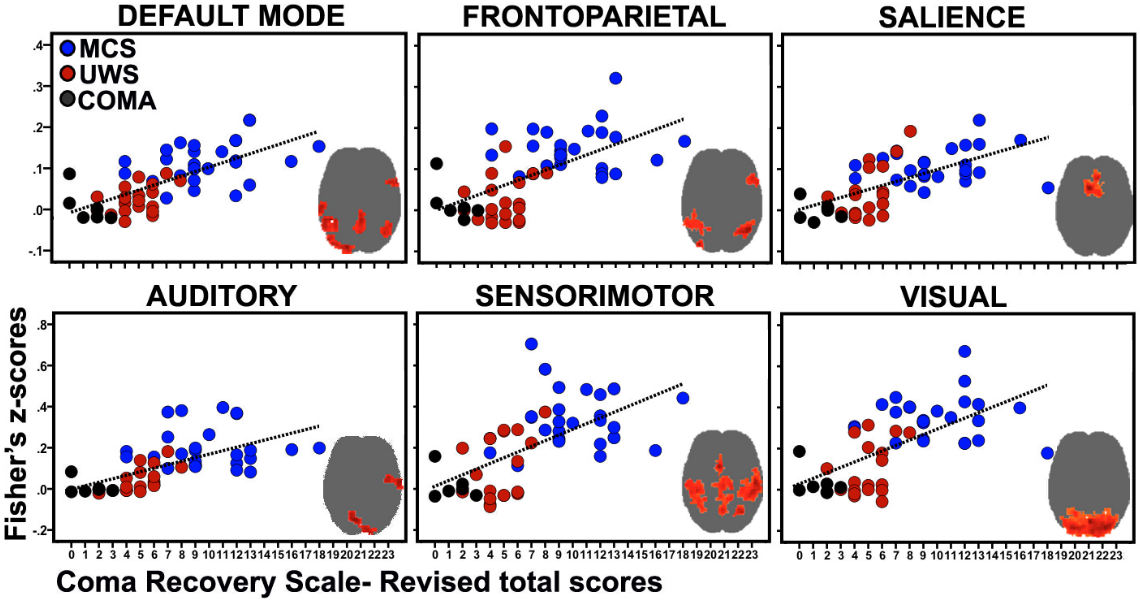

Figure 4.1 – Intrinsic connectivity networks are involved in consciousness-related processing. Functional connectivity of all studied networks (areas in red) correlate with the level of consciousness as determined by behavioural assessment with the Coma Recovery Scale-Revised (total scores) in patients in MCS, UWS and coma. Statistical maps are thresholded at FWE P<0.05 (cluster-level) and are rendered on a glass brain template (transverse view). Replicated from [180].

4.2 Differentiation between MCS and UWS within connectivity networks

Importantly for clinical practice, we further aimed at determining the capacity of each network to differentiate between patients in MCS and UWS. Differences in functional connectivity have been observed only at the group-level for the DMN [47, 96, 117, 181, 182], frontoparietal and auditory networks [182]. Here, we replicated these findings and further showed group differences in functional connectivity for the salience, sensori-motor and visual networks. Moving towards single-patient network-based differentiation, we found that all networks were able to differentiate patients with an acceptable accuracy (> 86%). Such a high rate of accuracy can be partly attributed to the fact that the network ranking was based on features extracted from the same population for which between-group differences were already known.

4.3 Single-subject classification of the auditory network

To avoid a double-dipping effect, we aimed at validating the most highly ranked network in two independently assessed patient data sets (Salzburg and New York) and across healthy controls. To that end, we opted for single-patient classification based on the connectivity strength of the auditory network (figure 4.2). Based on this network's connectivity, 20 of the 22 new patients were classified congruently (i.e., the clinical diagnosis matched the classification outcome). Of note is that the classifier positioned the independently assessed patients closer to the decision plane compared to patients included in the training set. This could be explained by the above mentioned favoring of the Liege training data set during the network ranking procedure, which might have led to a stricter classification of the validation set. Although the intrinsic connectivity networks have been shown to be robust independent of different scanning parameters [183], the different parameters employed in each of the three centers might also have influenced the classifier's estimation. Alternatively, the use of a relevance vector machine classifier [42], which returns probabilities of a patient belonging to a clinical condition instead of using a binary decision, could be a more sensitive way to classify patients less strictly. The classification results further highlight the challenges posed by behavioral examination [184] which in many cases underestimates patients' level of consciousness [21]. Here, the validation of the auditory network's classifier worked congruently for the majority of the included patients (20/22; figure 4.2). Interestingly, the patient who was misclassified as MCS had a profile of UWS on the day of scan but evolved to MCS 38 days later. The other patient was misclassified as UWS but had a clinical profile of MCS on the day of scanning based on the presence of localization to noxious stimulation (note that this behavior could not be elicited in any other evaluations). The validation of the classifier's outcome to the clinical evaluation was used as a starting point in our analysis. Therefore, a well-defined diagnostic baseline was critical for the subsequent patient classification. To that end, repeated clinical examinations with the CRS-R (average number of assessments n = 6 per patient) were performed. The clinical diagnosis was further confirmed with FDG-PET imaging, which has been shown to have high sensitivity in identifying patients in MCS [32]. Therefore, patients with an ambiguous profile on clinical assessment and neuroimaging data were not included in the analysis. Similarly, patients who received sedatives to minimize motion in the scanner were further excluded.

One explanation of why the auditory network (figure 3.1, 4.1) was identified as the system with the highest discriminative capacity could concern its underlying functional neuroanatomy. Apart from temporal cortices, the auditory network further encompasses regions in occipital cortex, pre- and postcentral areas, insula and anterior cingulate cortex [66, 67, 182, 185, 186].

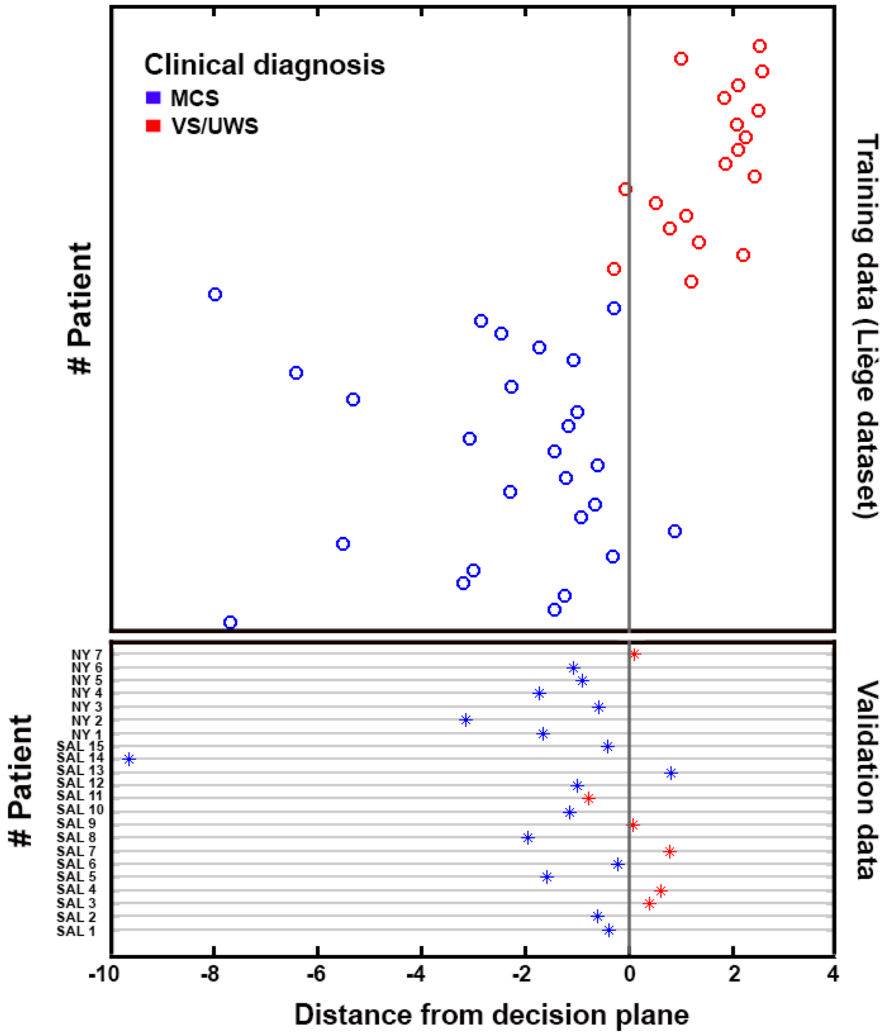

Figure 4.2 – Auditory-visual crossmodal functional connectivity discriminates single patients. The 3D space indicating connectivity between left auditory, right auditory and occipital cortex has been compressed into two dimensions to represent the distance of each patient (in circles) from the decision plane (arbitrary values). The upper panel plots the data of patients (in circles) who were used for the classifiers training (Liege data set, n= 45). The lower panel summarizes the classifiers decision on the validation data set including patients (in asterisks) independently assessed in Salzburg (n= 15) and New York (n= 7). Based on the crossmodal interaction, 20 of the 22 independently assessed patients were classified congruently, namely the behavioural diagnosis matched the classification outcome. Replicated from [180]

The direct comparison between patients in MCS and UWS restricted the identified areas to bilateral auditory and visual cortices. This pattern of auditory-visual functional connectivity has been previously described in normal conscious subjects during rest as well [187] and is in line with functional MRI results in consciousness research. For example, preserved functional MRI activity in temporal and occipital areas has been shown for healthy subjects during mental counting of auditory temporal irregularities; interestingly, this activation was identified only in those subjects who were attentive and aware of the auditory violations [188]. At a functional level, the auditory-visual functional connectivity, also referred to as cross modal interaction, is considered relevant for multisensory integration [189]. Multisensory integration has been suggested as a facilitator for top-down influences of higher-order regions to create predictions of forthcoming sensory events [190]. Such top-down connectivity was recently found with an EEG oddball paradigm that differentiated patients in MCS from UWS [191]. Interestingly, decreased cross modal auditory-visual interaction has been reported in healthy subjects with preserved structural connections but under pharmacologically-induced anesthesia [115]. In that study, recovery of consciousness paralleled the restoration of the cross modal connectivity suggesting a critical role of this connectivity pattern to consciousness level-dependent states. In our results, the cross modal interaction was more preserved in patients in MCS compared to UWS patients. The reduction in functional connectivity between the auditory-visual cortices in UWS could be partly attributed to disrupted anatomical connections, often encountered in post-comatose patients [97, 192–194].

Conclusions and future perspectives part I

In chapter 2 we aimed to directly investigate, in severely brain injured patients, the relationship between functional brain activity and structural connectivity within the DMN in an objective and combined fashion using both FDG-PET and MRI FA. We show that the function-structure relationship is present in both healthy controls and brain-damaged patients between functional metabolism of inferior-parietal, precuneus, and frontal regions and structural integrity of the frontal-inferior parietal, precuneus-inferioparietal, thalamo-inferioparietal and thalamofrontal tracts. When focusing on patients, we found a stronger relationship between structural integrity of thalamo-inferioparietal tracts and thalamic metabolism in patients who have emerged from MCS as compared to DOC patients.

We then reviewed data on resting state functional connectivity in physiological, pharmacological, and pathological unconsciousness in chapter 3. Resting state connectivity is altered under altered states of consciousness, such as sleep, sedation/anesthesia, hypnotic state, and clinical states of unconsciousness. These reductions are most studied in relation to the DMN, but are also present in other resting networks.

In chapter 4 we replicate the systems-level resting state functional MRI consciousness-dependent breakdown not only for the default mode network but also for the frontoparietal, salience, auditory, sensorimotor and visual networks. Furthermore, functional connectivity between auditory and visual cortices was the most sensitive feature to accurately discriminate single patients into the categories of MCS and UWS. Our findings point to the significance of multisensory integration and top-down processes in consciousness seemingly supported by cross modal connectivity.

The studies mentioned in this chapter have all looked at the brain function in (un)conscious states. The ultimate goal for this kind of research is to gain better understanding of consciousness and maybe even NCC. Although we are still very far from something like a correlate of consciousness, or a diagnostic tool easily implicated in clinical practise, several claims can be made based on these studies.

First of all, based on all three studies, and the many studies in literature, we know that brain function under unconscious states is reduced. This level of reduction seems different for each clinical entity, and differences can still be made between unconsciousness (UWS) and partial consciousness (MCS). Chapter 2 furthermore tells us that function and structure are linked, a positive function-structure connectivity exists. Such a structure-function connectivity can also be found between structural white matter and functional connectivity [174], and functional connectivity with PET metabolism [118]. From this we could conclude that general structure or function of the brain alone is necessary but not sufficient for conscious experiences to arise. Future research should thus focus its effort on multimodal studies to better understand brain function. While doing this, the here utilized lesion approach is a start, but brain function, and integration of information using multimodal studies [195] should be further explored before real conclusions can be drawn on the NCC.

Methodologically, future research can improve on several points. First of all, one problem with current resting state fMRI analysis in our patients is the inability to quantify if a subject fell asleep during our analysis or not [196]. Indeed, the current clinical setup only allows researchers to check patients in between sequences, and visual trackers are not yet available in the hospital where the patient data of this thesis was acquired. A second point to be mentioned here concerns the static nature of functional connectivity. Although informative, studies on dynamic functional connectivity have the potential to explore information not captured with classical resting state methods. The nature of the changes in connectivity strengths over time remains ill-defined in DOC. In addition, the methods for the assessment of deformed brains remain challenging. Normalisation issues and automatic segmentation can influence results, and should be taken into account.

Clinically, efforts need to be made to promote the feasibility of these relatively complex approaches in the clinical setting and promote the clinical utility of the resting paradigm for single-patient diagnostics. Furthermore, many subjects need to be sedated due to the presence of pre-scan motion (to reduce noise during data acquisition), and these subjects were not taken into account for classification of functional connectivity. The reason to exclude sedated patients in the previous part was because of our limited understanding of the potential effect of anesthetics on network connectivity [41]. Future investigations which will aim to disentangle between the variances of anesthetics and pathology in functional connectivity measures are certainly essential.

Part II

The passive paradigm: Sensory stimulation and diagnostic assessment

Introduction

As we have seen in the previous chapter, testing the brain at rest can provide information about the general structure and function of the brain. However, it cannot make inferences about how the brain reacts to incoming stimuli. Passive paradigms use such external stimulation, and thus try to indicated covert cognitive processing in these patients [197]. Using passive paradigms, differential activation patterns have been demonstrated in patients in UWS and MCS. For example, as a respond to sound, patients in UWS show activation limited to the primary auditory cortex [198], whereas patients in MCS demonstrate brain activation spreading to secondary auditory cortex, temporal and frontal areas [199]. More importantly for clinical management, during painful stimulation patients in MCS show similar brain activation compared to controls, while patients in UWS only show restricted activation in lower-level subcortical and primary cortical areas.

Furthermore, patients retain some form of cognitive processing, and are able to react to several kinds of stimuli like the own name [200–202], familiar voices [203], sensorimotor [204, 205], visual [204, 206, 207], and linguistic processing [192, 208, 209].

Behaviourally, besides the CRS-R scale, there are many more behavioural tests for bedside evaluation of patients. Some of these use these passive paradigms, usually sensory stimulation, to elicit reproducible, voluntary oriented responses and/or evaluate progress during sensory stimulation programs. Scales such as the Western Neuro Sensory Stimulation Profile (WNSSP) [210], the Sensory Modality Assessment Technique (SMART) [211], and the Disorders of Consciousness Scale (DOCS) [212] include the assessment of more sensory modalities compared to the CRS-R. More precisely they include tactile, olfactory and gustatory modalities. Few studies have assessed the specificity and sensitivity of these tests. Even so, it is recommended by the the American Congress of Rehabilitation Medicine to include such sensory stimulation [27].

Another personally relevant sensory stimulus is music. This stimulus conveys emotion [213], and might boost cognitive processes. These effects might be seen both in healthy [214], and pathological cerebral functioning [215, 216]. For example, auditory and verbal memory, focused attention, and mood improves in patients recovering from stroke when listening to music on a daily basis [217, 218]. In patients with visual neglect, visual attention is better when listening to preferred relative to non preferred music (i.e., patients showed enhanced visual awareness, also in the neglected side) [219]. Furthermore, music can decrease levels of anxiety and frequency of sedation during hospitalisation in the intensive care unit, in comparison with usual care or noise-cancelling conditions [220]. In DOC patients, music has not yet been extensively studied. Single case studies have indicated potential effects on behavioural responses in UWS patients [221–223], but were unable to draw firm conclusions due to the lack of quantified measures and/or control conditions.

The study in chapter 5 assesses sensitivity to provoke signs of consciousness using general sensory stimuli as used in several assessment scales. Chapter 6 will discuss the neuroimaging evidence indicating that personally relevant stimuli (preferred music) might increase the brains' functional connectivity. In chapter 7, we will return to the behavioural examinations assessing music and another sensory domain, namely olfaction. We will assess if this effect of music is due to preference of the stimuli, or an effect of acoustic properties.

Chapter 5

Sensory stimulation during behavioral examinations of consciousness

Based on:

Behavioral Responsiveness in Patients with Disorders of Consciousness
Heine L, Laureys S, Scnakers C.
in: Brain Function and Responsiveness in Disorders of Consciousness, *Springer International Publishing*, 2016

5.1 Tactile, olfactory and gustatory stimuli during diagnostic assessment

It has previously been shown that some sensory modalities are more sensitive to detect consciousness than others. In studies investigating misdiagnosis, oriented eye movements (i.e., visual pursuit and fixation) have been reported as the responses the most frequently missed during behavioral assessments [21, 25, 224]. In parallel, the visual modality of the CRS-R has been shown as the subscale allowing the highest detection of MCS as compared to the auditory, motor or verbal modalities [28, 225]. Oriented visual responses are particularly interesting to detect since it is one of the first signs of consciousness appearing during patients recovery which is also associated with good outcome [226–228]. Until now, the interest of other sensory modalities (such as tactile, olfactory and gustatory) when assessing consciousness are ill-described, even though several scales recommended by the the American Congress of Rehabilitation Medicine include such modalities [27].

In a preliminary study, we therefore decided to investigate the interest of tactile, olfactory and gustatory modalities in the assessment of consciousness. We assessed 38 patients (mean age=46, SD=16 years old, 17 traumatic, 21 chronic) diagnosed as being in a UWS (n=15) or in a MCS (n=23) by using the CRS-R. Tactile, olfactory and gustatory stimuli used in the WNSSP, the SMART and the DOCS have been administered in each patient in a randomized order. Tactile stimuli included tap on the shoulder, nasal swab, feather (applied on arms, fingers and face), air into the neck, hair touching, vibration on the arm, scrub (i.e., kitchen scouring pad applied over the arm) and firm hand pressure on the arm. Each of these stimuli was applied for ten seconds on both sides of the body on three consecutive trials. Olfactory stimuli included vinegar, syrup and ammonia which were held under the patients nose for ten seconds (patients mouth closed) on three consecutive trials. In case of tracheotomy, the entrance of the cannula was covered. Gustatory stimuli included vinegar and syrup. A stick soaked of this flavor was introduced into the patients mouth for ten seconds on three consecutive trials. Several recommendations had to be followed such as: applying the treatment while the patients were in a wakeful state with eyes open in a setting with minimal ambient noise and respecting a 30 minutes rest before each session (i.e., absence of nursing care). Oriented responses (e.g., eyes/head towards or away from the stimulus, hand towards or pushes away the stimulus, congruent facial expression, mouth opening or tongue pumping) was considered as present when it was clear and reproducible, meaning it was observed at least two out of three times to exclude reflexive behaviors. The oriented responses obtained using those tactile, olfactory and gustatory stimulations have then been compared to the diagnosis obtained using the CRS-R. Patients outcome were also collected at one year after assessment (n=27), using the Glasgow Outcome Scale (GOS) [229].

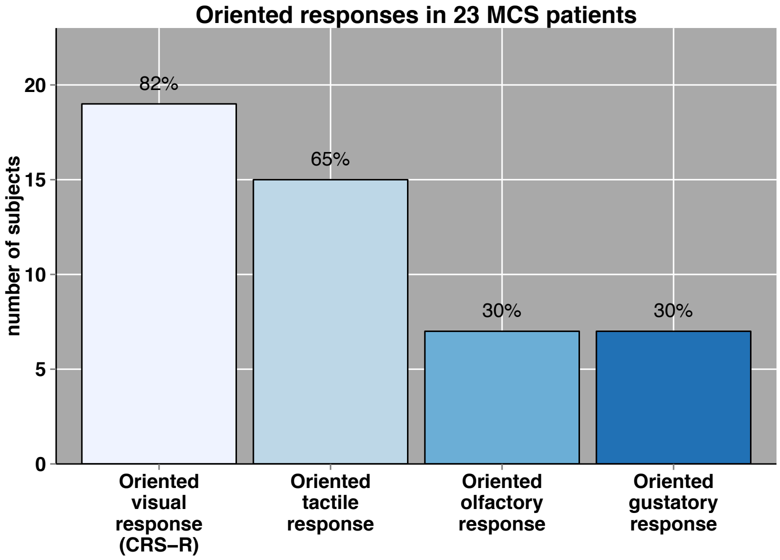

Figure 5.1 – Oriented responses in 23 MCS patients. Percentage of oriented responses in MCS patients in reaction to olfactory, gustatory and tactile stimuli, as well as the percentage of visual oriented responses as measured with the CRS-R.

In our dataset, 82% of MCS patients showed oriented eye movements, and 0% of UWS patients did so. Oriented eye movements consisted of visual following or fixation as measured using the CRS-R. Olfactory, gustatory, and tactile stimuli show different patterns. According to our results, a minority of patients diagnosed as being in a UWS (by using the CRS-R) showed oriented olfactory or gustatory responses (7% and 14%, respectively) (figure 5.2). The patients for whom we had outcome data (1 missing data) did not recover consciousness a year after assessment.

Additionally, oriented olfactory or gustatory responses were absent in a majority of patients diagnosed as being in a MCS by using the CRS-R (70%) and in a majority of patients who showed oriented eyes movements (61%) (figure 5.1). Using tactile stimuli, a higher percentage of patients diagnosed as being in a UWS showed oriented responses (40%) (figure 5.2). Oriented tactile responses were present in a majority of patients diagnosed as being in a MCS by using the CRS-R (65%) and in a majority of patients who showed oriented eyes movements (83%) (figure 5.1). When considering the stimulus leading to the most frequent oriented responses, the nasal swab helped to detect 80% of the oriented tactile responses present. However, only one of the UWS patients showing oriented tactile responses recovered consciousness a year after assessment (17%). The patient (50 years old, 50 days after

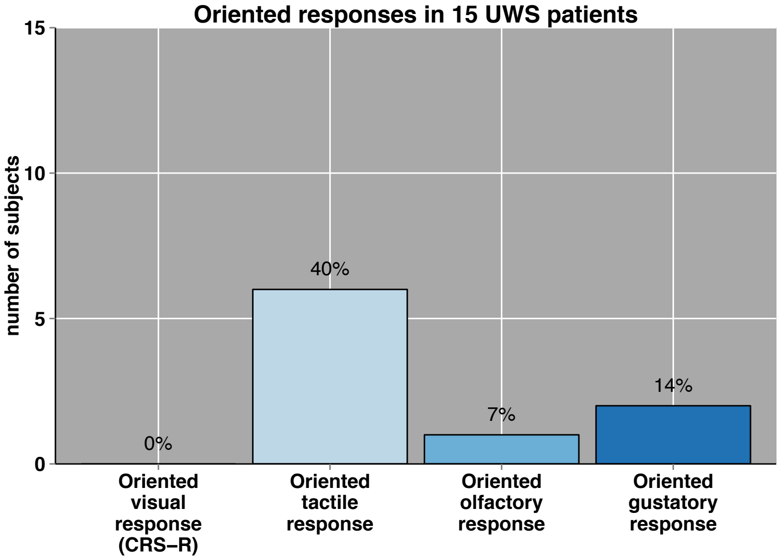

Figure 5.2 – Oriented responses in 15 UWS patients. Percentage of oriented responses in UWS patients in reaction to olfactory, gustatory and tactile stimuli, as well as the percentage of visual oriented responses as measured with the CRS-R.

non-traumatic injury) was able to localize a tactile stimulus using her hand. Repeated CRS-R assessments, at that time, showed only reflexive behaviors (i.e., auditory startle, blinking to threat, flexion to noxious stimulation, oral reflexive movements and arousal with stimulation). Two years after our assessment, the CRS-R indicated an EMCS. Finally, to test whether the outcome measured by the GOS differs according to the presence or absence of an oriented response, U Mann-Whitney tests were performed. There was no statistical difference for olfactory (U=51.5; p=0.61), gustatory (U=49; p=0.5) and tactile (U=76.5; p=0.51) modalities (figure 5.3).

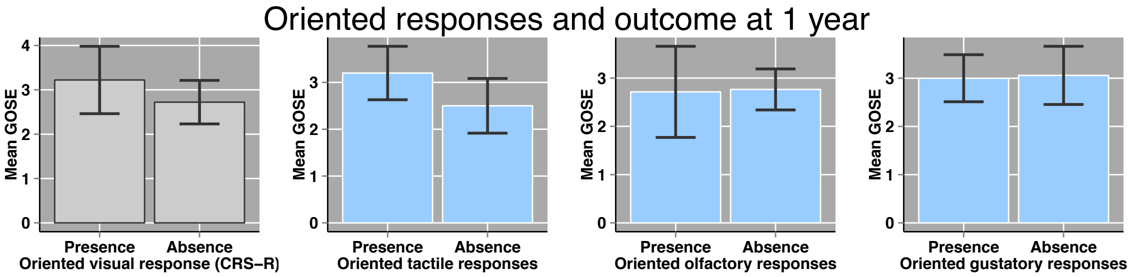

Figure 5.3 – Outcome at 1 year. Average Glasgow Outcome Scale Extended scores, and its standard error according to the presence or absence of oriented responses.

Considering our data, oriented responses to olfactory and gustatory stimuli without personal relevance do not seem to be linked to consciousness since they are not observed in the majority significant proportion of conscious patients and since they are not associated with consciousness recovery. Oriented tactile responses seem to be observed in most conscious patients but could be false positives as they are also observed in a large portion of unconscious patients. This preliminary study hence seems to indicate that adding sensory modalities such as olfactory, gustatory or tactile stimuli without personal relevance to the CRS-R does not constitute a further help when detecting consciousness in patients with severe brain injury.

5.2 Self-relevant stimuli might enhance cognitive processes

The previous section described the application of general sensory stimuli. These general stimuli might have induced a number of false negatives due to the lack of personal engagement. Indeed, especially familiar faces increase the likelihood of retrieving episodic information [230]. Furthermore, personalized stimuli enhance the probability to observe a cerebral response in DOC patients. For example, several behavioral studies have shown that a higher number of responses could be observed following self-referential stimuli, like the use of a mirror or the patients own name, as compared to neutral stimuli [28–30] or through the use of autobiographical and emotional stimuli [231].

Neurophysiological studies have shown that salient and emotional stimuli increase the probability of observing a cerebral response in patients with DOC. For example, the probability to observe a P300 event-related response (i.e., a brain response reflecting stimulus processing) is enhanced when the deviant stimulus is not a tone stimulus but the patients own name [201, 232]. Among these emotional, salient, and self-referential stimuli, music may be the most assessable and personally relevant. In patients with DOC, there is only a limited amount of research on the effects of music. Several single-case, or (uncontrolled) group studies, show that music improves patients interaction [222], emotional responses and cognitive capacity [221, 233, 234]. Very recently, it has also been shown that preferred music (i.e., an autobiographical and emotional stimulus) has an effect on cognitive processes of patients with DOC. Indeed, observing a P300 to ones own name was increased in patients with DOC after having been exposed to their preferred music compared to a control condition (i.e., acoustically similar noise) [235].

Chapter 6

Resting state functional connectivity during preferred music

Based on:

Exploration of Functional Connectivity During Preferred Music Stimulation in Patients with Disorders of Consciousness
Heine L*, Castro M*, Martial C, Tillmann B, Laureys S, Perrin F.
Frontiers in psychology, 2015, 6:1704

* Contributed equally

6.1 Preferred music

To explore whether the effect of music in severely brain-damaged patients with DOC is related to functional connectivity changes, we acquired functional MRI scans while participants were exposed to their preferred music as well as a control condition when they were exposed to the repetitive noise from the scanner (also present in the music condition). Using a functional connectivity parcellation [236], we assessed functional connectivity using seed regions in both primary auditory cortices. We also analysed network connectivity of the auditory network, the external network, and default mode network. The auditory network can be observed in 81% of healthy subjects, 46% in MCS, and is limited to 21% of UWS patients [182]. In fact, it has strong power to discriminate MCS and UWS patients, making automatic classification possible [180]. Another network that is also related to auditory processing [105] is the external network. This network is also related to external orientation, goal-directed behaviours, and cognitive processing of somatosensory [103], and visual [104] input. The external network is often named the dorsal attention network, or task positive network [47, 81]. It has been shown to be anticorrelated with an internal / default mode network [47, 81], implicated in self-awareness and stimulus-independent thoughts in healthy controls [78, 83]. Interestingly, auditory, external and internal/default mode networks include cortical regions that have been shown to be modulated by emotional sounds. Indeed, as compared to noise, meaningful sounds (infant cries or the patients own name) are associated to a widespread activation of the auditory cortex and medial cortical structures in DOC patients [237].

To create a sequence of preferred music, five musical excerpts were selected for each participant from a questionnaire on musical preference completed by family members or loved ones (for the patients) or the participant him/her self (for the healthy participants). These musical excerpts had a mean duration of 2 minutes and were all dynamic, musically coherent, and representative of the whole musical piece. The five excerpts were combined to create a musical stimulus of a duration of 10 minutes and 10 seconds, which overlaps with the duration of the functional scan. Fading in and fading out (around 2 sec.) was added to avoid rough transitions between the excerpts.

The functional scan was acquired twice during one MRI scanning session. Once with the participants preferred music (i.e., music condition), and once when participants were exposed to the repetitive noise from the scanner (i.e., control condition). This control condition is the same as used for the investigation of a classical resting state. Between March 2014 and April 2015, eight healthy participants (four female; mean age = 26 years, SD=3), and 5 patients (three MCS, two UWS; mean age = 50 years, SD = 10) were acquired and analysed.

6.2 Effect of music in healthy conscious subjects

We first assessed results in our group of healthy subjects alone. As there is a difference in age, these results were taken as a reference, but no statistical comparison was made between healthy subjects and our group of patients. In our healthy participants, the network of functional connectivity based on both primary auditory regions encompasses large parts of the auditory cortex, superior temporal gyri, insula, cingulate cortex, central areas (pre and post), supramarginal gyrus, and occipital areas, in both the music condition and the control condition. These are, as expected, part of the auditory network [66, 67, 88, 138, 147, 182]. To assess network integrity, mean network connectivity was assessed in the auditory network, external network, and default mode network (i.e., networks which are respectively linked to auditory processing, external orientation, and internal thoughts).

Network-based second level analysis of functional connectivity showed that the auditory network was clearly replicated in our healthy subjects during both the music and control conditions (figure 6.1, 3.2). This network has consistently been observed in previous resting state studies investigating not only healthy participants but also DOC patients [182]. In healthy participants it encompassed bilateral temporal gyri (including Heschls gyrus, opercular, insula, planum polare and superior temporal areas), extending to inferior frontal, precentral and angular areas, as well as clusters in anterior cingulate, pre- and post-central areas and the occipital fusiform gyrus [66, 67, 88, 138, 147, 182]. The external network has also been observed in healthy participants. It encompassed, as consistently observed in previous studies [47, 101], regions of bilateral inferior parietal sulcus and lobule, dorsolateral prefrontal, supramarginal gyrus, the frontal eye field, lateral occipital and precentral, as well as cerebellar and insular areas. The default-mode network showed functional connectivity in regions consistently observed in healthy participants and patient populations [238]. Most importantly, music did not show any increases in functional connectivity compared to the control condition for the seed-based and all three network-level analyses (figure 6.1). This result is consistent with [235], a study which observed that music (in comparison to noise) did not modify the event-related responses in healthy participants (while this was the case for the DOC patients). This observation suggests that the effects of music observed in previous research are possibly not present in healthy subjects (or that the cerebral responses could not be enhanced because they were already at ceiling). This finding could be due to the nature of our experimental material. Indeed [239] have shown functional connectivity differences (in the default mode network and between auditory brain areas and the hippocampus) between two music materials that strongly differ in terms of emotion, i.e., preferred and disliked music (in healthy participants). It is thus possible, that our control condition, which can be considered as rather neutral, was not disliked enough to warrant significant differences in functional connectivity with the preferred music condition.

6.3 Effect of music in DOC patients

Next, we analysed the data of our small group of patients. Seed-based analysis indicated that patients showed strongly limited functional correlations with the primary auditory cortices: activation was only observed around the seed areas and no long distance connectivity emerged within the auditory network (figure 6.2). This finding is in line with previous research showing a linear decrease in functional connectivity ranging from healthy participants to unresponsive patients [47, 48, 182]. In fact, many studies have shown that functional connectivity still exists in DOC patients, and other forms of decreased levels of consciousness [41]. Low-level activations in primary auditory cortices, without top-down feedback have also been observed in unresponsive patients [191, 198]. In fact, patients seem to have a general disconnection between brain regions, notably missing long range connectivity [175]. Our results are congruent with this observation as we observe mainly functional connectivity in the hemisphere of the seed. Furthermore, significant differences in the right precentral gyrus are observed during the preferred music condition compared to the control condition (figure 6.2). This finding is in agreement with a previous study investigating DOC patients and reporting activation in the right superior temporal gyrus during three 10-second blocks of musical stimulation based on a famous song [240].

The three network analyses further revealed significant differences in the auditory network and external network, but not the default mode network, during the music condition (figure 6.3). Patients showed a severely limited auditory network of functional connectivity during both conditions. During the control condition, activation was only seen in bilateral temporal areas. During the music condition, the auditory network was restricted to bilateral temporal gyri (only left including Heschls gyrus) and small clusters in the right inferior frontal gyrus and the left supramarginal gyrus, areas included in the temporal cluster for the healthy subjects. The right inferior frontal gyrus is implicated in auditory memory as well as the processing of musical syntactic-like structures [241–247]. When music was compared to the control condition, patients auditory network showed significantly more functional connectivity with the left precentral gyrus (Note that the seed-based analysis also revealed significant increased functional enhancement in the right precentral gyrus during music; see figure 6.3) and the left frontal pole. The precentral cluster overlaps with regions of the auditory network in healthy subjects. The lateral prefrontal cortex has also been linked to autobiographical memory [248, 249], and has also been implicated in rhythm perception [250]. The finding

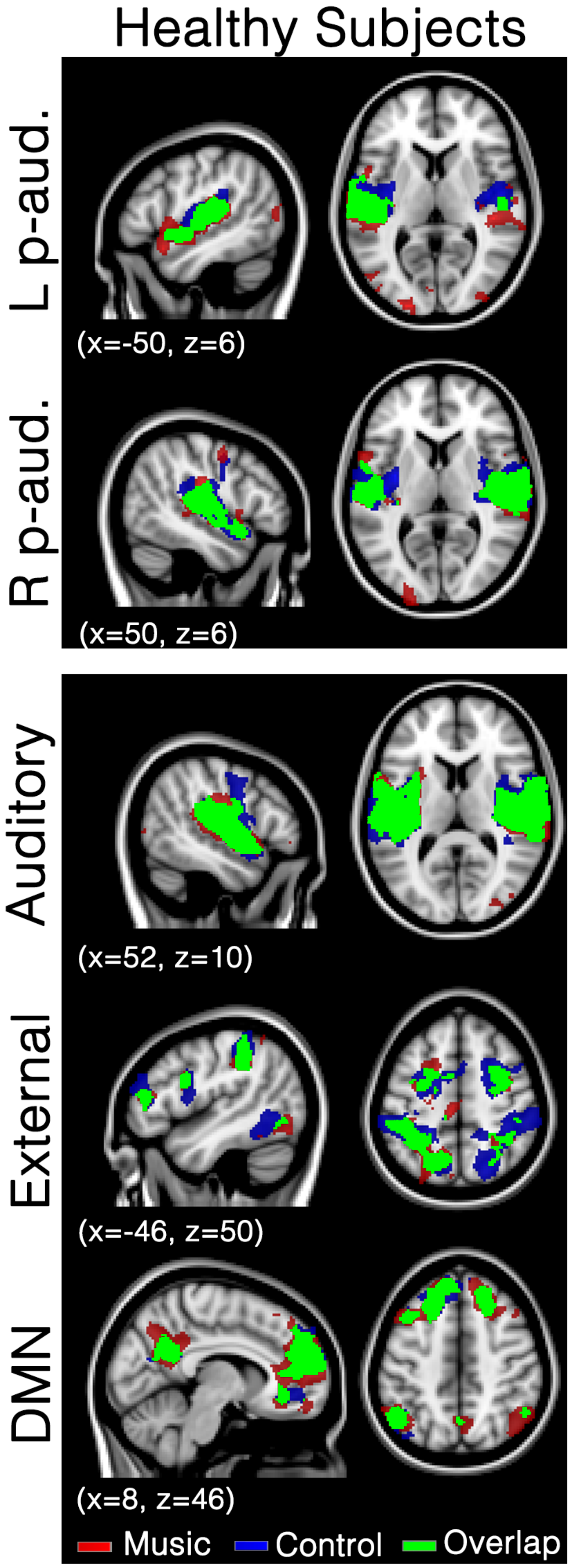

Figure 6.1 – Functional connectivity in healthy subjects during the music and control condition. Maps indicate healthy subjects (N=8) functional connectivity during favorite music exposure (Red) and the control condition (Blue), and regions where functional connectivity was present in both conditions (Green). The top two panels show seed-based analyses, the lower three panels show mean network connectivity. Note that there is no significant difference between music and control condition. Results were analyzed in a network-based manner and thresholded with a family-wise error corrected extended cluster level of $p<0.05$. Standardized MNI T1 2x2x2 template was used to render results. (x,y,z) value indicates MNI coordinates of represented sections.

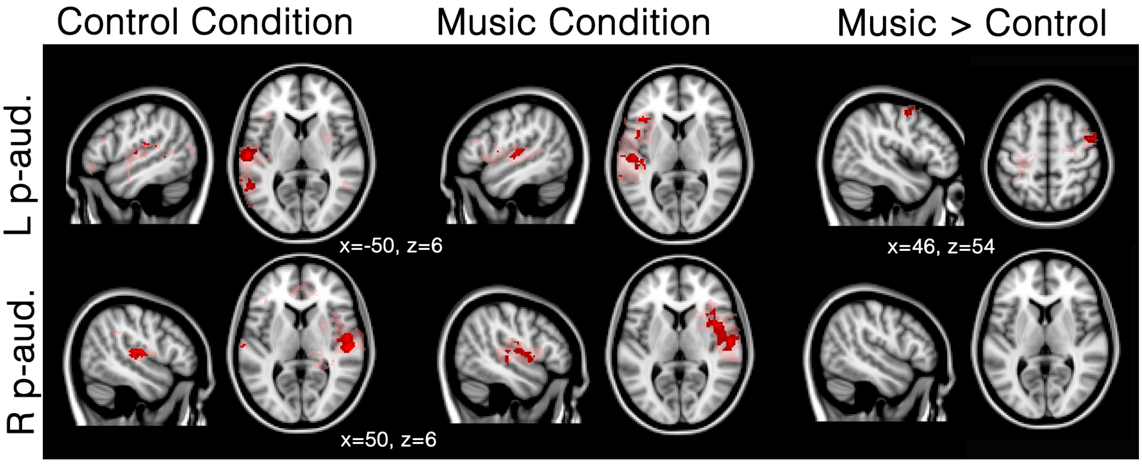

Figure 6.2 – Functional connectivity in patients using primary auditory seeds. Red/pink maps indicate patients (N=5) functional connectivity during the control condition (left) and favorite music exposure (middle) for both the left and right primary auditory cortex (L p-aud., and R p-aud.; respectively). Right maps show the regions that show significantly more functional connectivity during music condition compared to the control condition. Results were analyzed in a network-based manner and thresholded with a family-wise error corrected extended cluster level of $p<0.05$ (in red). For visualization a lowered threshold is indicated in pink (0.01 uncorrected height with family-wise error corrected extended cluster level of $p<0.05$). Standardized MNI T1 2x2x2 template was used to render results. (x,y,z) value indicates MNI coordinates of represented sections.

of increased functional connectivity in music compared to the control condition suggests that music has an effect on the auditory-related network in DOC patients, in whom short-term functional plasticity might appear following the lesions. In patients, the external network observed during the control condition was restricted to clusters of functional connectivity in inferior parietal sulcus and lobule, dorsolateral, middle frontal and supramarginal areas (figure 6.3). In the music condition, the external network showed besides these regions also connectivity with the region MT and parts of the frontal eye field. When directly compared to the control condition, the music condition showed more functional connectivity with the supramarginal/angular gyrus. This cluster overlaps with the supramarginal regions activated during spatial orienting in healthy subjects [251]. Interestingly, this region overlaps with disconnected areas in UWS patients [198]. Laureys et al., (2000) proposed that a lack of integration between primary regions (that activate after simple auditory stimulations in UWS), and higher order regions like the temporoparietal junction and superior temporal gyri (activated in MCS after simple auditory stimuli; [199]) makes conscious processing unlikely [198, 199]. Put differently, unconsciousness might be related to a disruption in feedback processing to the auditory regions [191].

Concluding, the effect of music on functional cerebral connectivity is reminiscent of previous findings which have shown effects of music in brain-damaged patients [218, 219, 234, 235]. For example, a recent EEG study investigating DOC patients has shown that the patients cerebral responses following the presentation of ones own name were increased after having been exposed to their preferred music [235]. A Mood and Arousal hypothesis, attributes the beneficial effects of music on cognition to an increase in mood and arousal [252, 253]. Within this hypothesis, the effects of music in DOC patients might be due to an overall cortical arousal in the cerebral structures that have been reported to be involved in emotional and mood states. A second hypothesis attributes the effect of music to autobiographical priming [235]. Interestingly, in the present study, an increased functional connectivity during the music condition (vs. the control condition) was shown in cortical structures linked to music perception, autobiographical memory and consciousness for DOC patients.

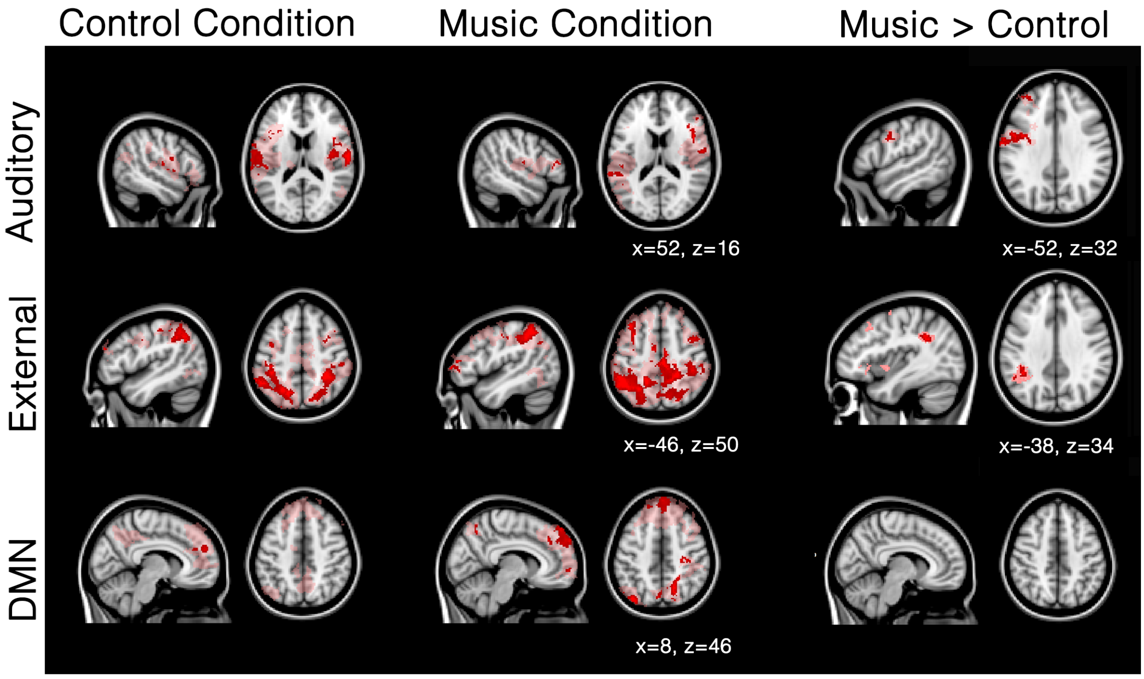

Figure 6.3 – Functional connectivity in patients using mean network connectivity. Red/pink maps indicate patients (N=5) functional connectivity during the control condition (left) and favorite music exposure (middle) for the auditory network, external network, and default mode network (DMN). Right maps show the regions that show significantly more functional connectivity during music condition compared to the control condition. Results were thresholded with a family-wise error corrected extended cluster level of $p<0.05$ (in red). For visualization a lowered threshold is indicated in pink (0.01 uncorrected height with family-wise error corrected extended cluster level of $p<0.05$). Standardized MNI T1 2x2x2 template was used to render results. (x,y,z) value indicates MNI coordinates of represented sections.

Chapter 7

Effect of preference or sound

Based on:

Effects of preference and sensory modality on behavioral reactions in patients with disorders of consciousness
Heine L, Tillmann B, Hauet M, Juliat A, Dubois A, Laureys S, Kandel M, Plailly J, Luauté J, Perrin F.
under review

Not only do the results of chapter 6 need to be confirmed in an extended group of patients, but the study also cannot disentangle the general effect of music (because of its acoustic and structural features) from its autobiographical effects (because of its emotional and meaningful contents in relation to the patients personal memory). Neither is there a clear consensus if this possible effect of music is translated to behavioural reactions (i.e., if sound is able to elicit higher scores on the most sensitive tool for diagnosis, the CRS-R).

To try to answer these questions, we aimed to test the potential beneficial context effects of preference (preferred vs. neutral) and of sensory modality (auditory, olfactory) on the performance to the CRS-R. Therefore, in 13 patients (7 MCS; 6 UWS), four stimulations were used as a testing context; preferred music, neutral music, preferred odor, and neutral odor. Which were followed by one of four items from the CRS-R (visual pursuit using a mirror, auditory localization of the own name, and two movements to command). Six patients were in an UWS during time of assessment (3 females, mean age=52, SD=11 years), and seven patients in a MCS (1 female, mean age=37 SD= 10 years) according to internationally established criteria [6, 8].

For each patient, four testing sessions were performed (separated by 3 to 7 days). Each session consisted of 4 trials, each including a 5-minute presentation of one of the four stimuli followed by one of the four CRS-R items. Each of the 16 stimulation-item combinations was presented once to each patient. Order of stimuli and items were randomized within- and between patients (figure 7.1).

Stimuli generation

For the selection of preferred sounds, patients legal guardian and/or loved ones were asked to fill in a questionnaire concerning patients preferred songs/pieces of music, preferred artists and preferred music styles. Six dynamic, musically coherent, and representative pieces of music were chosen. For the selection of preferred odors, patients legal guardian and/or loved ones were asked to indicate patients preferences on a list with 51 pre-defined essential-oils (herbs/flowers, fruits, foods, candy, drinks), of which 6 were chosen. These essential-oils were pre-testes by six people on how easily identifiable they are on a 0-3 scale, only odors with a score above 1,8 were used.

Six neutral sound excerpts were created. They were continuous music-like noise stimuli, i.e., six music (well-known songs of the genre classical, rap, rock, reggae, French variety, and pop) for which the overall phase spectrum was randomized the slow temporal envelope was deleted. Thus, they consisted of a spectral approximation of music, but did not share other acoustic characteristics (e.g., pitch, rhythm, envelope, or timbre). Six neutral odors consisted of artificially created smells that do not exist in nature (i.e., Citronellol, Rose oxide, Methyl

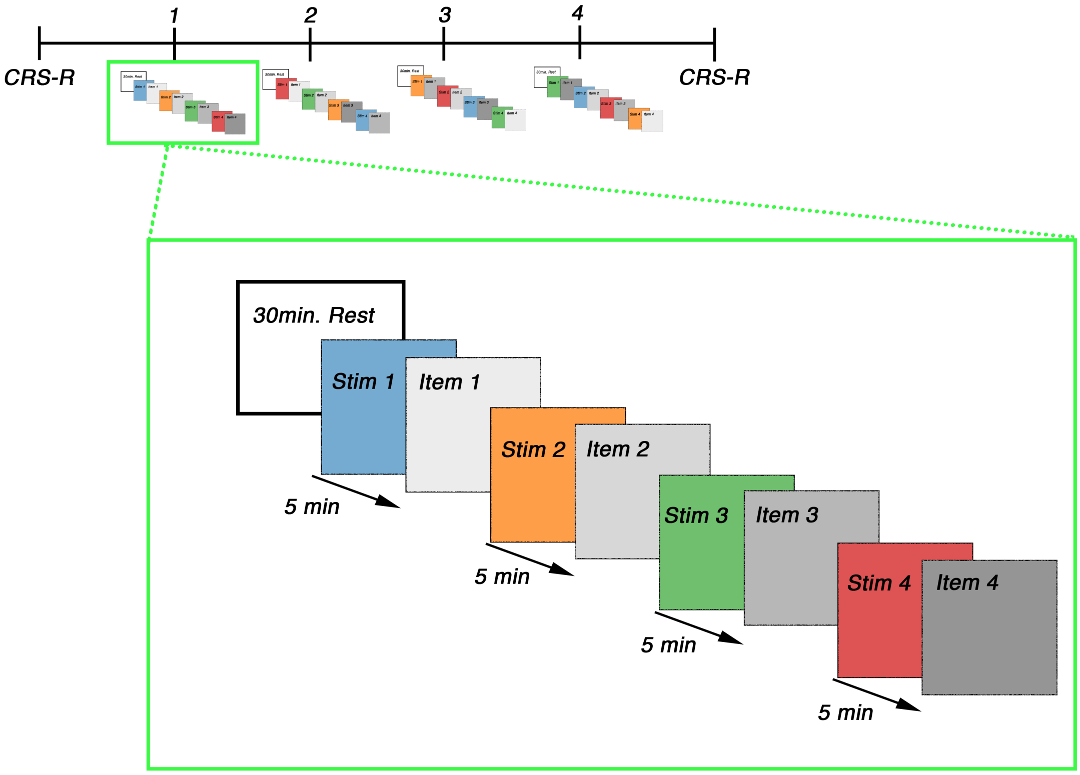

Figure 7.1 – Schematical representation of the behavioral protocol. For each patient, four testing sessions were performed (separated by 3 to 7 days). Each session consisted of 4 trials, each including a 5-minute presentation of one of the four stimuli followed by one of the four CRS-R items. Each of the 16 stimulation-item combinations was presented once to each patient. Order of stimuli and items were randomized within- and between patients.

octine carbonate, Ethyl acetyl acetate, Linalyl acetate, Cis-3-Hexenyl salicylate). The same sound and odor neutral stimuli were used for all patients.

Each stimulation had a duration of 5 minutes. For this, 3 musical experts were combined, and 3 odors were presented 3 times for duration of 30 seconds without direct repetition. The order within each stimulation was randomized to avoid habituation effects. Every musical excerpt and each odor was used during only two out of four sessions in the experimental protocol, and a session never consisted of a group of stimuli that were presented together before. The time between each stimulation and item was on average 10 seconds, and time from the end of one item until the start of a new stimulation was 30 seconds. Every session was preceded by 30 minutes silence (patient rested in his/her room without presence of others or sound/visual stimuli). The whole CRS-R was administered in the 48 hours before the first and after the last assessment.

Behavioral scoring

The behavior of the patient was filmed for subsequent quantitative and qualitative scoring, and scores were assigned during the experiment itself. For each item, the videos were blind-scored, that is without knowledge of the preceding stimulation, by three experimenters. Quantitative responses to the CRS-R item were scored and standardized by translating them into percentages of maximum score (i.e., 8 for visual pursuit, and 4 for localization to sound and response to command). Qualitative scoring was performed in order to acknowledge clinical signs of awareness that occurred during, as well as after the stimulation. A score of 2 was given when signs of consciousness were present [8] (i.e., voluntary participation, orientation, emotional reactions, intelligible speech, or automatic movements only observed during stimulation/item), while 1 indicated reactions which were not distinguishable from unintentional behaviors/reflexes [5] (i.e., short orientation, partial participation, not the requested behavior, agitation/grimaces. A score of 0 was awarded when no reaction was observed.

7.1 Effects of sensory stimulation on behavioral responsiveness

Quantitative and qualitative scores were used to assess potential effects of preferred and neutral contexts presented in the auditory and olfactory modalities on the scores of DOC patients for items of the CRS-R. Both scores showed that sound stimuli triggered higher responsiveness compared to olfactory stimuli. Qualitative scores also showed a main effect of preference (with better scores for preferred stimuli than neutral ones). In addition, qualitative scores revealed that scores were higher after preferred sounds than all other stimuli (figure 7.2, table 7.1).

Thus, this study establishes a hierarchy among the different types of stimuli, placing preferred music on top. This in in line with several studies reporting improved cognitive functioning after preferred music [40, 222, 233, 235, 254]. Systematical assessment using for instance the preferred music might thus be advised. Indeed, two UWS patients showed emotional and behavioural reactions to autobiographical/emotional stimulations (one of the criteria for diagnosis of MCS [8]); behaviours that were not observed during routine CRS-R assessments. One patient showed tears (during the stimulation period in two assessments) and was purposeful uncooperative (i.e., eyes firmly closed with head-averting), the other patient showed behavioral responses to the same response to command item only after preferred stimuli. This suggests that diagnostic assessment might be improved through the elicitation of meaningful (affective) behaviours due to testing context, which is crucial as misdiagnoses have consequences on treatment and end-of-life decisions.

No difference could be observed between scores obtained after preferred and neutral odors. These results suggest that auditory stimuli (and in particular the preferred music) are better than olfactory stimuli at enhancing cognition or arousal in these patients. However, this interpretation must be considered with caution; (1) Preferred auditory sound contained more changes (i.e., tone, rhythm, intensity), while olfactory stimulations had the same intensity and switched more gradual. (2) The preferred auditory stimuli could be sampled from patients preference while preferred olfactory stimuli were limited. Thus, the reminiscence power of autobiographical-memory might be higher for the preferred music. (3) Although the difference between preferred and neutral olfactory stimuli indicate that patients were not anosmic [255], we cannot exclude that there might be reduced olfactory abilities.

Although, this was not the main purpose of the study, it should be noted that the different types of stimulations used in this study did not modify the level of awareness on the long-term. Indeed, no difference was observed between pre- and post CRS-R.

In conclusion, the results showed that auditory stimuli triggered higher responsiveness compared to olfactory stimuli, as well as an effect of preference with better scores for preferred stimuli compared to neutral ones. This result pattern suggests that improving the testing context helps the expression of residual functions, and thus possibly improves diagnostic assessment.

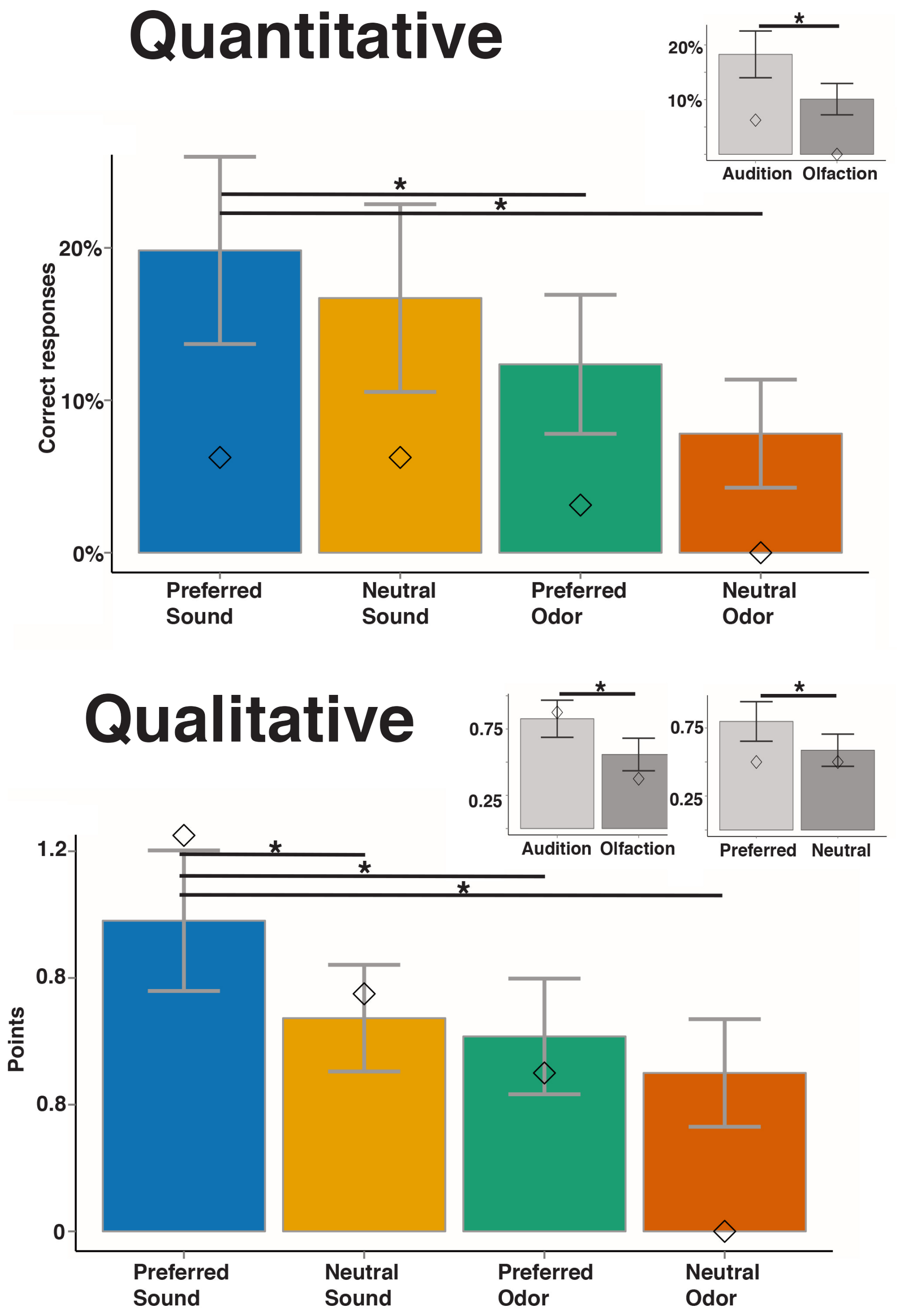

Figure 7.2 – Quantitative and qualitative scores. Left: Bar graphs represent mean standardized quantitative score on the CRS-R items for each stimulation. Right: Bar graphs represent mean qualitative score for each stimulation-item combination. Smaller figures in the top right show significant main effects of modality (auditory>olfactory) in both graphs, and a main effect of preference (preferred>neutral) in the qualitative scores. Lines and starts significant differences between stimulations. Error bars represent standard error; diamonds indicate median.

Table 7.1 – Results quantitative and qualitative analysis

	Quantitative	Qualitative
Friedmans ANOVA	X2(3) = 10.48, p=0.015	X2(3) = 13.73, p=0.003
	Wilcoxon signed rank tests	
Main effect of modality: auditory vs. olfactory	Music: 18,5% Olfactory: 10% p=0.01; r=-0.35	Music: 0.83 Olfactory: 0.56 p=0.006; r=-0.39
Main effect of preference: preferred vs. neutral	Preference: 17% Neutral: 12,5% p=0.07; r=-0.24 -> NS	Preference: 0.8 Neutral: 0.58 p=0.005; r=-0.38
Preferred music vs. preferred odor	Preferred music: 20% Preferred odor:12% p=0.035; r=-0.41	Preferred music: 0.98 Preferred odor: 0.62 p=0.014; r=-0.48
Preferred music vs. neutral music	Preferred music:20% Preferred odor:17% p=0.36; r=-0.18 -> NS	Preferred music: 0.98 Preferred odor: 0.67 p=0.024; r=-0.44
Preferred music vs. neutral odor	Preferred music:20% Preferred odor:8% p=0.011; r=-0.50	Preferred music: 0.98 Preferred odor: 0.5 p=0.006; r=-0.53

Conclusions and future perspectives part II

In this part we reported the results of three studies in which we looked at the effect of sensory stimulation. In chapter 5 we showed that various stimuli without personal relevance olfactory, gustatory, and tactile modalities do not increase the presence of signs of consciousness when added to standardized tests, such as the CRS-R. In chapter 6 we demonstrated that preferred stimuli, in this case tested with passive music listening, may alter functional connectivity. More precisely, we found that functional connectivity is stronger in music compared to the noise condition in regions of the auditory network that might be linked to autobiographical memory. In the final study (chapter 7) we showed that neural effects can be translated to increased behavioral responsiveness. We showed an effect of auditory stimulation and preference (i.e., preferred music) on patients behaviour, which may aid in the evaluation of DOC patients. Taken together these studies reinforce the notion that incorporating self-referential stimuli into the behavioural testing context could improve the expression of residual functions in patients, and increase the sensitivity of diagnostic assessments.

Future research should, in the behavioural domain, explore the potential cumulative effects of general and preferable stimuli. Although interesting, we were in chapter 7 (the study on behavioral responsiveness after auditory and olfactory stimuli), unable to compare the pre- and post CRS-R to the assessments in our experimental protocol. Indeed, CRS-R assessments include arousal protocols, nociceptive stimulations, and tactile as well as verbal stimulations to awaken the patient, none of which were present in the four experimental tests. Future studies testing the cumulative effects of general and preferable stimuli should also test for differences in patients responsiveness induced by nociception compared to preferred stimuli. If preferred stimuli (e.g., preferred music) were more effective, this would allow the avoidance of painful stimulations during repeated assessments.

It should be noted that all three studies assessed immediate effects of the sensory stimulation, and no inferences can be made about long term effects. These studies can therefore not be used as indicators for the effectiveness of sensory stimulation programs. These sensory stimulation programs mainly consist of presenting different types of environmental stimuli to the patient in order to optimize her/his levels of arousal and awareness. These multimodal sensory stimulation programs are supposed to constitute enriched environments which are assumed to improve brain plasticity, and accelerate the recovery from coma [256]. These programs have been tested before with unproven efficacy. For example, several studies claim an effect of multisensory stimulation on patients with DOC [208, 256]. Familiar stimuli might elicit even greater range of behavioral responses [257]. However, other studies could not support any beneficial effects of multisensory stimulation for severely brain injured patients (for reviews, see [222, 223, 258, 259]). The main problem is that none of these studies are able to differentiate spontaneous recovery from recovery due to treatment.

The results in this chapter instead imply that preferred sensory stimulation might have an immediate and short effect on behavioral responsiveness. It could thus be envisaged that if these stimuli are used in the context of diagnostic assessment of the highly fluctuating MCS, the likelihood that the patient is assessed at their best moment and thus able to show conscious behaviors is increased.

Part III

Functional connectivity of the senses

Introduction

The methods used in this thesis in relation to DOC patients can also be used to assess many very different pathologies and healthy brain functioning. For example, functional MRI can be used to assess the effect of senses. More specifically, vision is an important sense in daily lives, and learning how the loss of such a sense could be informative on the presence or absence of vision in brain damaged patients might have implications in patient assessment. The assessment of healthy conscious subjects without this sense could eventually lead to indications of (the absence of) vision in DOC patients. We therefore assessed functional connectivity in healthy but blind subjects.

Chapter 8

Congenitally blind

Based on:

Functional connectivity in visual, somatosensory, and language areas in congenital blindness
Heine L, Bahri A M, Cavaliere C, Soddu A, Reislev N, Laureys S, Ptito M, Kupers R.
Frontiers in Neuroanatomy, 2015, 138 (pt9):2619-31

The loss of vision from birth causes a myriad of compensatory plastic changes. At the behavioral level, congenitally blind subjects outperform their sighted counterparts in a wide range of non-visual sensory discrimination tasks ([260] for a recent review). For example, congenitally blind individuals show improved performance in tactile acuity at the finger tips [261] and perform better in pitch discrimination [262], syllable recognition [263] and sound localization [264]. Recent behavioral studies also indicate superior abilities in discrimination, identification and awareness of odors [265–267]. Compensatory plasticity is dependent on cross-modal reorganization of the brain in which the occipital cortex becomes recruited by various non-visual inputs [260]. Brain imaging studies have highlighted the pivotal role of the visual cortex in the ability of the blind to perform non-visual tasks [268]. Indeed, PET and fMRI studies have reported that congenitally blind individuals recruit their occipital cortex in tasks involving sound and tactile localization [269, 270], tactile and auditory motion detection [271–273], spatial navigation [274], odor perception [268], language [275–278] and memory processing [279].

Recent neuro-imaging studies also helped to illuminate the question how congenital blindness affects the structural organization of the brain, and through which pathways non-visual information reaches the occipital cortex. Structural brain imaging studies seem to concur that there are significant reductions in grey matter throughout the whole extent of the visual system. These include the optic chiasm, the lateral geniculate nucleus, the posterior pulvinar, and striate and extra-striate visual areas [280–282]. Regions of the ventral visual stream such as the inferior temporal gyrus and the lateral orbital cortex, as well as regions connected to the dorsal visual stream like the hippocampus also show volumetric reductions [283, 284]. In addition, cortical thickness is increased in the cuneus [268, 283], which is likely due to a reduction in cortical pruning during the early maturation process as a result of lack of visual input, and which may be indicative of alterations in connectivity. White matter changes in the visual pathways include atrophy of the optic tracts and the optic chiasm, reductions of the optic radiations, the splenium of the corpus callosum [280, 281, 285, 286] and microstructural changes within the ventral visual pathways [281].

Recent studies have also tried to elucidate functional changes in the blind brain. Brain activation studies [260, 273, 287–290] and transcranial magnetic stimulation (TMS) studies [291, 292] have found evidence for increased functional connectivity of the occipital cortex with auditory and somatosensory areas. Several of the available resting state studies reported stronger connections of the occipital cortex with somatosensory [293] and language areas [278, 294–296]. Other studies, however, concluded that the occipital cortex of the blind has a general reduced connectivity with somatosensory/auditory regions [297, 298], or even larger parts of the brain [296, 299]. Some of these differences may be due to small or inhomogeneous study populations, including both congenital and early blind subjects or subjects with and without residual light perception [294, 295, 300], or to the fact that the resting state scan was acquired after an active functional scanning paradigm [278].

To circumvent these issues, we analyzed resting state functional magnetic resonance imaging (rsfMRI) data of a homogeneous group of congenitally blind individuals lacking any residual light perception, using a priori defined regions of interest (ROIs) in areas with known roles in visual, somatosensory, auditory and language processing (figure 8.1). included twelve congenitally blind (CB; 5 females, 7 males; age: mean age=42, SD=14 years) and twenty healthy sighted controls (SC; 12 females, 8 males; mean age=42, SD=14 years). Using resting state functional MRI, we mapped out increases as well as decreases in functional connectivity in the congenitally blind brain, as compared to sighted controls.

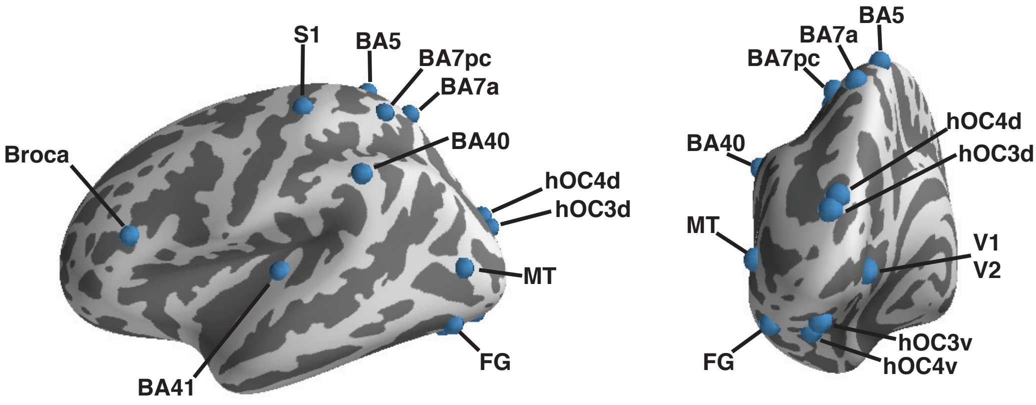

Figure 8.1 – A priori defined regions of interest. Regions of interest are shown on the left hemisphere of an inflated brain using PySurfer. Dark areas represent sulci, light gray areas gyri.

Functional connectivity changes in congenitally blind

Although our data revealed a mixture of increases and decreases in functional connectivity in the blind brain, the increases strongly prevailed. The most striking findings of this study were the increases in functional connectivity in the congenital blind brain within the ventral and dorsal visual streams, and between visual cortical regions and Brocas area. In sharp contrast, functional connectivity between dorsal and ventral visual areas was reduced. Results described are represented in figure 8.2, and figure 8.3.

Increased functional connectivity within the visual streams

Our data show evidence of increased functional connectivity in the ventral visual stream in congenitally blind subjects, more specifically between ventral stream areas hOC3v and fusiform gyrus and the inferior temporal gyrus (BA20). The ventral stream consists of a complex recurrent network between visual areas V1-V4 and the inferior temporal cortex [301]. In sighted subjects, this pathway is implicated in the processing of object quality, object representation or object category [301]. These processes are necessary for object and scene comprehension that form the contents of visual awareness. The fact that this pathway is preserved in blind subjects adds new evidence to the notion that the ventral visual stream holds representations of object shape which are supramodal in nature, and not necessarily visual [302]. For example, non-visual recruitment of the ventral temporal cortex was seen after haptic [303], non-haptic [304] and auditory [305] exploration of objects in congenital blind subjects.

Congenitally blind subjects also showed increased functional connectivity in the dorsal visual stream, more specifically between BA40 and the secondary visual cortex (V2), as well as between somatosensory areas (BA7pc) and BA40. In normal sighted individuals, the dorsal visual stream is heavily implicated in the visual guidance of action, and consists of a set of projections from the visual cortex to the superior parietal lobule. From there, the dorsal stream splits into the parieto-prefrontal, parieto-medial temporal and parieto-premotor pathway [306]. The parieto-prefrontal pathway connects the parietal cortex to prefrontal regions (e.g., BA46) and is important in top-down control of eye movements and spatial working memory. The parieto-medial temporal pathway, connecting to the posterior cingulate cortex via parahippocampal substructures, is implicated in spatial navigation. Finally, the parieto-premotor pathway connects to premotor regions and is involved in visually-guided actions such as reaching and grasping [306]. Our finding of increased functional connectivity between BA40 and V2, as well as between BA7pc and BA40, are indicative of a fast pathway for information processing from higher order somatosensory to lower level visual areas. This conjecture is in line with results of a recent MEG study indicating that somatosensory information reaches the occipital cortex in the blind via somatosensory and posterior parietal areas [287], and with results of functional activation studies showing occipital cortex activation following somatosensory stimulation in blind individuals [288]. Finally, applying TMS over the occipital cortex can induce tactile sensations in blind subjects trained in the use of a tactile sensory substitution device or in Braille reading [281, 292].

Our results of increased functional connectivity within both the dorsal and ventral visual streams in congenitally blind subjects are in line with results of a recent functional connectivity density mapping study [299]. Functional activation studies also support the finding of increased connectivity within the dorsal and ventral streams in the blind brain [302]. For instance, congenitally blind subjects trained in the use of the tongue display unit (TDU) showed stronger connectivity between the cuneus and areas within the dorsal and ventral streams [288]. In addition, a dynamic causal modelling study showed that the activation of the occipital cortex in blind individuals during an auditory discrimination task is mediated via enhanced corticocortical connections from the auditory to the occipital cortex [289]. These functional changes are probably due to anatomical reorganization of the pathways that funnel non-visual information to the visual cortex of the blind [302]. Thus, our rsfMRI data of increased connectivity in the visual streams are supported by results of various functional activation studies showing that the visual streams of the congenitally blind undergo compensatory plasticity and are able to process non-visual information in conjunction with the visual cortex [260, 307].

Decreased connectivity between the ventral and dorsal visual stream

In sharp contrast with the increase in functional connectivity within the visual streams, our data revealed decreases in connectivity between the two streams in blind participants. Connectivity of ventral areas hOC3v, hOC4v and fusiform gyrus with dorsal stream area hMT+ was decreased, as well as that between BA40 and the inferior temporal cortex (BA21). There is growing evidence that the dorsal and ventral streams are less independent than originally thought [308]. Although these streams have clear independent functional roles, there is functional and structural evidence that they do not function in an independent manner [308–312]. Our data suggest that in the congenitally blind brain the two streams are less interconnected than in the sighted brain. We hypothesize that this may be due to increases in functional connectivity within the two streams. An alternative explanation is that cross-modal non-visual sensory information processing in extrastriate cortex reduces the need for functional connectivity between the streams.

Connectivity of the primary visual cortex

We did not find evidence for changes in connectivity in primary visual cortex (V1 and V2). This is in agreement with several other functional connectivity studies [278, 293, 295, 298]. A recent study reported decreased functional connectivity density only in primary visual areas of late blind subjects, while congenitally blind showed increased connectivity between lower tier visual areas and somatosensory areas [299], overlapping with the small cluster of increased functional connectivity between BA40 and the primary visual areas observed in this study. However, the literature on changes in functional connectivity of primary visual areas in blind individuals is incongruent. Thus, several fMRI studies reported a correlation between damage to the optic radiation and an event-related fMRI response in visual areas [313], or decreased functional connectivity of primary visual areas with the rest of the brain [294, 296, 297, 314]. These results were explained by the general loss hypothesis. However, this proposed mechanism cannot explain the ubiquitous role of the primary visual cortex in non-visual perceptual and cognitive tasks [268, 270, 272–274, 276, 278, 288, 293, 315–320]. Nor can it explain enhanced effective connectivity with other regions [288, 289, 291]. Furthermore, a recent review on structural changes as measured with diffusion concluded that although the literature is inconsistent, it suggest that neither strength nor macro-scale topographic organisation is changed in blind individuals [321]. This is congruent with new research showing that functional connectivity based topographic organization of the visual cortices is indistinguishable from sighted controls, and increased functional connectivity to frontal and posterior temporal areas [322].

Visual cortex and language processing

Brocas area (BAs 44 & 45) was the cortical area with the largest amount of alterations in functional connectivity in congenitally blind participants. A total of five visual seeds, hOC3d, hOC3v, hOC4v, hMT+ and fusiform gyrus, showed increased functional connectivity with this area. In addition, Brocas area also showed stronger connectivity with ventral visual areas hOC3v, hOC4v, and with medial prefrontal cortical area BA 10. The current consensus is that the occipital cortex of blind individuals is involved in language processing, showing similar properties as classical language related areas [278]. Braille reading in blind subjects activates an extensive network of brain areas, including posterior and medial occipital areas, fusiform gyrus, area hMT+, inferior temporal gyrus, inferior frontal, prefrontal, intraparietal sulcus, and somatosensory motor areas [323]. More specifically, the increased functional connectivity between visual areas and Brocas area in congenitally blind individuals might relate to the role of the occipital cortex in semantic processing. Whereas semantic processing activates the inferior frontal cortex in both sighted and blind subjects, it activates additionally visual cortical areas in the latter group [276, 278, 293, 317, 324]. These results expand earlier findings of increased connectivity of the occipital cortex with Brocas area in congenital blindness [278, 293–296, 298, 325]. The co-activation with Brocas area extends to most of the occipital cortex [276, 325], and might next to language also functionally correlate to working memory [325]. These results also relate to findings of increased white matter volume within the tracts between prefrontal and occipital areas. More specifically in the fronto-occipital fasciculi [281, 321].

The increased functional connectivity between Brocas area and hMT+ might be explained by the role of tactile flow processing in Braille reading [326]. All our congenitally blind were

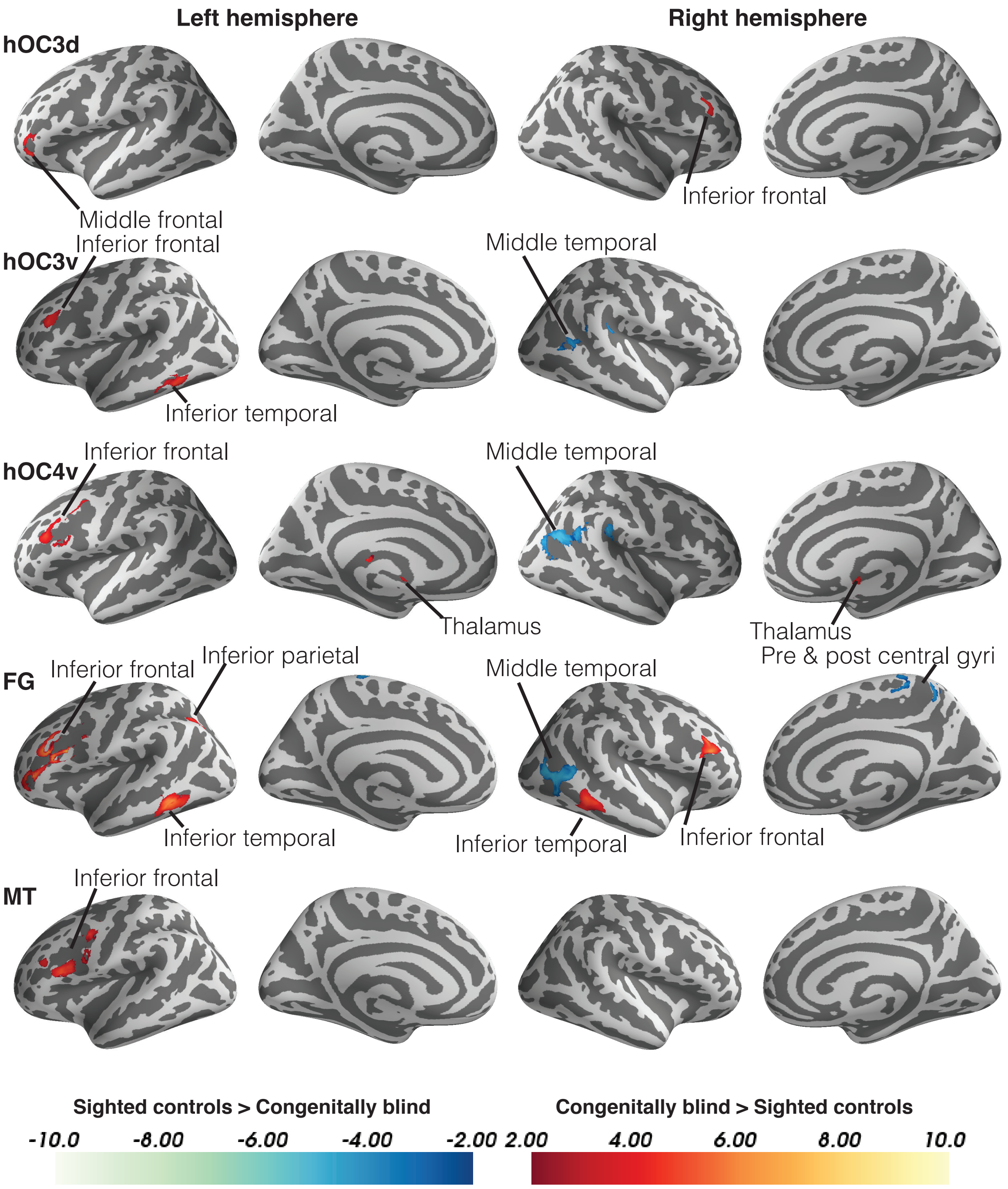

Figure 8.2 – Differences in resting state functional connectivity between blind and sighted controls (visual ROIs). Increases in functional connectivity in the blind group are indicated in red, whereas decreases in functional connectivity compared to controls are shown in blue. Cluster-level FWE-corrected p<0.05. Scale bars indicate Z-values. Abbreviations: CB = congenitally blind; SC = sighted controls.

reading Braille from early age (see Table 1 for speed of Braille reading). Burton et al., (2002 [323]) showed that area hMT+ is linked to Braille reading only in early bind subjects. The role of the occipital cortex in language processing is further supported by studies showing that rTMS over the mid-occipital cortex not only reduces accuracy of verb-generation [317], but also impairs Braille reading performance [327]. Finally, it is worth mentioning that a bilateral occipital stroke in an early blind patient resulted in the loss of Braille reading skills [328]. Future studies should hence more focus on the relationship between Braille reading and functional connectivity of Brocas area with hMT+ and with other brain areas in congenitally and late blind subjects. In line with previous results [278], congenitally blind subjects also showed increased functional connectivity between occipital area hOC4v and the thalamus. This finding suggests a thalamo-cortical implication in language processing in the congenitally blind, a conjecture that is supported by the observation that stimulation of left thalamic regions produces language deficits in blind subjects [329]. Our data also revealed a decrease in functional connectivity between Brocas area and its homologue in the right hemisphere. In sighted but not in congenitally blind individuals, the right inferior frontal area is also activated during language tasks [330]. Blind subjects might use the visually deprived occipital cortex instead because it is more cost-effective.

Somatosensory areas

Our results indicate increased functional connectivity between the supramarginal gyrus (BA40) and secondary visual cortex and area hMT+, and between SI and BA40. As stated above, the supramarginal gyrus, occipital, middle temporal and somatosensory cortices are activated by Braille reading [330–332]. We explain the co-activation of somatosensory regions by the tactile input of Braille reading. Indeed, tactile stimuli activate inferior and ventral temporal, as well as somatosensory regions in blind individuals [272, 288, 303, 304, 333]. This co-activation of parietal and visual areas may be at the basis of the superior tactile acuity in blind individuals [260], this might also be related to the increases in white matter volume found in somatosensory and motor areas [334]. Other rsfMRI studies have reported a decrease of functional connectivity between visual and somatosensory regions [278, 296, 297, 300]. However, this finding is at odds with results of several other activation studies indicating strong connectivity between these areas. For instance, functional connectivity was shown to be increased between hMT+ and somatosensory areas [273]. Furthermore, a recent MEG study revealed activation of the occipital cortex following median nerve stimulation in congenitally blind individuals [287]. A connectivity analysis further suggested that median nerve stimulation first activated primary somatosensory cortex, then the posterior parietal cortex and finally visual areas V3 and V5 [287]. Using somatosensory-evoked potentials, we reported that tactile

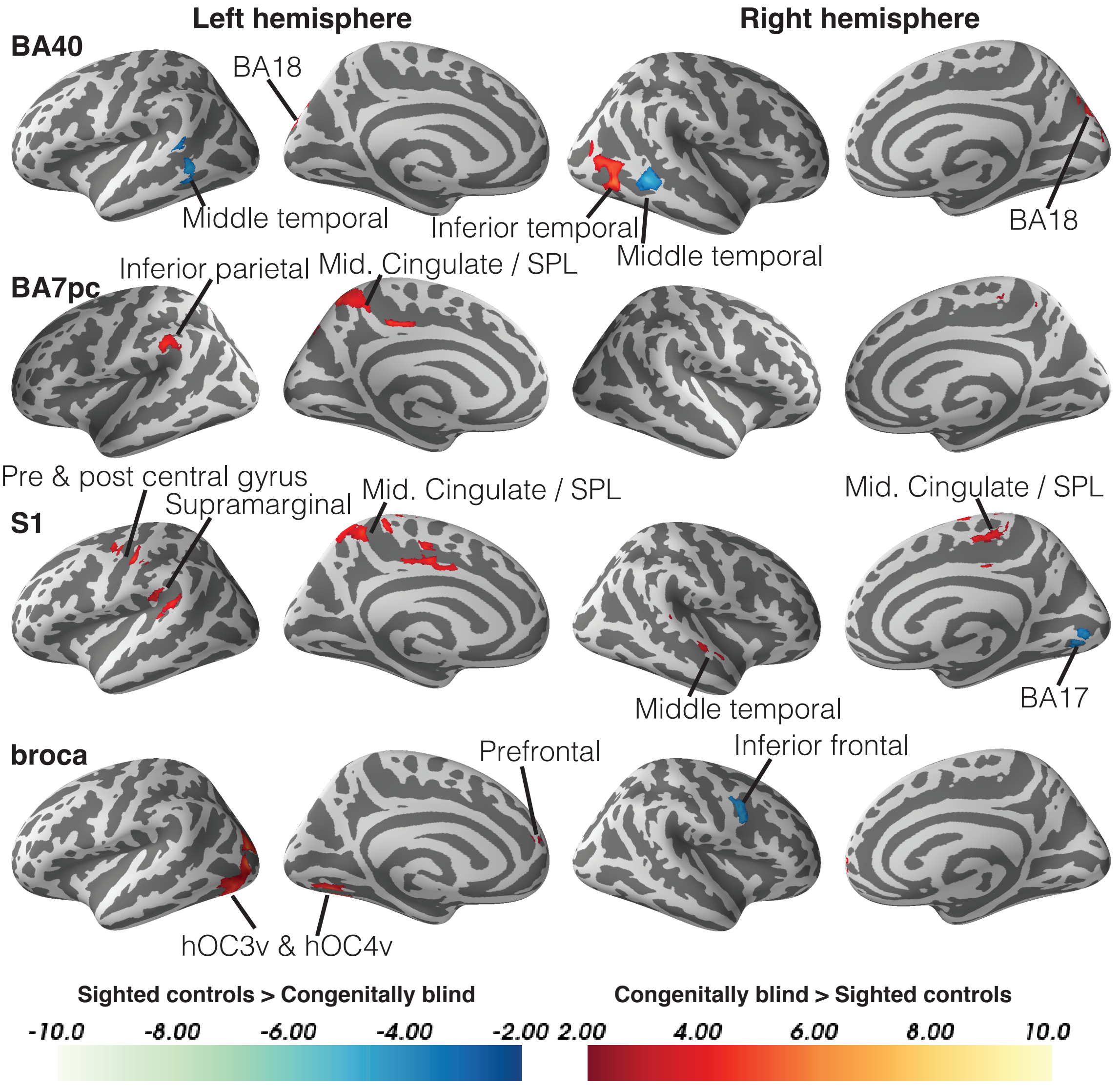

Figure 8.3 – Differences in resting state functional connectivity between blind and sighted controls (somatosensory and language ROIs). Increases in functional connectivity in the blind group are indicated in red, whereas decreases in functional connectivity compared to controls are shown in blue. Cluster-level FWE-corrected p<0.05. Scale bars indicate Z-values. Abbreviations: CB = congenitally blind; SC = sighted controls.

stimulation of the tongue in blind individuals trained in the use of the tongue display unit first activated the somatosensory cortex and then the occipital cortex [292]. Finally, a combined PET-TMS study showed that TMS of the primary somatosensory cortex leads to increased blood flow in the occipital cortex in congenitally blind subjects only [291]. Together, these findings argue in favour of an enhanced parieto-occipital connectivity in congenital blindness which is supported by the present rsfMRI data.

Auditory and motor areas

Although many studies have indicated superior auditory abilities in congenitally blind individuals [260], we did not find significant group differences in functional connectivity of auditory areas. Active tasks have indicated stronger cooperation between the auditory and occipital cortices in congenital blindness [289, 320]. It is possible that in the present study, scanner noise masked a purported increase in resting state functional connectivity between auditory and occipital cortices in blind individuals [335]. We found decreased functional connectivity between the fusiform gyrus and pre- and post-central areas. This is in agreement with several other studies that found decreases between visual areas and motor-related regions, a finding that was explained by the loss of eye-hand coordination in blind subjects [294, 296, 297]. Eye-hand coordination in sighted individuals leads to co-activation of visual and motor areas [336], which is reduced in conditions of congenital blindness.

Methodological considerations

Several rsfMRI studies have explored changes in functional connectivity in the blind brain. The reported results are not very consistent and sometimes even conflicting. These differences in results might be due to spurious samples or protocol bias. For instance, some studies included blind subjects with residual light perception [294, 298, 337], or had a mixture of congenitally blind and late-onset blind participants [295]. Our study cohort was a homogeneous group of congenitally blind participants without any light perception. Furthermore, contrary to some [296, 297], our study used subjects that are not previously used for any analysis, nor was there any active paradigm during the scanning session [278]. Another explanation for the inconsistency between studies relates to differences in used methodologies for assessing functional connectivity in rsfMRI data. Early studies used a more exploratory method with atlas-based regions of interest [293, 296, 299, 337, 338], or one or a few hypothesis-driven ROIs, mostly the primary visual area [297, 300, 337]. In contrast, our investigation focused on small areas that are not present in current atlases. Information about the time course (and therefore its functional correlation) of these small areas could also be missed when the time courses of all voxel in an atlas based area are averaged. Our research focused on brain areas with known functional or structural changes in blind subjects in the visual, somatosensory, auditory and language domain, and seed placement was done according to architectonical studies.

We combined the time-series of homologous areas from both hemispheres. For this reason we are unable to draw any conclusions on purported hemispheric differences in functional connectivity. Further, we excluded increased or decreased correlations in our second level analysis that were caused by anti-correlating time-series in our first level analysis. As with all resting state functional connectivity studies, we are only able to show correlations between different areas, and not any causality.

Conclusions and future perspectives part III

Data reveal increased functional connectivity within both the ventral and the dorsal visual streams in congenitally blind participants. However, connectivity between the two visual streams was reduced in blind subjects. In addition, our data revealed stronger functional connectivity in blind participants between the occipital cortex and areas implicated in language and tactile (Braille) processing such as the inferior frontal gyrus (Brocas area), the thalamus, the supramarginal gyrus and the cerebellum. Our results underscore the extent of cross-modal reorganisation and the supra-modal function of the occipital cortex in congenitally blind individuals.

In relation to DOC patients, all the problems that can influence behavioral assessment are important to quantify objectively. This seems most easy for sensory impairments like blindness, loss of smell, or deafness. Healthy conscious people who are able to transmay their conscious perception are good subjects to start to elucidate the changes in the brains connectivity after such a loss of a sense. There is still a huge work ahead of us before we can state from passive paradigms if a subject can see or hear, but maybe with this work, a start in this direction is made.

Concluding remarks

This thesis explores brain connectivity and sensory stimulation in patients with disorders of consciousness (DOC). These are serious conditions where massive brain damage can lead to a dissociation between arousal and awareness (e.g., UWS and MCS). The work described here explores several available methods for the assessment of consciousness. The main method utilized here is fMRI functional connectivity (Chapter 3, 4, 6, 8), while we also briefly go into PET and structural MRI (Chapter 2), as well as behavioral assessments (Chapter 5, 7). Study specific conclusions and future perspectives can be found in all separate parts of this thesis, Part I on page 51, Part II on page 79, and Part III on page 97.

Two general conclusions can be drawn from this thesis. First, concerning brain connectivity (as explored in Part I) we can conclude that brain function and structure are intimately related to each other, and to consciousness. Limited structural integrity is linked to a decrease in brain function. This decrease in brain function can be used to distinguish between the clinically indicated states of consciousness.

The second message which can be drawn from this work relates to the passive sensory stimulation in Part II. Preferred stimuli may have the power to momentarily enhance brain function, and therefore behavioral responses, to its maximal potential. Using preferred stimuli, such as music, as a testing context might optimize the diagnostic assessments of the fluctuating pattern of minimally conscious patients. The use of preferred stimuli might thus be advised in relation to diagnostic assessment when diagnostic doubts exist.

Appendix A

Paper I

Consciousness and disorders of consciousness

Heine L, Demertzi A, Laureys S, Gosseries O.

in: Brain mapping: An encyclopedic reference, *Elsevier*, 2015

Consciousness: And Disorders of Consciousness

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Glossary

Active paradigm Experimental condition that requires the subject to perform a specific task on request.

Coma Recovery Scale – Revised (CRS-R) Behavioral scale developed to assess the level of consciousness in patients recovering from coma. This scale has been specifically introduced to differentiate patients in VS/UWS from patients in MCS, and it tests auditory, visual, motor, oromotor, communication, and arousal functions.

Diffusion-tensor imaging (DTI) MRI technique that measures water molecule diffusion revealing the structural integrity of axon tracts in the brain.

Disorders of consciousness (DOCs) Refer to the altered states of consciousness as a result of severe acquired brain injuries and describe patients in coma, with vegetative state/unresponsive wakefulness syndrome, and in minimally conscious state.

Functional magnetic resonance imaging (fMRI) Noninvasive neuroimaging technique that measures neuronal activation based on blood oxygen level-dependent (BOLD) changes.

Locked-in syndrome (LIS) A clinical condition wherein patients are awake and aware, but with severe motor impairments, sometimes so severe that they cannot move any part of their body. The primary means of communication is through eye movements.

Minimally conscious state (MCS) A clinical disorder of consciousness wherein patients are awake but show fluctuating signs of awareness without being able to functionally communicate with their surroundings.

Passive paradigm Experimental condition during which there is the administration of external stimulations such as auditory, tactile, or visual stimuli while the subject is not asked to do anything in particular.

Positron emission tomography (PET) Invasive neuroimaging technique that measures brain metabolism energy turnover.

Resting paradigm Experimental condition during which no stimulation and/or tasks are administered to the studied population: subjects are only asked to relax and to let their thoughts pass without focus.

Vegetative state/unresponsive wakefulness syndrome (VS/UWS) A clinical disorder of consciousness wherein patients are awake but not aware of themselves and their surroundings.

Consciousness

At present, there is no definition of consciousness that is universal and covers all essential characteristics. In a clinical setting, consciousness is reduced into two main components: wakefulness (i.e., arousal) and awareness (Posner, Saper, Schiff, et al., 2007). Wakefulness refers to the level of vigilance and relies on the activity of the reticular formation, hypothalamus, and basal ganglia. Awareness is related to subjective experiences and can be subdivided into awareness of the external world (i.e., sensory or perception of the environment) and of the internal world (i.e., stimulus-independent thoughts, such as mental imagery and inner speech). Functional integrity of cortical frontoparietal connectivity with the thalamus is thought to be implicated in awareness.

Sleep is an illustrative example to describe the relationship between wakefulness and awareness: the drowsier we become as we move toward deep sleep, the less aware we are of our surroundings and ourselves. A disrupted relationship between these two components is observed in patients with disorders of consciousness (DOCs) following severe acquired brain injury. Anesthesia, epilepsy, and somnambulism (i.e., sleepwalking) are also states of diminished consciousness due to a dissociation between wakefulness and awareness (Figure 1).

Disorders of Consciousness

Coma

Coma may be a result of brainstem lesions and severe diffuse cortical or white matter damage. The main causes, however, are trauma, stroke, and anoxia (e.g., cardiac arrest). A coma is a transient condition: Patients' eyes remain closed even after painful stimulation, and hence, they remain unaware of the surroundings and of themselves. A coma must last at least one hour to be differentiated from fainting. Autonomous functions, such as breathing and thermoregulation, are reduced, which often requires respiratory assistance. Most patients recover from a coma within the first hours to weeks after injury. However, some evolve into other DOCs such as in a vegetative state/unresponsive wakefulness syndrome (VS/UWS) and minimally conscious state (MCS). Brain death can also be a result of severe brain injury and is defined by a permanent loss of all brain functions. Table 1 summarizes the diagnostic criteria for the clinical entities that can occur after a severe brain injury.

Vegetative State/Unresponsive Wakefulness Syndrome

Patients in VS/UWS recover arousal, meaning that they show spontaneous or induced eye opening. Autonomic functions are generally preserved, and breathing occurs usually without

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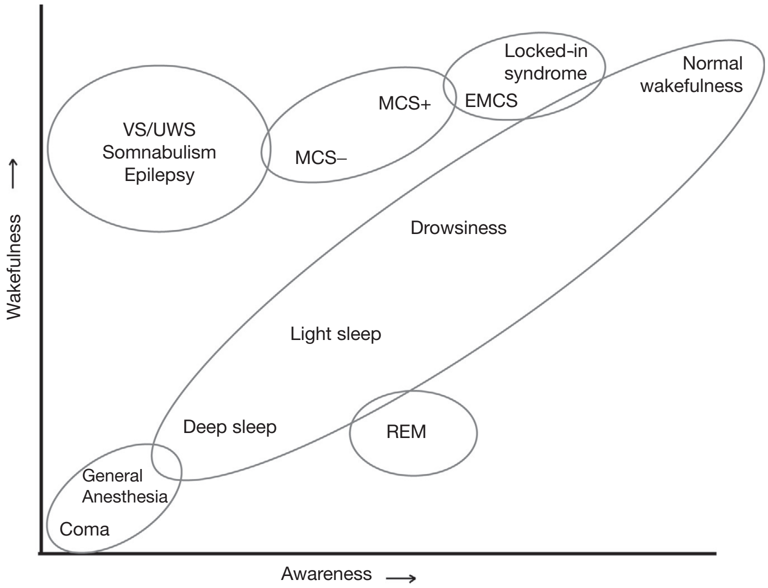

Figure 1 A clinical definition of consciousness. Interaction between arousal and awareness in different states of (un)consciousness. REM, rapid eye movement; EMCS, emergence from a minimally conscious state; MCS+, minimally conscious state plus; MCS–, minimally conscious state minus; VS/UWS, vegetative state/unresponsive wakefulness syndrome; LIS, locked-in syndrome.

Table 1 Diagnostic criteria for patients with severe brain injuries

Clinical entities	*DOC*	*Definition*	*Reference*
Brain death	No	Irreversible coma Evidence for the cause of coma Irreversible loss of all functions of the brain, including brainstem reflexes Apnea Absence of confounding factors (e.g., drugs, hypothermia, and electrolyte and endocrine disturbances)	Wijdicks (2001)
Coma	Yes	No wakefulness No awareness of self or environment Acute state (i.e., resolves in hours to maximum 4 weeks)	Posner, Saper, Schiff, et al. (2007)
Vegetative state/unresponsive wakefulness syndrome	Yes	Wakefulness No awareness of self or environment No sustained, reproducible, purposeful, or voluntary behavioral responses to visual, auditory, tactile, or noxious stimuli No language comprehension or expression Relatively preserved hypothalamic and brainstem autonomic functions (e.g., respiration, digestion, and thermoregulation) Bowel and bladder incontinence Variably preserved cranial nerve and spinal reflexes Acute and/or chronic state	The Multi-Society Task Force on PVS (1994) and Laureys, Celesia, Cohadon, et al. (2010)
Minimally conscious state	Yes	Wakefulness Awareness is inconsistent but definite Minus Visual pursuit Contingent behavior Reaching for objects Orientation to noxious stimulation Plus Following simple commands Intentional communication Intelligible verbalization	Giacino, Ashwal, Childs, et al. (2002) Bruno, Vanhaudenhuyse, Thibaut, Moonen, and Laureys (2011)
Emergence from minimally conscious state	No	Functional communication Functional object use	Giacino, Ashwal, Childs, et al. (2002)
Locked-in syndrome	No	Wakefulness Awareness Aphonia or hypophonia Quadriplegia or quadriparesis Presence of communication via the eyes Preserved cognitive abilities	American Congress of Rehabilitation Medicine (1995)

assistance. They show no voluntary interaction with their environment and no adapted emotional responses. The patient is able to perform a variety of movements, such as grinding teeth, blinking and moving eyes, swallowing, yawning, crying, and smiling, but these are always reflexive movements and not related to the context (The Multi-Society Task Force of PVS, 1994). 'Unresponsive wakefulness syndrome' was recently proposed as a replacement term for 'vegetative state' to avoid the negative 'vegetable-like' connotation and to provide a more neutral description of the behavior profile (Laureys, Celesia, Cohadon, et al., 2010). VS/UWS can be transient or permanent.

Minimally Conscious State

Patients in MCS show signs of fluctuating and reproducible remnants of nonreflexive willful behavior (Giacino, Ashwal, Childs, et al., 2002). For example, command following, visual pursuit as a direct response to moving or salient stimuli, localization of noxious stimulation, and contingent responses to emotional stimuli are considered signs of consciousness. Patients in MCS are more likely to experience pain and/or suffering (Boly, Faymonville, Schnakers, et al., 2008). MCS has been recently stratified into MCS+ (plus) and MCS− (minus) based on the complexity of behavioral responses (**Table 1**). When patients show reliable demonstration of 'functional communication' (i.e., accurate yes–no responses to situational orientation questions) and/or 'functional object use' (i.e., demonstration of the use of two different objects), the patient emerges from an MCS (EMCS) (Giacino, Ashwal, Childs, et al., 2002).

Locked-In Syndrome

Patients suffering from a locked-in syndrome (LIS) can be easily misdiagnosed as a DOC due to ventral brainstem lesions that damage the corticospinal tract, which severely affects motor behavior but leaves cognitive abilities intact. The primary mode of communication is via eye movements or blinking (American Congress of Rehabilitation Medicine, 1995).

Clinical Assessment of Consciousness

Correct diagnosis is highly important in DOC for prognostic, therapeutic, and ethical reasons. The prognosis of patients in MCS is relatively better than those in VS/UWS (Luauté, Maucort-Boulch, Tell, et al., 2010); 12 months after brain injury, about half of the patients in MCS had improved, compared with a very small percentage of patients in VS/UWS (Giacino & Kalmar, 1997). In terms of therapeutic choices, the medical team may choose to apply pharmacological (e.g., with amantadine, zolpidem, or palliative medication) and/or nonpharmacological interventions (e.g., deep brain stimulation) (Schiff, Giacino, Kalmar, et al., 2007) or choose to withdraw artificial life support. In ethical issues regarding end-of-life decisions, legal precedence in several countries has established the right of the medical team to withdraw artificial nutrition and hydration from patients in VS/UWS but not in MCS.

At present, behavioral assessment remains the gold standard for the assessment of consciousness. Clinically, wakefulness is assessed by spontaneous or stimulus-induced eye opening, whereas awareness is measured by command following or other nonreflexive purposeful behaviors. The examination of awareness is challenging and may lead to a high rate of misdiagnosis (up to 40%) if patients are not assessed carefully by a standardized scale. Indeed, motor reactions can be inconsistent, very small, or easily exhausting. Impaired cognition (aphasia and apraxia), sensory impairment (blindness and deafness), pain, pharmacological sedatives, sleep disturbances, and/or medical complications can all interfere with the assessment of consciousness (Schnakers, Vanhaudenhuyse, Giacino, et al., 2009). The Coma Recovery Scale – Revised (CRS-R) (Giacino, Kalmar & Whyte, 2004) is currently the most reliable and sensitive tool for the differential diagnosis of DOC (Seel, Sherer, Whyte, et al., 2010). It was developed to differentiate VS/UWS from MCS and uses visual, motor, auditory, oromotor, communication, and arousal subscales. The use of self-referential stimuli such as one's own name and one's own face (using a mirror) should be used to increase the patient's responsiveness. Neuroimaging methods are starting to assist behavioral assessments with the challenging task of differential diagnosis.

Ancillary Testing of Consciousness

Neuroimaging methods such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and electroencephalography (EEG) have offered the possibility to objectively study covert cognitive processes. Spontaneous brain function can be assessed in resting paradigms where the subject receives no external stimulation and is instructed to let their mind wander. Passive paradigms use external stimulation to assess brain function and measure the spread of information within the cortex. Active paradigms use willfully modulated brain signals, for example, by using mental imagery tasks, to detect command following similar to command response tests done at the bedside.

Positron Emission Tomography

PET gives an approximation of functional tissue integrity by measuring cerebral glucose consumption. In resting conditions, this method has shown a decrease in brain metabolism in VS/UWS of up to 40% of normal value. However, the loss of consciousness is not related to a global dysfunction in cerebral metabolism, but rather to regional decreases. Indeed, patients suffering from DOC show decreased metabolism in a widespread frontoparietal network, encompassing the lateral prefrontal and posterior parietal areas and midline anterior cingulate/mesiofrontal and posterior cingulate/precuneal associative cortices (Nakayama, Okumura, Shinoda, et al., 2006) (**Figure 2(c)**). The lateral areas of this frontoparietal network are considered to be implicated in external awareness, whereas the midline regions have been linked to internal awareness (Vanhaudenhuyse, Demertzi, Schabus, et al., 2010). According to this scheme, patients in MCS show higher metabolism in precuneus than patients in VS/UWS (Thibaut, Bruno, Chatelle, et al., 2012) and are characterized by the metabolic restoration in the frontoparietal network and the connections between

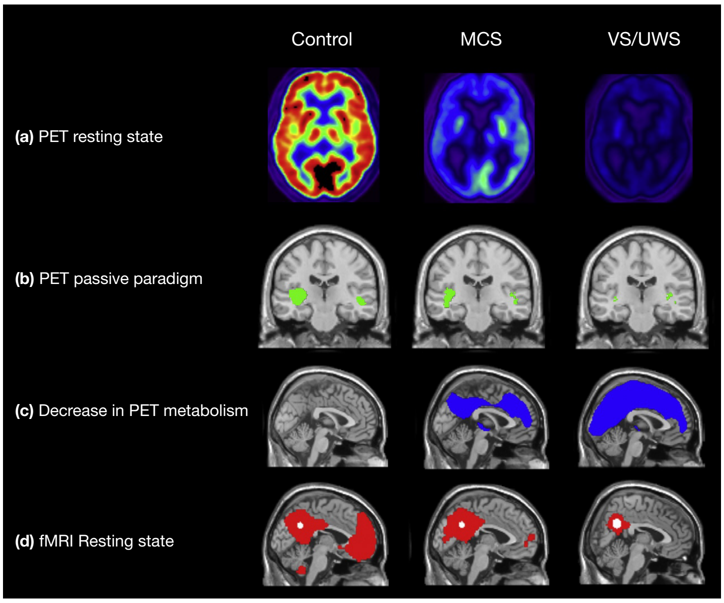

Figure 2 Group-level studies using PET and fMRI in healthy condition and in disorders of consciousness. Resting and passive paradigms in healthy controls and in patients in a vegetative state/unresponsive wakefulness syndrome (VS/UWS) and minimally conscious state (MCS). (a) Cerebral metabolism as measured with PET in the three groups. Images are shown using the same color scale. (b) Cerebral metabolism activation during auditory paradigm with PET. Healthy controls show activation of the primary and secondary auditory cortices, whereas patients in MCS and VS/UWS show a severely decreased activity in these areas. (c) Areas with significant metabolic impairment in patients in MCS and VS/UWS compared to healthy controls are found in the frontoparietal network. (d) Resting-state fMRI BOLD of the default-mode network is preserved in healthy conditions and partially preserved in patients with DOC (white spot indicates the seed region with which the other brain areas in red are functionally connected). Both PET and fMRI statistical maps are thresholded at a family-wise error correction rate for multiple comparisons ($p < 0.001$).

prefrontal and central thalamic areas (Laureys, Faymonville & Luxen, 2000). Notably, the decreased metabolism in the thalamus seems to be related to impaired consciousness (Lull, Noé, Lull, et al., 2010). The recent proposal to subcategorize the MCS into MCS− and MCS+ was confirmed by resting-state PET analysis, where differences in language and sensorimotor areas are observed between patients in MCS− and MCS+ (Bruno, Majerus, Boly, et al., 2011).

Using passive paradigms, differential activation patterns have been demonstrated in patients in VS/UWS and MCS. For example, as a response to sound (**Figure 2(b)**), patients in VS/UWS show activation limited to the primary auditory cortex (Laureys, Faymonville, Degueldre, et al., 2000), whereas patients in MCS demonstrate brain activation spreading to the secondary auditory cortex as well as temporal and frontal areas (Boly & Faymonville, 2004). More importantly for clinical management, during painful stimulation, patients in MCS show similar brain activation compared to controls, while patients in VS/UWS only show restricted activation in lower-level subcortical and primary cortical areas.

Magnetic Resonance Imaging

Anatomical MRIs help to assess the extent of structural damage. Diffusion-tensor imaging (DTI) is a measure of the directionality of water molecules that indicates white matter tracts (**Figure 3(a)**). DTI has been shown to correctly classify patients in VS/UWS versus MCS with a 95% accuracy in a group of 25 patients (Fernández-Espejo, Bekinschtein, Monti, et al., 2011). fMRI visualizes brain function derived from blood oxygen level-dependent (BOLD) changes. During rest, the brain shows spontaneous oscillating patterns of BOLD low-frequency neuronal activity, allowing the brain to get organized in distinct functional networks (Damoiseaux, Rombouts, Barkhof, et al., 2006). In healthy subjects, ten resting-state networks can be reliably detected, such as the default-mode, visual, auditory, salience, sensorimotor, and executive control networks (Smith, Fox, Miller, et al., 2009). The robustly detected default-mode network (mainly encompassing the midline anterior cingulate/mesiofrontal and posterior cingulate/precuneal regions and lateral parietal areas) has been linked to conscious processes. Such consciousness-related default-mode functional connectivity has been shown to decrease as a function of the level of consciousness in patients with DOC (**Figure 2(d)**; Heine, Soddu, Gomez, et al., 2012). Sub cortical, subcortical connectivity may also be informative for patients in DOC. Indeed, it has been recently suggested that, instead of decreased connectivity, patients with DOC present *hyper*connectivity in the subcortical limbic system (Di Perri, Bastianello, Bartsch, et al., 2013).

Passive fMRI paradigms also indicate that auditory, visual, and somatosensory activation is restricted to lower sensory regions in patients in VS/UWS, while brain activation is widespread in MCS (Di, Yu, Weng, et al., 2007). Unlike PET, fMRI

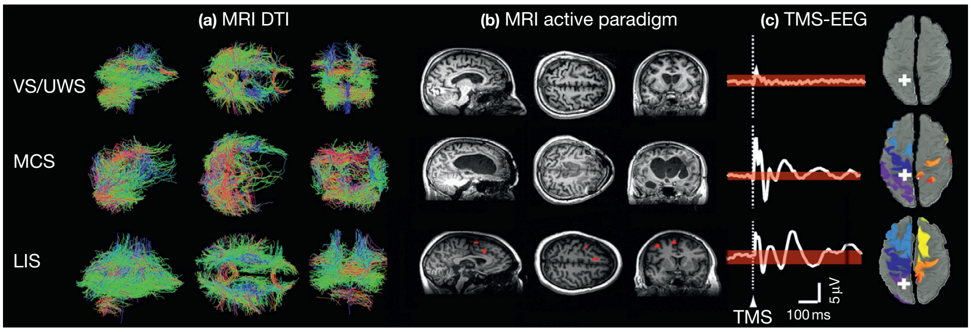

Figure 3 Multimodal neuroimaging in three patients with disorders of consciousness. Neuroimaging methods at the single-patient level: One patient is behaviorally diagnosed with vegetative state/unresponsive wakefulness syndrome (VS/UWS), one diagnosed with minimally conscious state (MCS), and one diagnosed with locked-in syndrome (LIS). (a) MRI diffusion-tensor imaging (DTI) indicates the amount of damage in structural connectivity (i.e., white matter tracts, color-coded by axis). (b) fMRI active paradigms allow for motor-independent response to command (here, tennis imagery). (c) Transcranial magnetic stimulation (TMS) combined with EEG assesses cortical excitability and effective connectivity. EEG evoked response under the stimulation area (left image) and the subsequent widespread of the activation (right, color-coded by brain area). When the result of multimodal neuroimaging assessment converges, greater confidence can be achieved in the assessment of the level of consciousness.

allows the use of active paradigms to show voluntarily modulated responses to command. Patients can be asked to perform a mental imagery task such as playing tennis or navigating through their house that activates supplementary motor areas and parahippocampal areas, respectively (Owen, Coleman, Boly, et al., 2006; **Figure 3(b)**). This study, as well as multiple others, has shown that some patients behaviorally diagnosed as VS/UWS are able to correctly perform these imagery tasks. Therefore, the absence of responsiveness at the bedside does not always correspond to an absence of consciousness. This method has even been used for binary communication in a patient with DOC (Monti, Vanhaudenhuyse, Coleman, et al., 2010). Although active paradigms do not give positive results in all (partially) conscious patients, new methods for communication are being created (Naci, Cusack, Jia, et al., 2013).

Electroencephalography

Resting-state measures of electrical brain activity can also aid diagnosis with the advantage of being performed at the bedside (Gosseries, Schnakers & Ledoux, 2011). For instance, studies using quantitative and connectivity EEG measures have demonstrated the ability of this technique to differentiate between patients in MCS and those in VS/UWS at the group level. EEG alpha activity is decreased in all patients with DOC, whereas delta power is increased in VS/UWS only (Lechinger, Bothe, Pichler, et al., 2013). Several studies using passive paradigms have assessed event-related potentials (ERPs) as a response to stimulations. The presence of an ERP response to stimuli (e.g., N1) and to odd stimuli within a sequence (mismatch negativity (MMN)) serves as predictors of outcome. The P3 ERP response to unexpected stimuli also aids prognosis and can be used as a response to a command paradigm by showing higher ERP when used in an active condition as compared with a passive situation (Schnakers, Perrin, Schabus, et al., 2008). As in fMRI active paradigms, some patients who are behaviorally diagnosed as VS/UWS have been shown to be able to perform active mental imagery tasks (Cruse, Chennu, Chatelle, et al., 2011).

EEG in combination with transcranial magnetic stimulation (TMS) is used to stimulate a brain region and assess cortical excitability (i.e., the amplitude of the initial response to TMS) and effective connectivity response (i.e., causal interaction between the stimulated area and the subsequent activated cortical regions). This technique has been shown to successfully differentiate patients in VS/UWS from MCS (**Figure 3(c)**). Indeed, MCS patients demonstrate complex long-lasting widespread activation patterns, whereas patients in VS/UWS show stereotyped and local slow-wave responses that indicate a breakdown of effective connectivity (Rosanova, Gosseries, Casarotto, et al., 2012).

Multimodal Assessment

The different neuroimaging methods reviewed in the preceding text provide information on the structural location and extent of brain lesions (e.g., via MRI DTI) and their functional impact (e.g., metabolic FDG-PET, hemodynamic fMRI, and EEG measurements). Although there have been many studies indicating differences between patients with DOC and healthy controls and between patients in VS/UWS and MCS, almost all of them have reported results at the group level (**Figure 2**). Nevertheless, when these methodologies are applied separately in patients, they can demonstrate challenges in diagnostic and prognostic terms. Multimodal assessment may shed more light on the individual brain function because it highlights the complementarities of these neuroimaging methods in the study of DOC (Bruno, Fernández-Espejo, Lehembre, et al., 2011). When the results of multiple neuroimaging assessments converge, then greater confidence can be achieved in the assessment of the level of consciousness (**Figure 3**).

Conclusion

Patients with DOC offer the possibility of studying consciousness using a lesion approach. This has taught us that consciousness is not an all-or-none phenomenon and should be considered as a continuum, as shown by the different DOCs with varying levels of awareness. Neuroscientific findings should also be viewed together in a theoretical framework of consciousness to ultimately lead to a unification of passive and active paradigms in a coherent diagnostic approach. Several hypothesis about consciousness and the neural correlates of consciousness have been developed (Boly & Seth, 2012). For example, the global workspace theory states that consciousness is an emergent property of the frontoparietal network (Dehaene & Changeux, 2005). The information integration theory of consciousness indicates that consciousness is related to a system's capacity for information integration. Each causal mechanism is capable of choosing among alternatives that generate information, and information is integrated to the extent that it is generated by a system above and beyond its parts (Tononi, 2008). The next step is therefore to combine neuroscientific findings related to conscious and unconscious states, as well as theory, in order to study how stimuli access conscious processing, the phenomenon of consciousness itself, and the neurological basis and measures of dynamical complexity. All in all, neuroimaging approaches are showing promise for the assessment of patients with DOC, and the study of this patient population might aid the quest for the neural correlates of consciousness.

See also: **INTRODUCTION TO ACQUISITION METHODS**: Anatomical MRI for Human Brain Morphometry; Basic Principles of Electroencephalography; Diffusion MRI; Functional MRI Dynamics; Molecular fMRI; Positron Emission Tomography and Neuroreceptor Mapping *In Vivo*; **INTRODUCTION TO ANATOMY AND PHYSIOLOGY**: Cytoarchitectonics, Receptorarchitectonics, and Network Topology of Language; Functional Connectivity; Insular Cortex; Lateral and Dorsomedial Prefrontal Cortex and the Control of Cognition; Posterior Parietal Cortex: Structural and Functional Diversity; Somatosensory Cortex; Thalamus: Anatomy; The Brain Stem; The Resting-State Physiology of the Human Cerebral Cortex; **INTRODUCTION TO CLINICAL BRAIN MAPPING**: Brain Mapping Techniques Used to Guide Deep Brain Stimulation Surgery; Imaging Alzheimer's Disease: The Evolution of Biomarkers; Recovery and Rehabilitation Poststroke; **INTRODUCTION TO METHODS AND MODELING**: Dynamic Causal Models for Human Electrophysiology: EEG, MEG, and LFPs; Effective Connectivity; Resting-State Functional Connectivity; **INTRODUCTION TO SOCIAL COGNITIVE NEUROSCIENCE**: Action Perception and the Decoding of Complex Behavior; Body Perception; The Default Network and Social Cognition; **INTRODUCTION TO SYSTEMS**: Large-Scale Functional Brain Organization; Network Components; Pain: Acute and Chronic.

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Relevant Websites

http://www.alis-asso.fr – Association for the Locked-in Syndrome.
www.comascience.org – Coma Science Group.

Appendix B

Paper II

Imaging correlations in non-communicating patients.

Heine L, di Perri C, Soddu A, Gomez F, Laureys S, Demertzi A.

in: Clinical neurophysiology in disorders of consciousness:
Brain function monitoring in the ICU and beyond

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Imaging Correlations in Non-communicating Patients

12

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Steven Laureys, and Athena Demertzi

Contents

Abstract

The diagnosis and medical management of patients with acute or chronic disorders of consciousness (DOC) are challenging. Motor-independent functional neuroimaging technologies are increasingly employed to study covert cognitive processes in the absence of behavioural reports. Studies with functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) performed in this patient population have utilized active, passive and resting-state paradigms. Active paradigms refer to mental imagery tasks that measure wilful modulation of brain signal in specific brain areas, aiming to detect command-following. Passive paradigms are used to measure brain responses to external sensory stimulation (e.g. auditory, somatosensory and visual). Alternatively, in resting-state paradigms, spontaneous brain function is assessed while subjects receive no external stimulation and are instructed to let their mind wander. Independently from each other, these methods have shown differences between healthy controls and patients, as well as among patients with DOC. However, these techniques cannot yet be used in clinical settings before robust information at the single-subject level will be provided: it is expected that multimodal research will improve the single-patient diagnosis, shed light on the

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prognostic biomarkers, and eventually promote the medical management of patients with consciousness alterations.

12.1 Introduction

In the 1950s the intensive care unit welcomed the mechanical ventilator, which allowed patients to sustain heart function and systemic circulation after traumatic and non-traumatic brain injuries. Ever since, some of the surviving patients were found to suffer from altered states of consciousness that were never encountered before. Patients could now lie in a coma for hours to weeks with eyes closed and were hence considered unaware of the surroundings and of themselves. In cases where they opened their eyes but remained unresponsive to any external stimulation, they were considered to be in a vegetative state (VS; Jennett and Plum 1972) or, as most recently coined, unresponsive wakefulness syndrome (UWS; Laureys et al. 2010). It was only in 2002 that the medical community recognized another entity which characterized patients showing signs of fluctuating yet reproducible remnants of nonreflexive behaviour; these patients were said to be in a minimal conscious state (MCS; Giacino et al. 2002).

The diagnosis and medical management of patients with acute or chronic disorders of consciousness (DOC) are challenging, mainly because these patients are unable to communicate. For example, pain[1] in patients with DOC can only be inferred through behavioural observation (Schnakers et al. 2012). Interestingly, such observations are not unanimous among clinicians. As evident by a recent survey, more than half of medical doctors (56 %) thought that patients in a VS/UWS feel pain (Demertzi et al. 2009), despite official criteria denouncing such experience from these patients (The Multi-Society Task Force on PVS 1994). Similarly, clinicians' opinions differed when more ethically challenging issues were concerned, such as the limitation of life-sustaining therapies. Indeed, clinicians appeared more reluctant to withdraw treatment from patients in MCS (28 % agreed) than from those in UWS (66 % agreed) (Demertzi et al. 2011). The agreement with withdrawal of life-sustaining treatment in patients in a UWS is also supported by others (Kuehlmeyer et al. 2012). Taken together, these studies show that the medical management of pain as well as discussions regarding end-of-life decisions is highly influenced by the diagnostic category. As such, the need for valid and sensitive tools to improve accurate diagnosis of patients with DOC is increasing. To date, diagnostic assessment is mainly based on observing motor and oro-motor behaviours at the bedside. As these assessments are prone to false-negative diagnosis (Schnakers et al. 2009), motor-independent neuroimaging technologies may aid the search for residual cognitive function of non-communicating patients.

12.2 Neuroimaging Can Aid Diagnosis

Functional neuroimaging methods have offered the possibility to objectively study cognitive processing in the absence of behavioural reports. Functional magnetic resonance imaging (fMRI) quantifies brain function derived from blood-oxygen-level-dependent (BOLD) changes. Position emission tomography (PET) measures different aspects of metabolic function according to the type of the administered radioactive tracer. The structural properties of the brain can also be revealed by means of anatomical MRI, while diffusion tensor imaging (DTI) measures white matter integrity. Below we will refer to experimental paradigms that have been most frequently adopted to infer covert cognitive abilities in non-communicating patients suffering from DOC (Fig. 12.1).

12.2.1 Active Paradigms

Active paradigms refer to mental imagery tasks which measure wilful modulation of brain signal in specific brain areas, aiming to detect command-

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[1] The unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Loeser and Treede 2008).

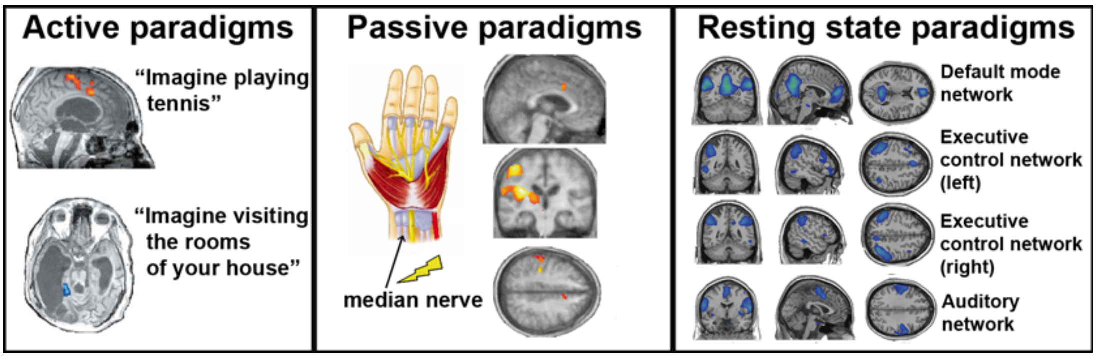

Fig. 12.1 Neuroimaging paradigms in assessment of residual cognitive processes in DOC

following. Command-following in patients with DOC is of major clinical importance because, according to standardized behavioural assessment, this behaviour differentiates patients in MCS from patients in UWS (Bruno et al. 2011b; Giacino et al. 2004). At the bedside, command-following is tested by asking patients to follow simple object-related and the non-object-related commands. Those patients that can follow the commands "consistently"[2] or "reproducibly"[3] are said to be in MCS. In the fMRI environment, two mental imagery tasks have been shown to encompass reproducible cortical activations across healthy controls, namely, thinking about playing tennis (encompassing primarily supplementary motor area) and imagining visiting the rooms of one's house (encompassing primarily parahippocampal cortex; Fig. 12.1, left panel) (Boly et al. 2007). When this paradigm was employed in a behaviourally unresponsive patient, her brain activity was indistinguishable from healthy controls. Since this patient was able to comprehend and execute the mental imagery commands in a sustainable manner, the behavioural diagnosis was challenged, and the patient was no longer considered as in UWS (Owen et al. 2006). In a larger cohort ($n=54$) of patients with DOC, the same paradigm confirmed that not all behaviourally assessed VS/UWS patients were unresponsive (Monti et al. 2010). Indeed, command-following was observed in five patients, two of whom showed no signs of awareness when evaluated behaviourally. Interestingly, one patient was able to further use this technique to provide yes/no responses to autobiographical questions which could not be elicited at the bedside. Based on the command-following rationale, other mental tasks for evidencing response to command in patients with DOC have been employed. For example, with a silent picture-naming task, complete and partial preservation of the object-naming brain network was observed for all patients in MCS and two in VS/UWS (Rodriguez Moreno et al. 2010). By instructing patients in VS/UWS to move their hand, voluntary behaviour was also evidenced for two patients who activated premotor cortex (consistent with movement preparation) in the absence of overt muscle activity (Bekinschtein et al. 2011). Similarly, using selective auditory attention, all 3 assessed patients (2 MCS and 1 VS/UWS) were able to convey their ability to follow commands, and 2 of them (1 MCS and VS/UWS) were even able to use attention to correctly communicate answers to several binary questions (Naci and Owen 2013). Residual cognitive capacities in patients DOC has also been assessed using a hierarchical fMRI approach, starting from command-following tasks similar to those described above, to question tasks using binary choice responses and eventually multiple choice responses (Bardin et al. 2011).

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[2] When the patient follows both object-related and non-object-related commands in all eight administered trials (i.e. four trials per command).

[3] When the patient shows three clearly discernible responses over the four trials on any one of the object-related or non-object-related commands.

A criticism of using mental imagery tasks to unfold cognition and/or to communicate relies on patients' limited short-term memory resources and restricted attention span. As a result, relatively long scanning intervals might be necessary to increase the signal-to-noise ratio, which in turn contributes in patients' fatigue and ultimately in lack of their vigilance (Naci et al. 2013). Additionally, patients may suffer from other cognitive (i.e. aphasia, apraxia) and sensory impairments (i.e. blindness, deafness), from small or easily exhausted motor activity, pain, sedative medication, sleep disturbances and/or medical complications (such as infections), which can interfere with the direct assessment of command-following. In these cases, however, absence of responsiveness does not necessarily correspond to absence of awareness (Sanders et al. 2012). Alternatively, residual cognitive function in patients with DOC has been further assessed by means of passive and resting-state paradigms which overcome the above-mentioned limitations.

12.2.2 Passive Paradigms

Passive paradigms measure brain responses to external sensory stimulation (e.g. auditory, somatosensory and visual), while the subject is not performing any mental task. Using PET, the administration of simple auditory clicks in patients in VS/UWS was shown to activate primary auditory cortices (Laureys et al. 2000c), whereas patients in MCS demonstrated more widespread activation in the secondary auditory cortex, as well as temporal and frontal areas (Boly and Faymonville 2004). More recently a difference between patients in VS/UWS and MCS was observed in the impairment of backward connectivity from frontal to temporal cortices, as evidenced by an EEG mismatch negativity paradigm using auditory clicks (Boly et al. 2011) (see also Chap. 9). When more complex auditory stimuli were administered, differential brain responses between patients and controls, as well as between patient groups, were also observed. For example, sentences which were manipulated at different levels of auditory intelligibility[4] and semantic ambiguity[5] were presented to a patient in VS/UWS. The patient presented consistent and similar-to-controls responses in the auditory cortex as a response to intelligible speech stimuli as well as partially intact responses to semantically ambiguous stimuli (Owen and Coleman 2005). Also, in an fMRI task of passive listening to narratives played forward and backward, one patient in VS/UWS (out of three) and one patient in MCS (out of four) showed cerebral responses very similar to healthy controls (Fernández-Espejo et al. 2008). Another salient auditory stimulus which has been preferred because of its attention-grabbing properties is the patient's own name. With the own-name paradigm, it was shown that one patient in VS/UWS exhibited activation in medial prefrontal cortex, left temporoparietal and superior frontal cortex, which is again similar to activation observed in controls (Staffen et al. 2006). With a similar own-name paradigm, two out of seven patients in VS/UWS and all four patients in MCS showed activation not only in the primary auditory cortex but also in higher-order associative temporal areas, which are thought to be implicated in the conscious processing of the incoming stimuli. Interestingly, these two patients in VS/UWS subsequently recovered to MCS as observed 3 months after their fMRI scan, highlighting the prognostic value of this paradigm (Di et al. 2007). This prognostic utility was further supported by data of seven out of eight patients in VS/UWS who progressed to MCS and showed speech-specific or semantic responses to sentence stimuli 6 months earlier in their fMRI assessment (Coleman et al. 2009).

In the somatosensory modality, painful electrical stimulation of the median nerve of the wrist encompassed the entire "pain matrix" (including the anterior cingulate cortex and insular areas; Fig. 12.1, middle panel) in patients in MCS. On the other hand, patients in VS/UWS

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[4] Speech in noise was used as a form for distortion by adding a continuous pink-noise background to sentences.

[5] Sentences containing at least two ambiguous words, either homonyms or homophones.

showed restricted activation to lower-level subcortical and primary cortical areas (Boly et al. 2008; Laureys et al. 2002), indicating that MCS patients are more likely to experience the administered stimuli as painful.

In the visual modality, when pictures of different emotional valences were presented to patients in MCS by means of fMRI, visual activation similar to healthy controls was found for patients (Zhu et al. 2009). A more recent case study on visual cognition used a battery of tests in a MCS patient. Specifically, the battery first assessed passive visual processing whereas, at the final level, it assessed the ability to voluntarily switch visual attention though the focus on competing stimuli. This approach revealed appropriate brain activations, undistinguishable from those seen in healthy and aware volunteers suggesting that the patient retained the ability to access his own visual representations (Monti et al. 2013).

Taken together, the rationale behind passive experimental paradigms is that an indistinguishable response between patients and controls is indicative of covertly preserved cognitive processing in these patients (Owen 2013). Generally, these paradigms have shown that auditory, visual and somatosensory activation is restricted to lower-level sensory regions in patients in VS/UWS, while brain activation is widespread in MCS reaching higher-level associative areas. The limitations of using this approach stem from patients' pathologies and technical requirements. Indeed, patients can present variant clinical picture, ranging from visual problems, motor spasticity, somatosensory hypersensitivity and cortical auditory deafness, which can prevent the administration of external stimuli. Also, the technical setup of these examinations are not always straightforward, and therefore cannot be widely used across medical and research institutions.

12.2.3 Resting-State Paradigms

Alternatively, increasing attention is being paid to resting-state paradigms (Soddu et al. 2011). In these paradigms, spontaneous brain function is assessed while subjects receive no external stimulation and are instructed to let their mind wander. As such, this approach surpasses the limitations which are raised by the other two types of experimental tasks.

Using PET at rest, it was shown that patients in VS/UWS exhibit decreased brain metabolism up to 40 % of normal value (Laureys et al. 2000b; Tommasino et al. 1995). Nevertheless, recovery from the VS/UWS does not coincide with the resumption of global metabolic levels. It rather seems that some areas are more critical to consciousness than others. Indeed, patients suffering from DOC show decreased metabolism in a widespread network encompassing frontoparietal areas, such as lateral prefrontal and posterior parietal regions as well as midline anterior cingulate/mesiofrontal and posterior cingulate/precuneal associative cortices (Nakayama et al. 2006; Silva et al. 2010). Importantly, recovery from the VS/UWS parallels the restoration of connectivity in these areas (cortico-cortical) but also between these regions and the thalamus (thalamo-cortical) (Laureys et al. 2000a, 1999). More recently, it was shown that patients in MCS retain metabolism in the lateral frontoparietal areas, whereas midline regions are highly dysfunctional (Thibaut et al. 2012). Broadly speaking, the midline cortices are assumed to mediate self-related cognition, whereas frontoparietal cortices are thought to mediate awareness of the environment (for a short review, see Demertzi et al. 2013a). As such, these data suggest that patients in MCS are characterized by altered self-awareness besides their abilities to, at least to a certain extent, interact (but not communicate) with their surroundings.

In fMRI, the resting-state network approach has been used lately to quantify various higher-order and sensory-related systems (Damoiseaux et al. 2006; Laird et al. 2011; Smith et al. 2009). These networks show differential connectivity alterations under different states of unconsciousness (Heine et al. 2012), highlighting their importance when assessing consciousness levels. In a recent investigation of fMRI resting-state connectivity in patients with DOC, it was found that, among the long-range systems, the default mode network (DMN, which encompasses precuneus, medial prefrontal cortex and bilateral temporoparietal junctions) and bilateral executive control or frontoparietal networks were severely disturbed in patients with DOC (Fig. 12.1, right panel) (Demertzi et al. in press). This implies that these systems might be important to sustain consciousness-related processes. Interestingly, it has been found that the resting brain is characterized by a switch between the dominance of the DMN (linked to "internal" or self-awareness) and the bilateral frontoparietal network (linked to "external" or environmental awareness; Fox et al. 2005; Fransson 2005). More recently, it was found that such alternating pattern not only happens spontaneously in the brain but also has a behavioural counterpart. In other words, behavioural reports of "internal awareness" were linked to the activity of the DMN, whereas subjective ratings for "external awareness" seem to correlate with the activity of lateral fronto-parieto-temporal regions (Vanhaudenhuyse et al. 2010), which are part of the so-called executive control network, exhibiting increase of activity during attention-demanding cognitive tasks. These findings imply that the anti-correlated pattern between the internal (DMN) and external (executive control network) awareness systems is of functional relevance to the phenomenological complexity of subjectivity (Demertzi et al. 2013b). This assumption is further supported by evidence from patients who show severely disrupted connectivity in one or both systems. For instance, in a brain dead patient, functional connectivity of the DMN was absent (Boly et al. 2009), whereas in patients with DOC, albeit preserved, functional connectivity of the DMN showed consciousness level-dependent decreases (Soddu et al. 2012; Vanhaudenhuyse et al. 2010).

Next to the investigation of reduced connectivity, the presence of pattern of hyper-connectivity might also be informative of patients' brain function. Indeed, it was recently showed that the subcortical limbic system (including the orbitofrontal cortex, insula, hypothalamus, and the ventral tegmental area) exhibits paradoxically increased fMRI connectivity with the DMN in patients with DOC (Di Perri et al. 2013). These results were considered as suggestive of a persistent engagement of residual neural activity in self-reinforcing neural loops, which, in turn, could disrupt normal patterns of connectivity in patients.

12.3 Conclusions

Functional neuroimaging has been employed to test imaging correlations in patients not able to communicate as a result of severe brain damage. Nevertheless, parallel to function, information about the brain's anatomy also sheds light on the differential neuropathology of patients with DOC. For example, recent studies suggest that the structural connectivity assessment by means of DTI is severely disrupted in patients in coma. Specifically, the connections from the brainstem to the thalamus, also known as the ascending arousal system, have been shown to be seriously impaired in patients (Edlow et al. 2012). Although one could expect that patients with such severe structural damage show poor recovery rates, this is not necessarily the case, especially when the assessment of axonal injury is shortly after the accident. For example, structural imaging of a patient suffering from altered states of consciousness 8 weeks after a traumatic accident showed severe damage in the corpus callosum, brainstem and bilateral white matter; nevertheless, 1 year after injury, this patient had regained consciousness and reintegrated in the community (Edlow et al. 2013). In more chronic patients, it has been shown that structural connectivity assessment could correctly classify patients in UWS versus MCS with a 95 % accuracy in a group of 25 patients (Fernández-Espejo et al. 2011). These studies imply that the assessment of structural connectivity is of salient clinical importance especially when it is known that structure is linked to function (Sui et al. 2014). Ideally, maximal information about patients' clinical picture can be obtained by combining different technologies (Bruno et al. 2011a; Gantner et al. 2013); the use of functional imaging together with electroencephalography is very promising, as recently done with transcranial magnetic stimulation and EEG (TMS/

EEG; Casali et al. 2013; Rosanova et al. 2012) (see also Chap. 10). Multimodal assessment of patients using the aforementioned neuroimaging methods is expected to bring us closer to patient-specific underlying neuropathology, which in turn could aid diagnosis and prognosis (Bruno et al. 2011a).

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Appendix C

Paper III

Technology-based assessment in patients with disorders of consciousness

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Technology-based assessment in patients with disorders of consciousness

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Abstract

Introduction. A number of studies highlight the difficulty in forming a diagnosis for patients with disorders of consciousness when this is established merely on behavioral assessments.

Background. Positron emission tomography (PET), functional magnetic resonance imaging (fMRI), diffusion tensor imaging (DTI), and electroencephalography combined with transcranial magnetic stimulation (TMS-EEG) techniques are promoting the clinical characterization of this challenging population. With such technology-based "objective" tools, patients are also differentially able to follow simple commands and in some cases even communicate through modified brain activity. Consequently, the vegetative state and minimally conscious state have been revised and new nosologies have been proposed, namely the unresponsive wakefulness syndrome, the minimally conscious state plus and minus, and the functional locked-in syndrome.

Aim. To our mind, an integration of different technical modalities is important to gain a holistic vision of the underlying pathophysiology of disorders of consciousness in general and to promote single-patient medical management in particular.

Key words
- disorders of consciousness
- functional neuroimaging
- electroencephalography
- vegetative state/unresponsive wakefulness syndrome
- minimally conscious state

INTRODUCTION

Disorders of consciousness (DOC) are among the most challenging and poorly understood conditions of modern medical care. These clinical conditions have increasingly appeared in the clinical setting since emergency medicine and lifesaving technologies had been introduced back in the 1950s [1]. Ever since, the advance of such tools contributed to the raising number of patients who survived after sustaining extreme brain damage. At the same time, the introduction of medical imaging in the 1970s, and its rapid development ever since, boosted a deeper understanding of brain function in states of unconsciousness [1, 2]. Here we aim at providing an updated overview of the application and contribution of neuroimaging and electrophysiological techniques for the assessment of patients with DOC. Such technology-based approach seems to be imperative for diagnosing these patients, especially when one considers that standard neurological and behavioral assessment is not always accurate [3]. We will discuss the information we obtain about the brain's metabolic capacities (with positron emission tomography – PET), its hemodynamic function (with functional magnetic resonance imaging – fMRI), the metabolic and biochemical activity (with MRI spectroscopy), its structural properties (with diffusion weighted MRI), and the dynamics of cortical excitability (with electroencephalography recordings of transcranial magnetic stimulation evoked pulses – EEG/TMS). To our mind, by combining the temporal and spatial properties which characterize these different techniques, one gains a holistic vision of the underlying pathophysiology of patients with DOCs in general and of the single patient under care in particular.

THE CLINICAL ENTITIES OF CONSCIOUSNESS

What differentiates DOC from other states of unconsciousness, such as due to pharmacological anesthesia, sleep and epileptic seizures, is the prolonged impaired awareness following severe brain damage. After an acute brain insult, a patient may spend some time in coma. Patients in coma are not awake, as evinced by eye closure even when intensively stimulated [4] and presumably they are not aware of themselves and their environment. Coma may arise after structural or metabolic lesions of the brainstem reticular system or after widespread bilateral cerebral damage [4]. The condition of coma usually does not last longer than 4 weeks, after which patients either evolve to brain death (*i.e.*, permanent loss of brainstem functions) or may completely recover consciousness or evolve to a vegetative state (VS) [5].

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The VS, recently termed as "unresponsive wakefulness syndrome" (UWS; [6]), is a condition of wakefulness without awareness [7]. This means that patients in VS/UWS open their eyes but they exhibit only reflex behaviors [8]. Therefore, they are considered unaware of themselves and their surroundings. Neuropathological findings seem to associate this condition to profound damage to the subcortical white matter and the major relay nuclei of the thalamus [9]. It has been further shown, in a large post-mortem series of patients who sustained a blunt traumatic brain injury, that clinical prognosis seems to be related to the location and extent of brainstem damage. Patients with lesions in central parts of the rostral brainstem, frequently associated with extensive diffusion axonal injury (DAI), showed no recovery from coma or VS/UWS, which occurred only in the patients with damage to the dorso-lateral brainstem tegmentum or pontine basis [10]. It has been proposed that the VS/UWS is permanent after 12 months following traumatic brain injury and 3 months following non-traumatic insults, and therefore chances for recovery are slim [11]. However, patients with late spontaneous recoveries challenge these proposed time boundaries and as such the futile connotation of the vegetative state has been revisited [6]. Patients from VS/UWS may die or evolve into a minimally conscious state (MCS), which may be the endpoint of their improvement or a temporary stage on the way to further recovery of consciousness [12].

Patients is MCS exhibit sings of discernible non-reflex behaviors which occur reproducibly (yet inconsistently) as a response to visual, auditory, tactile, or noxious stimuli [12]. However, patients in MCS are not able to communicate accurately with their environment although some may show intentional sings of communication [13]. The heterogeneity of MCS has been recognized and recently it has been proposed to subcategorize this entity into MCS PLUS and MINUS [14]. The differentiation was based on the level of complexity of the observed behavioral responses, such as the ability to following simple commands [15]. Patients from MCS may die, regress back to VS/UWS, or emerge out of EMCS once they regain the ability to reliably communicate and/or use objects in a functional manner [12]. The temporal limits of irreversibility have not been proposed yet for MCS. Although there is some evidence suggesting that patients in a MCS have better chances of recovery than patients in VS/UWS, at present we are not in a position to refer to possibly chronic MCS [16].

Some patients, most frequently after a focal brainstem lesion, may evolve from a coma to a locked-in syndrome (LIS). This is not classically a DOC, but it is worth to be mentioned here as it often can be misdiagnosed as a DOC. In classic cases, patients in LIS have a fully recovered consciousness but have lost voluntary motor control, except for small eye movements making it possible for them to answer yes-no questions. More rarely they loose the control of all their voluntary muscles, including extrinsic eyes muscles (complete LIS), making more challenging or impossible to communicate with them [17]. In these patients, the only evidence for preserved consciousness may be their ability to communicate via assisting technologies (*i.e.*, fMRI, EEG or evoked potentials). As such, the term "functional LIS" has been recently proposed to describe those patients with a dissociation between extreme behavioral motor impairment and the identified preserved higher cognitive abilities only detectable by functional imaging techniques [14].

Differential diagnosis of the above mentioned clinical DOC entities raises important ethical and medical issues, including end-of-life decision and pain treatment [18]. Nowadays, the gold standard to assess the level of consciousness is the clinical assessment, based on patients' behavioral responsiveness. Because responsiveness represents only an indirect evidence of consciousness (*i.e.* the lack of responsiveness does not necessarily imply lack of consciousness) reliance on these behavioral markers presents significant challenges and may lead to misdiagnoses. Clinical studies have shown that up to 40% of patients with a diagnosis of VS/UWS may in fact retain some level of awareness [3, 19, 20], and the main causes of misdiagnosis are associated with patient's disabilities, such as paralysis and aphasia, fluctuation in arousal level, difficulty differentiating between reflexive and voluntary movements, the presence of drugs' side effects and the non-use of standardized and sensitive clinical scales such as the Coma Recovery Scale-Revised (CRS-R) [13]. Furthermore, conventional brain structural imaging studies have shown highly variable and heterogeneous results in patients with DOC, suggesting that a specific brain region cannot be unequivocally related to awareness [21]. This knowledge has lead to the search for other non-clinical assessment techniques in order to better understand brain function in these patients and to overcome the limits of behavioral assessment in the detection of possible retained consciousness in unresponsive patients.

Positron emission tomography (PET)

Positron emission tomography (PET) is a nuclear medical imaging technique for assessing brain activity and function by recording the emission of positrons from radioactively labeled molecules. If the chosen molecule is fludeoxyglucose (FDG), an analogue of glucose, the concentrations of tracer imaged will indicate tissue metabolic activity by virtue of the regional glucose uptake and hence neural activity whereas the O labelled water PET, due to the short half-life of this molecule, is usually used to detect activation during active and passive paradigms [22].

When PET is used to investigate brain death, the so-called "hollow skull sign" is present, accounting for the absence of glucose uptake in the brain and therefore metabolic activity. In these cases the only region showing an uptake of glucose is the skin surrounding the skull [4]. In comatose patients, PET studies showed on average a reduced grey-matter metabolism up to 50-70% of normal range in patients of traumatic or hypoxic origin [4]. After recovery from post-anoxic coma, cerebral metabolic rates for glucose show no drastic increase, exhibiting glucose rates only up to 75% of the normal values [23]. Global cerebral metabolism was shown to correlate poorly with the level of conscious-

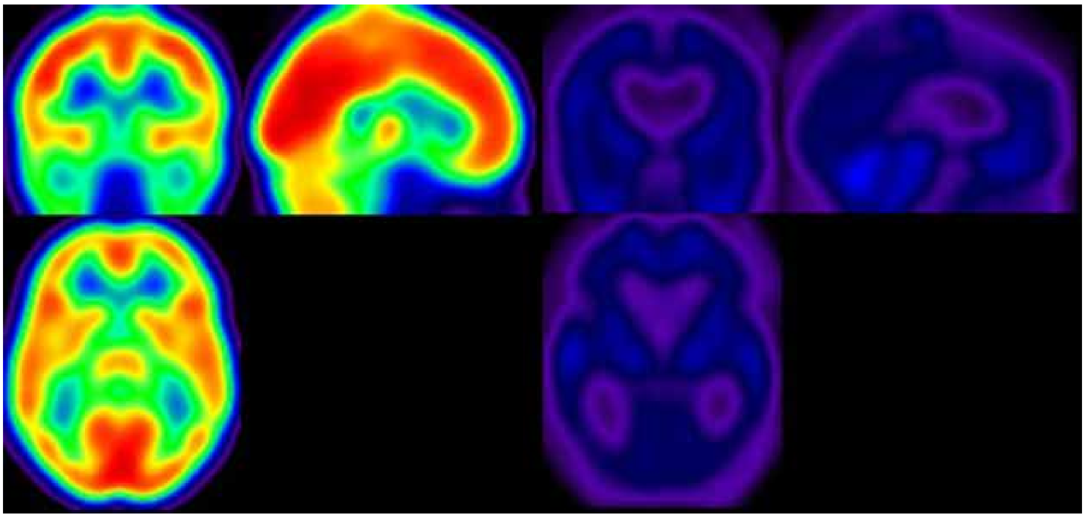

Figure 1
Global brain metabolism detected by FDG-PET in a healthy control (left) and in a patient in an unresponsive wakefulness syndrome (right). Red colorscale indicates regions with high consumption of glucose; blue colorscale indicates regions with low consumption of glucose.

ness, as measured by the Glasgow Coma Scale, in patients studied within the first month after head trauma [24]. Furthermore, no established relation between cerebral metabolic rates of glucose or oxygen as measured by PET and patient outcome has been found. A global decrease in cerebral metabolism, in fact, is not unique to coma, as it can be associated to several different situations with temporary loss of consciousness, such as deep sleep [25], general anesthesia [26] and Cotard's syndrome [27].

In VS/UWS, where awareness is impaired whilst wakefulness is spared, PET studies showed that unresponsive patients are characterized by reduced global metabolism compared with healthy subjects *(Figure 1)*. Interestingly, recovery of consciousness from VS/UWS does not necessarily coincide with resumption of global metabolic activity [17]. PET voxel-based studies have indicated that impairment of awareness is related to impairments in specific brain areas, consisting of a large-scale fronto-parietal network encompassing the polymodal associative cortices [28]. The higher-order associative fronto-parietal network mentioned above has been recently functionally subdivided into two different networks: extrinsic awareness network and intrinsic awareness network [29]. The extrinsic awareness network (also known as executive control network – ECN) encompasses the lateral fronto-parietal brain regions and it is related to the sensory awareness or awareness of the environment. The intrinsic awareness network (most widely known as the default mode network - DMN), encompasses mainly the medial prefrontal cortex, the precuneus and the bilateral posterior parietal cortex, and it is related to awareness of self and self-related processes, such as mind-wandering and autobiographical thinking [30]. Patients in VS/UWS have shown metabolic impairment of both the internal and external awareness networks [31], and to their connections with thalamic nuclei. This latter evidence stems from the recovery of this thalamo-cortical activity in a VS/UWS patient who had subsequentially recovered consciousness [32]. PET studies using passive auditory and noxious stimulation [33, 34] have furthermore demonstrated a peculiar disconnection in VS/UWS patients between the primary sensory areas and these large-scale associative fronto-parietal cortices, which are thought to be required for conscious perception [7]. In contrast, function in known arousal structures including the reticular formation in the brainstem, the hypothalamus, and the basal forebrain appeared to remain relatively intact [4]. In line with their clinical condition, patients in MCS show a partial preservation of this large-scale associative fronto-parietal network [35]. In particular, it has been demonstrated recently that MCS patients show a better preservation of the external awareness network rather than internal awareness network, which could suggest an impairment of self-awareness otherwise difficult to detect at the bedside [31]. In contrast to VS/UWS, PET studies employing passive stimulations have further confirmed a partial preservation of the associative fronto-parietal cortices thought to be required for conscious perception. More interestingly, PET studies employing passive noxious stimuli, have elicited the activation of association areas related to pain processing in MCS patients in a similar network as in normal controls, suggesting therefore a potential pain perception capacity in this patient category [22]. This information supported the idea that MCS patients might need analgesic treatment and has further led to the validation of the Nociception Coma Scale, which assesses behavioral responses to nocistimulation in patients surviving coma [36].

In terms of diagnostic accuracy, cerebral metabolic information obtained by PET has shown to be able to specifically and reliably differentiate VS/UWS from LIS patients and healthy controls [37]. It was based on PET investigation of brain function in MCS that the subcategorization into MCS PLUS and MCS MINUS was suggested. In particular, patients in MCS PLUS exhibited preserved metabolism in language comprehension related area, which is in accordance with their capacities to follow simple commands and/or communicate intentionally [15].

Functional magnetic resonance imaging (fMRI)

Functional magnetic resonance imaging (fMRI) relies on the natural diamagnetic properties of oxygenated hemoglobin and paramagnetic properties of desoxygenated hemoglobin to analyze changes of blood oxygenation in the brain associated with neural activity (blood oxygenated level dependent –BOLD- signal). As it does not require the use of X-rays and radiotracers injection, and for its superior spatial and temporal resolution in comparison to PET, it has been increasingly used for detecting brain activity changes. In the last decade, fMRI has been largely used in patients with DOC in order to detect brain activity related to residual cognition and awareness, and in some cases even established two-way communication, without requiring any behavioral output from patients [38]. For instance, a recent fMRI study using mental imagery tasks (imagining playing tennis vs. spatial navigation around one's house) showed that in a large cohort of 54 patients with DOC, five (3 patients in VS/UWS and 2 patients in MCS- two of them who did not show any signs of consciousness at behavioral assessment) were able to willfully modulate their brain activity; furthermore, one behaviorally patient in VS/UWS was able to use this technique to correctly respond with yes (by imagining playing tennis) or no (by imagining visiting the rooms of his house) to autobiographical questions during the fMRI scanning. This study showed that 17% of patients diagnosed as in VS/UWS after behavioral assessment can follow commands when such commands need a change in blood oxygenation level dependent response, rather than overt motoric behavior. Similarly, a further study using selective auditory attention showed that 3 behaviorally unresponsive patients out of 3 (2 in MCS and 1 in VS/UWS) were able to convey their ability to follow commands, and 1 of them in VS/UWS was even able to use attention to correctly communicate answers to several binary questions [39]. This evidence shows how different tasks, which allow to overcome the motor unresponsiveness of brain injured patients by tackling different cognitive aspects, might detect residual covert awareness in this patient category.

It is worth to stress that absent command-related brain activation does not allow to infer that awareness is not present [40]. Indeed, out of 31 patients in MCS described in the study by Monti *et al.* [38], only one was able to willfully modulate his brain activity. This could be related to several reasons. For example, a patient may not have understood the task instructions because of deafness or aphasia, or because of its fluctuating level of consciousness, or simply because he was not willing to perform the task, leading to possible false negatives results [41]. In this context, resting-state fMRI is a non invasive technique used to investigate the spontaneous temporal coherence in BOLD fluctuations related to the amount of synchronized neural activity (*i.e.*, functional connectivity) existing between distinct brain locations, even in the absence of input or output tasks [42]. This technique has been increasingly used in the analysis of patients with DOC, mainly because it is non invasive and does not require any effort or feedback from the patient. Among the several functional networks that have been detected so far [43], the DMN has attracted most attention. As stated above, the DMN is defined as a set of areas, encompassing posterior-cingulate/precuneus, anterior cingulate/mesiofrontal cortex, temporoparietal junctions and hippocampi [5], that show more activity at rest than during attention-demanding tasks. Because of its link to internally-oriented cognitive content, DMN has been thought to be implicated in consciousness processes [30, 44, 45]. Across the groups of patients in coma, VS/UWS, MCS, it was shown that DMN areas exhibited reduced functional connectivity which was correlated to the degree of consciousness [46]. Recently, more networks at resting state have been investigated in DOC, such as the bilateral fronto-parietal or executive control networks, salience, sensorimotor, auditory, visual systems, and the cerebellar network. It was found that the DMN, the bilateral executive control networks and the auditory system were significantly less identifiable (in terms of spatial and neural properties) in patients with DOC compared to healthy controls and showed consciousness-level dependent decreases in functional connectivity across the spectrum of DOC (*Figure 2*). Eventually, with machine learning classification trained on the identification of these ten networks as neuronal or not, it was able to accurately separate healthy controls from patients in DOC with 85% accuracy [47]. The potential prognostic value of DMN connectivity was shown in a cohort of patients in the acute stage of coma for whom the presence of DMN functional connectivity was paralleled to subsequent reversibility of coma [48].

Decreased DMN connectivity, however, is not unique to DOC, as it can be associated to several different conditions such as other physiological and pharmacological loss of consciousness (sleep and general anesthesia [49]) and pathological conditions such as Alzheimer Disease [50] and drug related states such as alcohol [51] and amphetamine [52]. Nevertheless, the persistence of coherent DMN connectivity in some patients in VS/UWS as well as in a known case of anesthetized monkey [53], in contrast with its complete absence in case of brain death [54], suggests that further phenomena may modulate the interplay between consciousness and the DMN. Concurrently with decreased DMN connectivity, we recently reported paradoxical hyperconnectivity in limbic structures in DOC patients (11 VS/UWS and 7 MCS patients) compared to healthy controls, and more strongly in VS/UWS than in MCS patients [55]. This hyperconnectivity may represent an epiphenomenon of global decrease in neural projections in patients, indicating more resistant connection between DMN and limbic structures. Alternatively, it

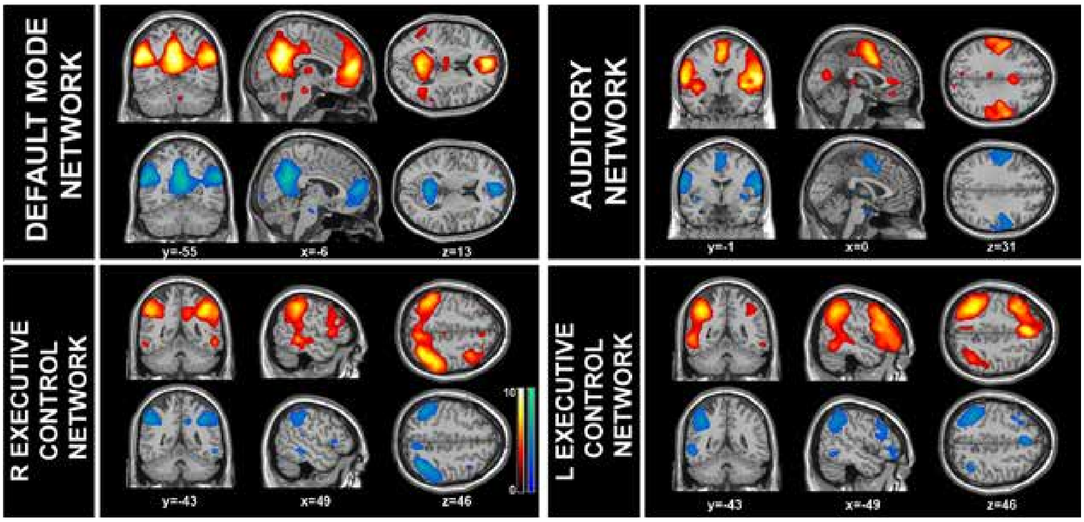

Figure 2
fMRI resting state connectivity in long-range (*i.e.,* default mode network, right and left executive control) and sensory cortical networks (*i.e.,* auditory) is disrupted in patients with disorders of consciousness (blue areas) compared to healthy controls (in red). Images are shown on triplanar anatomical slices.

may reflect the persistent engagement of residual neural activity in self-reinforcing neural loops which may disrupt normal patterns of connectivity [55]. This complex picture is in line with previous theories explaining diffuse (extralimbic) connectivity reduction in patients with DOC, and might delineate a condition in which dysfunctional hyperconnectivity may impair awareness by permanently engaging critical neural resources. This scenario would suggest a much more complex and multifaceted brain connectivity architecture in DOC patients than previously thought. This being also recently supported by the detection of hyperconnectivity patterns also in states of pharmacological coma, such as general anesthesia [56].

Along with intra- and inter-network connectivity, the importance of anti-correlation among different networks has further attracted scientific attention. There is now increasing evidence that the DMN network, or internal awareness network, and the executive control network, or external awareness network, show routinely an anti-correlation, *i.e.* when one is active the other is not and vice-versa. [30]. A decreased anti-correlation of these two networks in shown in unconscious states, such as anesthesia [57], deep sleep [58] and VS/UWS patients [54]. These findings highlight, at least partially, that the anti-correlated pattern between DMN and the executive control network may be of functional significance to conscious cognition of subjectivity [59].

However, the study of resting state activity in DOC can be challenging to both clinical and methodological issues. For example, patients that show pre-scan motion activity might need to be anesthetized during the scan in order to reduce the motion artifacts. In this case, the effect of anesthesia will need to be accounted during the processing of the acquired data. An example of a methodological problem is the spatial normalization of these often severely severely deformed brains. This issue has been partially tackled in previous studies, where a mid-template [55] or study-template [37] was generated by taking into account the mean size of the group of patients and healthy controls, in order to use the same level of deformation in both patients and controls' groups. Finally, identified resting state connectivity patterns need to be interpreted according to the studied population. The future challenges will be to interpret resting-state patterns according to studied population, for example, in order to unravel the relationship (correlation and anti-correlation) between the resting state networks in different level of consciousness, and to better comprehend its functional and clinical meaning in general and at single subject level. A further challenge will be to move from static functional connectivity measurements to the assessment of temporal dynamics of connectivity, namely looking at the changes of correlation and anti-correlation among networks across time. This becomes imperative when considering the dynamic nature of intrinsic connectivity, which characterizes most areas of the brain beyond the DMN [49].

However, in practice fMRI is not suitable for all patients because it requires placing the subject into an MRI scanner. Therefore, its use is virtually impossible for those patients carrying non-compatible MRI devices (pace-makers, metallic implants etc), often present in an intensive care setting. Similarly, fMRI is not suited for patients at home as a "communication device". In such cases, the use of electrophysiological recordings is more practical and appropriate.

Electroencephalography and transcranial magnetic stimulation (combined with electroencephalography)

Like fMRI, electroencephalography (EEG) recordings in patients with DOC can evaluate different aspects of cognitive residual function and provide means to communicate with the outside world without motor output. Standard recordings in the neurological department offer a first global view of the electrogenesis of a patient and can detect abnormal activity and therefore guide treatment [60]. In resting conditions, various EEG paradigms have made an effort to differentiate between the clinical entities of patients with DOC. EEG is routinely used to confirm the diagnosis of brain death and can be of diagnostic importance in some cases of complete LIS patients [61]. Generally speaking, following a severe brain injury, whether it is of traumatic or anoxic origin, the EEG can be altered and display abnormalities. A visible main effect is a slowing of the brain activity proportional to the severity of the injury. Therefore, the predominant rhythm is no longer posterior alpha (related to the awake stages in adult healthy adult individuals) but diffuse theta or delta (normally present in the slow stages of sleep in healthy adult individuals). In some cases alpha or theta activity can be observed, but its activity differs from a normal adult alpha activity [61].

Regarding DOC patients, measures of signal complexity such as the bi-spectral index (a measure of the depth of anesthesia) were shown to discriminate between patients in VS/UWS and patients in MCS, at the group level [62]. The bi-spectral index was also positively correlated with behavioral scores of awareness at the time of testing and was associated with outcome at 1-year post-trauma.

However, at the single-subject level, establishing a diagnosis solely based on a single standard EEG is difficult since the patterns are not specific of the etiology and the same subject can have varying patterns in short intervals. A study based on patients in persistent VS/UWS concluded that there was no possible diagnostic use of EEG due to its heterogeneous and varying aspects [63]. Despite the limited diagnostic role of standard EEG recordings, prognostic statements are possible but challenging as the same pattern can be found in encephalopathy of different origins. Furthermore, the outcome does not depend uniquely on the brain affection itself but on the overall condition of the patient. EEG information needs, therefore, to be backed-up by etiology in order to have insights on the prognosis [60].

In this context, active command mental paradigms combined with EEG [64-66] or electromyography [67] appear to be more convenient in the diagnosis of DOC. They have, in fact, allowed both detection of voluntary brain function in VS/UWS and functional communication in patients with complete LIS. A recent study showed that out of 16 studied patients, 3 of them who seemed to be entirely in VS/UWS on the basis of repeated specialist behavioral assessment, were found to be aware and capable of substantially and consistently modulating their EEG responses upon command [66]. However, as pointed out above, we cannot infer any diagnostic information from a negative result, as it does not necessarily imply the lack of consciousness. In fact, as mentioned earlier, patients with minimal consciousness might still not be able to understand and follow instructions. In this context, EEG combined with TMS (TMS-EEG) may be especially useful to assess the level of consciousness in DOC patients, because it does not rely on a subject's ability to process sensory stimuli, to understand and follow instructions, or to communicate. TMS-EEG allows to non-invasively stimulate a subset of cortical neurons, and to measure the effects produced by this perturbation in the rest of the brain [68, 69]. For patients in VS/UWS, when stimulating a superficial region of the cerebral cortex, TMS either induced no response or triggered a simple, local EEG response, indicating a breakdown of effective connectivity (*i.e.*, the influence that one brain region exerts on another [70, 71]) similar to the one observed in deep sleep and anesthesia [69, 72]. In contrast, for patients in MCS, TMS triggered complex EEG activations that sequentially involved distant cortical areas, similar to activations recorded in patients in LIS and healthy awake subjects. Interestingly, a patient in MCS assessed during a period of no responsiveness still showed complex and widespread brain responses to TMS, even though no conscious behavior could be observed at the bedside [71]. Furthermore, an empirical measure of brain complexity, the perturbational complexity index (PCI), which gauges the amount of information contained in the integrated response of the thalamocortical system to a direct TMS perturbation, has recently been introduced [68]. The PCI was tested on a large data set of TMS-evoked potentials recorded from healthy subjects during wakefulness, dreaming, non-rapid eye movement (NREM) sleep, and different levels of sedation induced by different anesthetic agents (midazolam, xenon, and propofol) as well as from brain-injured patients who had emerged from coma (overall, 208 sessions in 52 subjects). Empirically, PCI showed to provide a data-driven metric that can discriminate level of consciousness in single subjects under different conditions: wakefulness; dreaming; the LIS; the MCS; the EMCS; intermediate levels of sedation; NREM sleep; midazolam-, xenon-, and propofol-induced loss of consciousness; and the VS/UWS [68]. Because this technique is handy, not invasive, does not require patients' cooperation and works at the single-subject level, it appears to be a promising tool for the diagnosis of patients with DOC [68].

STRUCTURAL MRI

Diffusion tensor imaging

Diffusion tensor imaging (DTI) is an extension of diffusion weighted imaging which is based on the principle that water molecule movement is restricted by barriers to diffusion in the brain depending on tissue organization. The diffusion of water protons is higher along fiber tracts than across them in the white matter, which allows for directional measurement of diffusion and, hence, measurement of structural integrity. DTI differs from diffusion weighted imaging for the higher number of directions taken into considerations when studying the water flow (> 6). DTI data can be used to compute the fractional anisotropy as well as to track fibers *(Figure 3)*. The fractional anisotropy quantifies anisotropic diffusion in the brain, which is related to the density, integrity, directionality and crossings of white matter tracts [73, 74]. DTI evaluates the architectural organization of white matter fibers and is a powerful

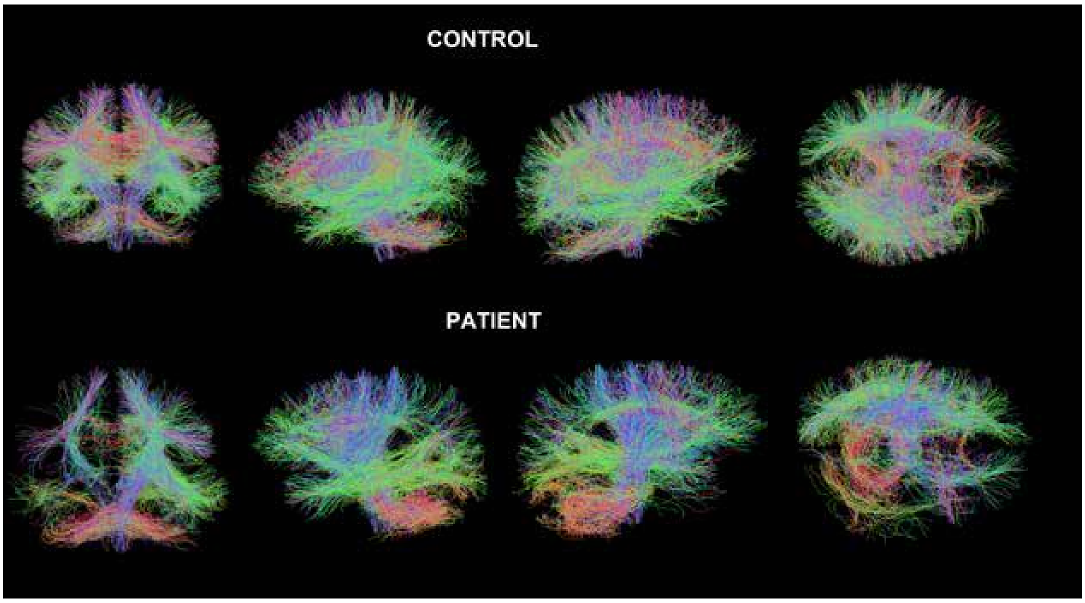

Figure 3
Neural tracks obtained with DTI in a healthy control (top) and a patient in MCS (bottom) confirms the structural damage which is evident in the temporo-parietal regions of the right hemisphere (bottom right - T1 MRI structural image). Colors indicate directionality of water diffusion: red = left-right; green = anterior-posterior; blue = superior-inferior.

technique for *in vivo* detection of diffuse axonal injury after brain trauma [75].

One of the advantages of DTI is the possibility to evaluate brain trauma even in sedated patients, as its values are theoretically not influenced by sedatives or hypnotics, such as during clinical exams or fMRI studies. So far, DTI has been mostly used in the attempt to assess the prognosis in traumatic brain injured (TBI) patients.

In TBI patients a significant negative correlation has been reported between fractional anisotropy in the splenium of the corpus callosum and in the internal capsule and Glasgow Coma Scale (GCS [76]) score at discharge [77]. A first study evaluating the combination of DTI and magnetic resonance spectroscopy (MRS) as a tool for predicting long-term outcome of traumatic patients, [78] showed that fractional anisotropy was significantly lower in patients who did not recover at all measurement sites, except in the posterior pons. The prediction of non-recovery after 1 year could be calculated with up to 86% sensitivity and 97% specificity when taking into account both DTI and MRS values. Non-recovery of traumatic patients was also shown to be correlated with decreased fractional anisotropy in cerebral peduncle, posterior limb of the internal capsule, posterior corpus callosum, and inferior longitudinal fasciculus [79].

Recent multicentric studies have further shown that white matter assessment with quantitative DTI increases the accuracy of long-term outcome prediction compared with the available clinical/radiographic prognostic score both in TBI and anoxic patients following cardiac arrest [80, 81] As regards diagnostic accuracy, a recent study used DTI to assess the neuropathology of 25 patients in VS/UWS and MCS *in vivo* and to identify measures that could potentially distinguish the patients in these two groups [82]. The MCS and unresponsive patients differed significantly in subcortical white matter and thalamic regions, as measured by means of diffusivity (MD) maps, but appeared not to differ in the brainstem. DTI results predicted scores on the CSG and successfully classified the patients in to their appropriate diagnostic categories with an accuracy of 95% [82]. Furthermore, DTI showed to be helpful for characterizing etiologic differences in patients in VS/UWS. While there was evidence of marked, broadly similar abnormalities in the supratentorial grey and white matter in a group of both traumatic and anoxic patients, discordant findings were found in the infratentorial compartment, with DTI abnormalities in the brainstem confined to the TBI group [83].

These studies confirm the relevance of using DTI as biomarker for consciousness recovery after a traumatic brain injury. The available data support the possible benefit of this biomarker for early classification of patients and suggest the possibility to provide objective method for classifying these patient populations and therefore to complement the behavioral assessment.

Magnetic resonance spectroscopy (MRS)

Magnetic resonance spectroscopy (MRS) is a noninvasive method that is able to measure resonances of different metabolic compounds in the brain. Thereby, it can potentially provide useful metabolic information on brain damage that may not be visible on structural MR images [84]. Most often, proton MRS (1H-MRS) is used because of the abundance of protons in the human body and its central nervous system. However, phosphorus MRS (31P-MR) is on the increase and may provide specific information on the energy metabolism, in particular. 1H-MRS at intermediate or long echo time (135-288 ms) yields excellent signal-to-noise ratio for the main metabolites choline (Cho), creatine (Cr), N-acetylaspartate (NAA), and lactate (La). Cho is a metabolic marker of membrane synthesis and catabolism, in particular. Its concentration is higher in white than in gray matter and increases when there is an increased membrane turnover or breakdown due to cell proliferation or inflammatory processes. Cr is considered as a marker of the aerobic energy metabolism. Under the assumption that it remains fairly constant across different pathological conditions, it is widely used for calculating metabolite ratios such as NAA/Cr and Cho/Cr ratios. However, this assumption must clearly be challenged. Alternatively, the water resonance peak can be used for referencing. However, in TBI with brain atrophy cerebrospinal fluid contamination of the spectroscopically measured volume of interest will be increased and partial volume needs to be corrected for. This pertains to any of the metabolites measured in DOC. NAA is found in both gray and white matter in approximately equal quantities as a marker of neuronal density and viability produced in the mitochondria of the neurons and transported into the neuronal cytoplasm and the axons [21]. It is, on the other hand, also found in glial components of the central nervous system. La is a marker of anaerobic glycolysis. Normally, La remains below or just around the reliable limits of detectability in the normal brain, which are commonly determined by the Cramer-Rao lower bounds of the metabolite quantification. La may increase in the course of hypoxic, ischemic or severe post-traumatic brain injuries. Most often, these increases will be transient but detecting them at the right time may be of prognostic value. These issues have not yet been investigated systematically.

In order to assess brain metabolism in coma survivors, it is suggested that a comprehensive MRS protocol should include an axial chemical shift imaging (CSI) multivoxel spectroscopy at the level of the basal ganglia covering the thalamus, insula, and periventricular white matter for the supratentorial assessment and a single-voxel point-resolved 1H spectroscopy (PRESS) placed on the posterior two-thirds of the pons for the infratentorial assessment [21]. Previous investigations have indicated that 1H-MRS may be a valuable tool to predict patient outcome. In particular, NAA/Cr ratios seem to be correlated with recovery of TBI patients while no clear link with other metabolite ratios such as Cho/Cr has been observed [85]. Other studies demonstrated that metabolic changes in TBI patients are detectable by MRS even in the immediate days early after the trauma [86]. Here, NAA was found to be decreased. Its level was correlated with the initial GCS and the outcome at 3 months. Notably, NAA/Cho ratios were not suited to disentangle patients who regained consciousness from those who did not recover [87]. Other investigations have pointed to a significant correlation between NAA/Cr ratio and outcome of TBI patients in the gray and white matter of occipito-parietal [88, 89], frontalbrain areas [90], the splenium of corpus callosum [91], and thalamic brain regions [92]. In addition, pontine MRS recorded in the acute phase after a trauma may allow to separate patients who recover from patients left with severe neurological impairments, in VS/UWS or those that actually die [93]. Three distinguishable pontine MRS profiles have been proposed after head trauma: 1) a normal profile (with higher peak of NAA than Cho and Cr); 2) the neuronal-loss profile with decreased NAA peaks (going down nearly to the level of the Cr peak); and 3) the gliosis profile with an increased Cho peak, no change in the Cr or NAA peak and the associated metabolite ratios. Overall, NAA/Cr ratios seem to be of a better predictive value than NAA/Cho ratios in evaluating traumatic patients and its decrease appears to be a quite reliable index of unfavorable outcome [84]. NAA does indeed decrease immediately after a severe brain trauma. Subsequently, it seems to decline to a minimum within 48 h. After that, NAA levels remain stable within the first month after the injury. Therefore, MRS assessments during the second or third week after TBI can be considered valid markers of the degree of the traumatic impact on the brain [94, 95]. Between 6 weeks and 1 year after the insult, the evolution of the NAA/Cr ratio is much more heterogeneous. Here, NAA levels have been shown to decrease or increase but partial volume corrections have not been consistently carried out which may be important to improve the sensitivity and specificity of the findings in this period following a TBI.

As indicated above, the use of metabolite ratios may be problematic insofar that their common denominator Cr is very likely not to be unaffected by TBI. Cr resonances may be reduced in hypermetabolic and raised in hypometabolic states [96, 97] and this may well bias recordings obtained in mild-traumatic-injured patients [98]. Therefore, accurate MRS quantification and repeated longitudinal measurements will be the clue to improve MRS performance and our insights, which we can gain from this technology. Extension to 31P-MRS may be supplement further investigations as a biomarker of the altered energy metabolism associated with DOC.

CONCLUSIONS

In the last decade, we have witnessed the development and the validation of standardized behavioral scales and neuroimaging/EEG techniques to better understand the variable conditions of patients with DOC. The need to objectively measure phenomena associated with DOC has, in fact, boosted an increased use of the neuroimaging/EEG techniques. Here we have reviewed the basic principles of how each of the different techniques (PET, FMRI, EEG, TMS-EEG) provides us with unique information about brain function in DOC patients. We have alerted the reader to the possible drawbacks of the single techniques. For example, PET, which investigates brain metabolism based on radiotracer uptake, is not suitable as a functional communication device. fMRI, giving indirect measure of neural activity by BOLD measure, requires patients to be placed in an MR scanner, making it also not easily suited or impractical as a communication devices, at least in broader application. Furthermore, activation tasks can easily lead to false negative results (*i.e.* if we get positive results we can infer about the presence of brain activity in patients but we can not infer much from a negative result). DTI and MRS provide indirect measures, respectively, of the structural integrity of WM tracts and of brain metabolism. EEG is a direct recording of neural activity, but establishing a diagnosis solely based on a single standard EEG is difficult since the patterns are not specific of the etiology. Finally, the statistical analysis of such data may require substantial training. Data analysis is further challenged by the specific brain injuries and their sequelae in these particular type of patients which often suffer from profound atrophy, focal brain lesions etc. Similarly, inferring from group-level analysis results to a single given patient is not yet possible but would be what matters in the clinical context. As one single technique can give partial information on the patient's diagnosis and prognosis, we believe in the need of combining structural/functional neuroimaging and neurophysiological techniques in order to obtain a more holistic vision of the disease per se and of the single subject. We believe that in a far reach perspective a wide integration of the neurophysiological and neuroimaging available technique may drastically improve our diagnosis and subcategorization of DOC even at single patient level. Eventually, this may allow us to translate the results of such studies into clinical decisions relevant to the individual patient under our care.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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Appendix D

Paper IV

Function-structure connectivity in patients with severe brain injury as measured by MRI-DWI and FDG-PET

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Stender J, Martial C, Wannez S, D'Ostilio K, Amico E,
Antonopoulos G, Bernard C, Tshibanda F, Hustinx R, Laureys S.

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Function–Structure Connectivity in Patients with Severe Brain Injury as Measured by MRI-DWI and FDG-PET

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Abstract: A vast body of literature exists showing functional and structural dysfunction within the brains of patients with disorders of consciousness. However, the function (fluorodeoxyglucose FDG-PET metabolism)–structure (MRI-diffusion-weighted images; DWI) relationship and how it is affected in severely brain injured patients remains ill-defined. FDG-PET and MRI-DWI in 25 severely brain injured patients (19 Disorders of Consciousness of which 7 unresponsive wakefulness syndrome, 12 minimally conscious; 6 emergence from minimally conscious state) and 25 healthy control subjects were acquired here. Default mode network (DMN) function–structure connectivity was assessed by fractional anisotropy (FA) and metabolic standardized uptake value (SUV). As expected, a profound decline in regional metabolism and white matter integrity was found in patients as compared with healthy subjects. Furthermore, a function–structure relationship was present in brain-damaged patients between functional metabolism of inferior-parietal, precuneus, and frontal regions and structural integrity of the frontal-inferiorparietal, precuneus-inferiorparietal, thalamo-inferioparietal, and thalamofrontal tracts. When focusing on patients, a stronger relationship between structural integrity of thalamo-inferiorparietal tracts and thalamic metabolism in patients who have emerged from the minimally conscious state as compared with patients with disorders of consciousness was found. The latter finding

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Additional Supporting Information may be found in the online version of this article.

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was in line with the mesocircuit hypothesis for the emergence of consciousness. The findings showed a positive function–structure relationship within most regions of the DMN. *Hum Brain Mapp 00:000–000, 2016.* © 2016 Wiley Periodicals, Inc.

Key words: FDG-PET; DWI; disorders of consciousness; function–structure coupling; default mode network

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INTRODUCTION

Massive brain trauma can result in a disorder of consciousness (DOC), such as the unresponsive wakefulness syndrome (UWS) [Laureys et al., 2010], or minimally conscious state (MCS) [Giacino et al., 2002]. Patients who have emerged from MCS (EMCS) are able to functionally communicate and/or functionally use objects, but remain severely handicapped and dependent on full-time care. A substantial body of literature exists on grey matter metabolic (e.g., Fluorodeoxyglucose PET [FDG-PET]) and white matter structural (e.g., MRI-DWI [diffusion-weighted imaging]) brain characteristics in this patient group. Both these methods independently show severe impairments in DOC and EMCS patients [for review see Laureys and Schiff, 2012]. At a global level, functional measures show that metabolism in DOC patients is decreased by up to 40%–50% from their normal value [De Volder et al., 1997; Laureys et al., 1999b; Laureys et al., 2004; Rudolf et al., 1999; Tommasino et al., 1995]. Regional metabolic dysfunction is seen in a widespread frontoparietal, thalamocortical network. The medial part of this frontoparietal network, often called the default mode network (DMN), encompasses midline anterior cingulate/mesio-frontal and posterior cingulate/precuneal associative cortices as well as posterior parietal areas [Nakayama et al., 2006; Thibaut and Bruno, 2012]. In patients with disorders of consciousness, metabolic activity as well as MRI functional connectivity are reportedly more reduced in these regions than in the rest of the brain [Boly et al., 2009; Demertzi et al., 2014; Soddu et al., 2012; Vanhaudenhuyse et al., 2010a).

While the DMN is defined in terms of functional connectivity, there are indications of clear structural underpinnings [Greicius et al., 2009; Van Den Heuvel et al., 2009]. In patients with DOC, these structural connections are known to be damaged. For example, fractional anisotropy (FA), a measure of directionality of water diffusion assumed to be related to myelination of white matter, is specifically reduced in the DMN [Fernández-Espejo et al., 2011, 2012; Gomez et al., 2012].

The cerebral metabolic reductions in DOC are proposed to result from widespread neuronal injury [Thibaut and Bruno, 2012] or disruption of central excitatory drivers [Schiff, 2010]. The latter mesocircuit hypothesis proposes that large-scale dysfunction is due to an important reduction of thalamic excitatory output to the cortex. The observations of impaired metabolism suggest that axonal deafferentiation may be a key driver. We here aim to explore this DMN function–structure relationship in severely brain-damaged patients with varying levels of consciousness as measured by metabolism (standardized uptake value [SUV]) and white matter structural integrity (fractional anisotropy [FA]).

METHODS

Population

PET and MRI data from patients and 25 healthy controls were acquired at the University Hospital of Liège, Belgium. Patients were excluded from this study when preinsult neurological illness, non-compatibility with either MRI or PET was present, or when less than 18 years. Behavioural diagnosis was determined by multiple coma recovery scale revised [CRS-R's; Giacino et al.,] assessments, including assessments on both MRI and PET scan dates. Written informed consent was taken from each healthy subject and the legal guardians of each patient in accordance with the Declaration of Helsinki. The Ethics Committee of the University Hospital of Liège approved the study.

Data Acquisition

MRI data was acquired using a 3 Tesla scanner (Siemens Trio, Siemens Medical Solutions, Erlangen, Germany). Structural MRI T1 data were obtained with T1-weighted 3D gradient echo images using 120 slices, repetition time = 2,300 ms, echo time = 2.47 ms, voxel size = $1 \times 1 \times 1.2$ mm^3, flip angle = 9°, field of view = 256×256 mm^2. Diffusion-weighted images were acquired at a b-value of 1,000 s/mm^2 using 64 encoding gradients that were uniformly distributed in space by an electrostatic repulsion approach [Jones et al., 1999]. Voxels had dimensions of $1.8 \times 1.8 \times 3.3$ mm^3, field of view = 230×230 mm^2, repetition time = 5,700 ms, echo time = 87 ms, and volumes were acquired in 45 transverse slices using a 128×128 voxel matrix. A single unweighted ($b = 0$) image preceded the diffusion-weighted volumes and the 64-volume diffusion imaging sequence was repeated twice.

Five days prior to MRI, an ^{18}F-FDG PET scan was performed 52 ± 13 minutes after intravenous injection of 300 ± 47 MBq of FDG using a Gemini TF PET-CT scanner (Philips Medical Systems). A low-dose CT was acquired

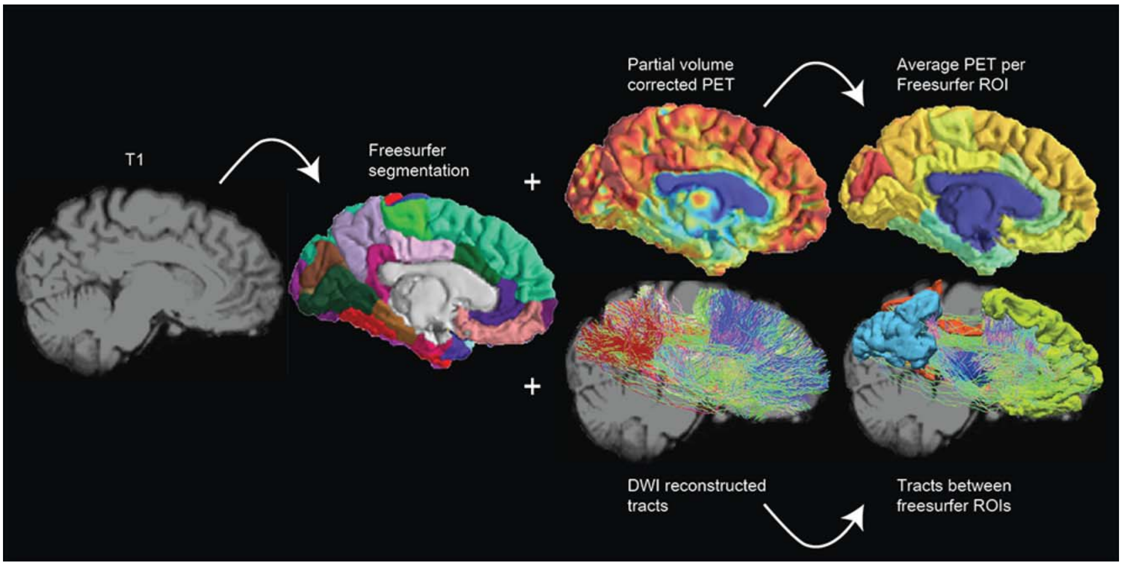

Figure 1.

Schematic representation of the processing pipeline. Data was assessed in subject space where the T1 MRI was segmented using Freesurfer. PET glucose metabolism was estimated by calculation of mean partial volume corrected standardized uptake values within the default mode network ROIs. FA was extracted of the voxels that the DMN tract passed through. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

for attenuation correction, followed by a 12-minute emission scan. The studies were reconstructed using a LOR-OSEM algorithm and reconstructed images had 2 mm^3 isotropic voxels in a 256 × 256 × 89 voxel matrix.

Data Processing

The images of each subject were manually reoriented to the orientation of the MNI 152 1 mm^3 template. Next, each subject's T1-weighted image was automatically labelled using the Desikan–Killiany atlas via the processing pipeline of Freesurfer v 5.3.0 [Desikan et al., 2006]. Several preselected region labels were combined to produce eight regions of interest representing the DMN in both hemispheres. Specifically, regions of interest (ROIs) in the left and right thalamus, inferior parietal cortex, mesio-frontal cortex (encompassing: medial orbital frontal, superior frontal, and rostral anterior cingulate cortex), and precuneus/posterior cingulate cortex (encompassing: precuneus, isthmus, and posterior cingulate cortex) were targeted. Figure 1 shows an example of the processing protocol adopted. The Freesurfer processing pipeline was also used to segment the entire cerebrum into distinct grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) images.

Because the processing of damaged brains is slightly inconsistent across neuroimaging toolboxes, we additionally segmented each T1-weighted image into whole-brain WM, GM, and CSF masks using FAST, part of FSL (FMRIB Software Library v 5.0) [Smith et al., 2004]. A robust cerebral white matter mask was obtained for each subject by multiplying the WM masks produced by the FAST and Freesurfer toolbox. Subsequently, a reliable cerebral brain mask for tractography termination was produced by multiplying the cerebrum mask calculated by Freesurfer (including all cortical brain matter) with the inverse of the FAST cerebrospinal fluid mask. This procedure helped to minimize contamination of masks with non-brain tissue that had been incorrectly labelled.

Diffusion-weighted images were corrected for subject motion by rigid registration of the weighted volumes to the unweighted volume. Rotations applied to the diffusion-weighted volumes were also applied to the corresponding gradient directions [Leemans and Jones, 2009]. Distortion artefacts induced by eddy currents were then corrected by affine registration of the diffusion-weighted images to the unweighted volume. All registrations were performed with FLIRT, part of FSL. The data of some subjects was contaminated by table vibration artefacts which have previously been reported for this model of MR scanner [Gallichan et al., 2010]. The artefact manifested as extraordinarily high diffusion in the left-to-right direction that was clearly visible in calculated RGB-FA images. In our sample it was found primarily in posterior brain areas, though its effects occasionally appeared throughout the brain. We reduced the effect of the artefact by removing volumes in which the absolute value of the x-component of the encoding gradient vector exceeded a manually selected threshold. This threshold was chosen by repeatedly examining the RGB-FA image at distinct thresholds by at least two assessors.

For both analysis and preprocessing diffusion tensors were fit at each voxel using non-linear least squares fitting. Tensor eigenvalues were constrained to positivity by taking their absolute value. This method is known to be robust against noise [Koay, 2009]. FA, tensor mode [Ennis and Kindlmann, 2006], and RGB-FA images were computed. Tensor mode provides a method for quantifying the type of anisotropy (e.g., planar = two fibre populations, or linear = one fibre population) found in the voxel. Mode ranges between −1 (planar anisotropy) and +1 (linear anisotropy) with 0 representing orthotropy. All tensor calculations were performed with Dipy [Garyfallidis et al., 2014].

Affine registration was performed between each subject's white matter mask (in T1 space) and a thresholded FA image ($FA > 0.2$) using FLIRT (nearest neighbour interpolation, mutual information cost function), part of FSL. The translation component of the transformation matrix was modified by adding half of the difference between the fields of view of the DWI and T1-weighted images. This allows the transformation matrix to be used to register T1-derived masks to those in DWI orientation without downsampling. The transformation matrix was applied to the T1-derived white matter mask, cerebral track termination mask, and ROI label map.

A set of voxels with unidirectional diffusion (or a "single fibre population") was identified by eroding and thresholding ($0.8 < FA < 0.99$) the FA image and multiplying this by a map of the thresholded tensor mode ($mode > 0.9$). These operations were performed with fslmaths, part of FSL. Binary single fibre population masks were manually revised to select only voxels that were clearly inside the corpus callosum and corticospinal tracts. These high FA and high mode voxels were used to estimate the diffusion-weighted signal response for a single fibre population. Next, non-negativity constrained spherical deconvolution was performed and fibre orientation distribution functions within each voxel were estimated. A maximum harmonic order of 4 was used for both response estimation and spherical deconvolution. Probabilistic tractography was performed using randomly placed seeds within the subject-specific white matter masks described above. Fibre tracking settings were as follows: number of tracks = 1,000,000, FOD magnitude cutoff for terminating tracks = 0.1, minimum track length = 10 mm, maximum track length = 200 mm, minimum radius of curvature = 1 mm, tracking algorithm step size = 0.2 mm. Streamlines were terminated when they (i) extended out of the cerebrum track termination mask, or (ii) could not progress along a direction with FOD magnitude or curvature radius higher than the minimum cutoffs. A connectivity matrix for the eight-region connectome was computed using the streamline origin and termination points and the ROI label mask. For each streamline the FA was averaged over all the voxels it passed through; meaning that we extracted the FA within the 12 tracts connecting each of our DMN regions of interest. Constrained spherical deconvolution and fibre tracking were performed with MRtrix 0.2.12 [Tournier et al., 2012]. Computation of the connectivity matrices was performed with MRtrix 0.3.

FDG-PET images for each subject were first manually reoriented toward the T1-weighted image using Statistical Parametric Mapping 8 (SPM8; www.fil.ion.ucl.ac.uk/spm) in order to ease later automated registration tasks. FDG-PET images underwent partial volume effect (PVE) correction using the Muller–Gartner–Rousset method [Müller-Gärtner et al., 1992; Rousset et al., 2007] in PVElab v 2.2 [Quarantelli et al., 2004; Svarer et al., 2005]. Partial volume correction aims to remove the spillover of signal to regions that are known to be without activity (e.g., CSF), in order to prevent underestimation of signal in regions with activity (e.g., GM). Grey matter, white matter, and cerebrospinal fluid partial volume estimate images were obtained, as earlier, using FAST. A rigid-body transformation was obtained between the uncorrected FDG-PET and the subject's T1-weighted image to bring the T1 image to PET space (trilinear interpolation, correlation ratio cost function; in six patients, cost function was changed to normalized mutual information to improve registration). The inverse of this transformation matrix was applied to the GM, WM, and CSF partial volume images to bring them into the space of the PET image. The point spread function was modelled by 3D Gaussian function with in-plane full-width at half maximum (FWHM) values of 8 mm. Finally, following PVE correction the PET image was transformed into T1 space and the mean SUV value was extracted within each ROI using in-house software.

For all subjects it was necessary to manually check the performance of the automated labelling and registration procedures. In six patients there were voxels that were clearly mislabelled and required correction. The ROI labels were evaluated and adjusted when deemed necessary by at least two researchers. Processing pipelines were developed in Python using Nipype [Gorgolewski et al., 2011] and are freely available online (https://github.com/GIGA-Consciousness/structurefunction). A figure showing ROIs and tracts can be found in the Supporting Information Fig. 1.

Statistical Analysis

Statistical analysis was done using R [R Core Team, 2014]. First, to test for demographic differences (age and gender) between our two groups (healthy controls and brain-injured patients), we used independent two-sample *t*-tests or chi-square test, respectively. To test for possible differences between left and right brain function/structure we assessed laterality differences using a two sample *t*-tests within brain-injured patients and controls with a "logit" transform of FA and log transformation of SUV to account for parametric test-assumptions. Subsequently, SUV and FA of the left and right hemisphere regions were averaged for each subject. Next, to test the differences between the two groups in mean SUV and FA values two-sample *t*-tests with Bonferroni correction ($\alpha = 0.012$ and $\alpha = 0.008$ respectively) were used. Using a χ^2 test the number of volumes removed during vibration artefact correction was evaluated to identify any potential group bias. Subsequently, multivariate linear regression analysis with group and FA as regressors was performed to model how group and structural integrity (FA) of DMN tracts relate to metabolism (SUV) in adjacent regions. SUV was log scaled to meet normality assumptions. Furthermore, to take into account the differences in variance we weighted the regression function by the inverse of the FA of the corresponding connection. Type II ANOVA's were used to assess significant main and interaction effects. As each SUV ROI is tested three times, Bonferroni correction was used with $\alpha = 0.016$.

We then focused on patients alone to better understand the structure–function relationship in the brain-injured group. Multiple linear regression models were used to investigate how demographic factors as diagnosis (DOC vs. EMCS), aetiology (TBI or non-TBI), disease duration (subchronic vs. chronic), gender, and age influence the function–structure relationship in the patient population. Type II ANOVA's were used to assess significant main and interaction effects. Within the control population we tested for an effect of FA on SUV using a simple linear regression with FA as regressor and SUV as outcome measure.

To assess a possible global effect of SUV and FA we used a multivariate linear regression analysis to model how group and whole-brain structural integrity of white matter (FA over all white matter voxels) relate to whole-brain grey matter metabolism (SUV). Furthermore we performed an analogous regression analysis for the patient and control group separately.

RESULTS

We obtained MRI data of 163 adult, (sub-) chronic (>30 days after injury) patients without pre-existing comorbidities and 14 healthy control subjects between November 2009 and October 2013. Stringent exclusion criteria were applied as visualized in Fig. 2. Patients were excluded because of technical difficulties in either MRI or PET ($N = 25$), more than 5 days between exams ($N = 5$), severe deformations consisting of more than one-third of one hemisphere (e.g., enlarged ventricles, haemorrhage, severe atrophy), metal/drain artefacts ($N = 79$). Data of 54 patients were preprocessed, although 29 subjects had to be excluded because Freesurfer was unable to complete segmentation. Thus, the final cohort consisted of 25 patients and 25 healthy controls. The patients did not differ from control subjects in age ($P = 0.214$; mean age = 36.3 for brain injured and 40.9 for healthy controls) or gender ($P = 0.756$; 13 male against 11 male in brain injured and healthy groups, respectively).

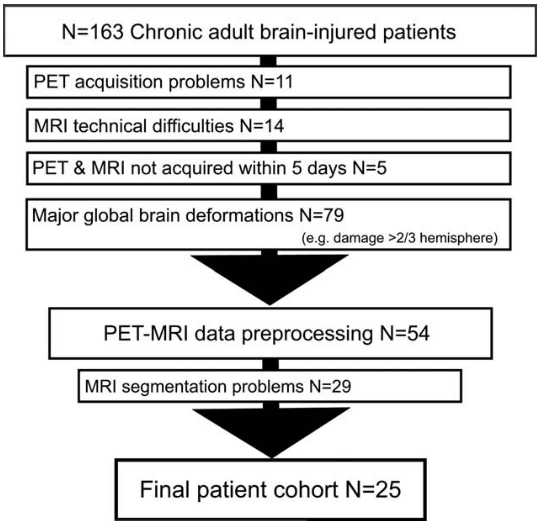

Figure 2.

Flowchart of exclusion criteria. This study was conducted using stringent exclusion criteria. Of the 163 MRI examinations performed at the Coma Science Group between November 2009 and October 2013, 109 were excluded before image analysis. Data preprocessing was done for 54 patients, of which additionally 19 subjects had to be excluded due to inaccurate segmentation. Finally, we included 25 patients in the statistical analysis.

DOC patients had been clinically diagnosed as in an unresponsive wakeful state (UWS, $n = 7$) or a minimally conscious state (MCS, $n = 12$), and diagnosis was consistent with the diagnosis of the day of the PET and MRI scan. Six subjects were diagnosed as emerged from a minimally conscious state (EMCS, $n = 6$). The patient cohort consisted of subacute ($n = 10$, between 30 days and 3 months after onset) and chronic patients ($n = 15$, >3 month after onset) with a mean time since onset of 1.8 years (SD = 1.9 years). Patients suffered from traumatic brain injury (TBI, $n = 12$), anoxia ($n = 11$), both (mix, n = 1), or infection ($n = 1$). For demographic and clinical details see Table I.

The vibration artefact affected 14 of 25 patients and 5 out of 25 controls. On average, 39 diffusion-weighted volumes (30%) had to be removed from patient data and 32 (25%) out of 128 volumes from control subjects. We did not find a difference between the amount of affected volumes between patients and controls [$\chi^2(1, N = 50) = 3.14$, $P = 0.076$].

TABLE I. Patient demographics

Diagnosis	Age	Gender	Etiology	Days (Onset)	GOSE	CRS-R						CRS-R total
						A.	V.	M.	O.	C.	Ar.	
EMCS	33	F	TBI	388	NA	4	5	6	3	2	3	23
EMCS	30	M	TBI	881	3	4	5	6	3	2	3	23
EMCS	37	M	Anoxia	284	3	4	5	6	3	0	2	20
EMCS	45	F	Anoxia	38	3	4	5	6	3	1	1	20
EMCS	31	F	TBI	439	3	4	5	5	2	2	2	20
EMCS	22	M	TBI	2,424	3	3	4	6	3	1	1	18
MCS+	28	M	TBI	589	3	1	2	2	1	0	1	7
MCS+	45	M	TBI	533	3	3	3	3	1	0	2	12
MCS+	47	F	Anoxia	210	NA	3	0	1	1	0	2	7
MCS+	19	M	MIX	1,236	3	3	4	1	2	0	2	12
MCS+	23	M	TBI	752	3	3	4	5	1	0	2	15
MCS+	48	F	Anoxia	292	NA	3	1	2	2	0	1	9
MCS+	49	M	Anoxia	674	1	3	1	3	1	0	2	10
MCS-	29	F	TBI	569	3	1	2	1	2	0	1	7
MCS-	28	M	TBI	634	2	0	1	2	1	1	1	6
MCS-	36	F	Anoxia	549	3	1	3	2	2	1	1	10
MCS-	45	F	Anoxia	259	1	0	0	2	1	0	1	4
MCS-	48	M	Anoxia	1,100	3	1	3	1	2	0	1	8
UWS	40	M	Anoxia	2,890	3	1	0	1	2	0	1	5
UWS	21	M	TBI	196	2	1	0	2	2	0	2	7
UWS	44	F	Anoxia	101	1	0	0	2	1	0	1	4
UWS	54	F	Infection	51	1	0	0	2	1	0	1	4
UWS	20	M	TBI	31	7	0	1	2	1	0	1	5
UWS	48	F	Anoxia	129	1	1	0	0	1	0	2	4
UWS	37	F	TBI	1,192	3	1	0	1	1	0	2	5

EMCS, Emergence from minimally conscious state; MCS, minimally conscious state; UWS, unresponsive wakefulness syndrome; M, male; F, female; TBI, traumatic brain injury; MIX, anoxia and traumatic brain injury; GOSE, Glasgow outcome scale extended; CRS-R, Coma recovery scale – revised; A., Auditory; V., visual; M., motor; O., oromotor; C., communication; Ar., arousal.

To assess if there is an effect of laterality, the difference between SUV values in our ROIs and FA values for connections in the left and right hemisphere were assessed. No differences could be observed within SUV values for controls ($P = 0.794$) or patients ($P = 0.691$), nor in FA values between the left and right tracts (controls; $P = 0.156$, patients; $P = 0.053$). The values of each hemisphere were averaged for subsequent analysis so that each subject was not tested twice.

Functional and Structural Integrity

Metabolism (SUV values) was significantly ($P < 0.001$) lower in brain-injured patients compared with controls (Fig. 3). The average reduction in SUV of patients was 42%. The reduction was strongest in the precuneus (44%) and weakest in the frontal cortex (39%).

FA was also significantly ($P < 0.001$) lower in brain-injured patients compared with controls (Fig. 4). The average reduction of FA was 17%, with the biggest reduction in the fronto-precuneus tract (23%) and the smallest reduction in the thalamo-precuneus radiation (13%). A table indicating all two-sample *t*-tests with Bonferroni correction can be found in the Supporting Information.

Regression Analysis

Explaining SUV through FA and group

Multiple linear regression (Fig. 5; Supporting Information Fig. 2; Tables (II–V) for *P*-values) including the two groups (patients and controls) showed a main effect of FA and of group (with Bonferoni correction of $P < 0.016$). The main effect of group is significant for all the regions and adjacent tracts with a higher SUV for healthy controls than for patients, except for SUV in the inferioparietal cortex with FA from the frontal to inferioparietal tract where only a trend can be observed.

The main effect of FA can be found in five tracts (Fig. 5). This is the case for SUV in the inferioparietal cortex and FA from all assessed tracts toward this ROI (thalamo-interioparietal $P < 0.0001$, frontal-inferioparietal $P = 0.012$, precuneus-inferioparietal $P < 0.0001$), SUV in the precuneus and precuneus-inferioparietal FA ($P < 0.0001$), and SUV of the frontal cortex and thalamo-frontal FA

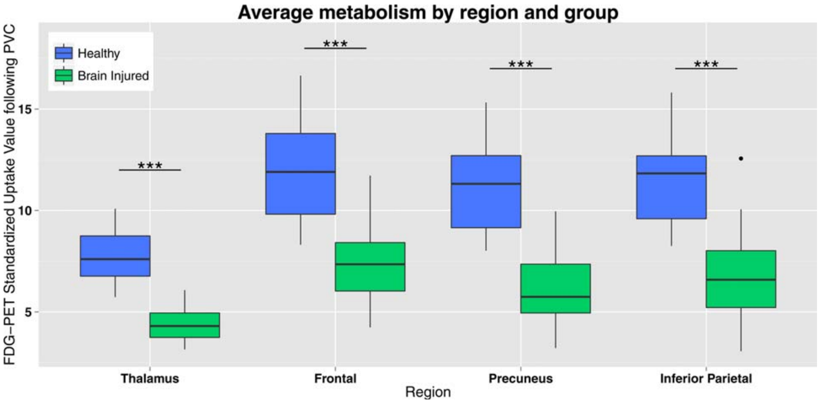

Figure 3.

PET glucose uptake in DMN regions. Standardized uptake value following partial volume correction (PVC) in the default mode network regions (average of standardized uptake values of left and right hemisphere) for healthy controls and brain injured patients. Brain injured patients show a decreased standardized uptake value compared with controls in all default mode network regions. $*** = P < 0.001$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

($P = 0.009$) (Tables II–V). The relationship between FA and SUV within each of the two samples has been further investigated and the results are discussed in the following paragraph.

Explaining SUV through FA and demographic factors

Within patients, we did not find any significant effect of aetiology (TBI or non-TBI), duration, gender, or age in the multiple linear regression model. An interaction effect between group (EMCS vs. DOC) and FA was found (with Bonferoni correction $\alpha < 0.016$) on thalamic SUV and thalamo-inferioparietal FA ($P = 0.006$). Trends were observed for thalamic SUV and the other two tracts toward this ROI (thalamo-frontal $P = 0.04$, thalamo-precuneus $P = 0.017$) (Supporting Information Fig. 4). Furthermore, apart from one, all observed main effects of FA seen in the previous analysis were also observed in the current analysis between the two patient populations. SUV in the inferioparietal cortex is explained by thalamo-

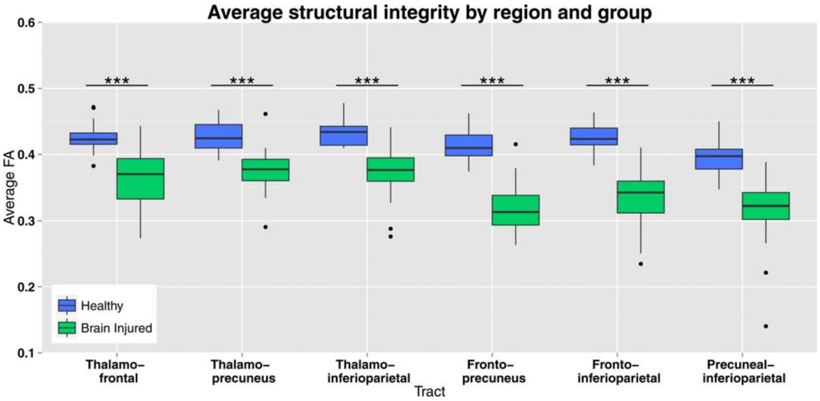

Figure 4.

MRI white matter structural integrity in DMN tracts. Structural integrity of tracts between the default mode network regions represented with FA of the voxels that the tracts pass through, in healthy controls and brain injured patients (average of left and right hemispheres). Brain injured patients show lower FA values compared with healthy controls subjects in all tracts. $*** = P < 0.001$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

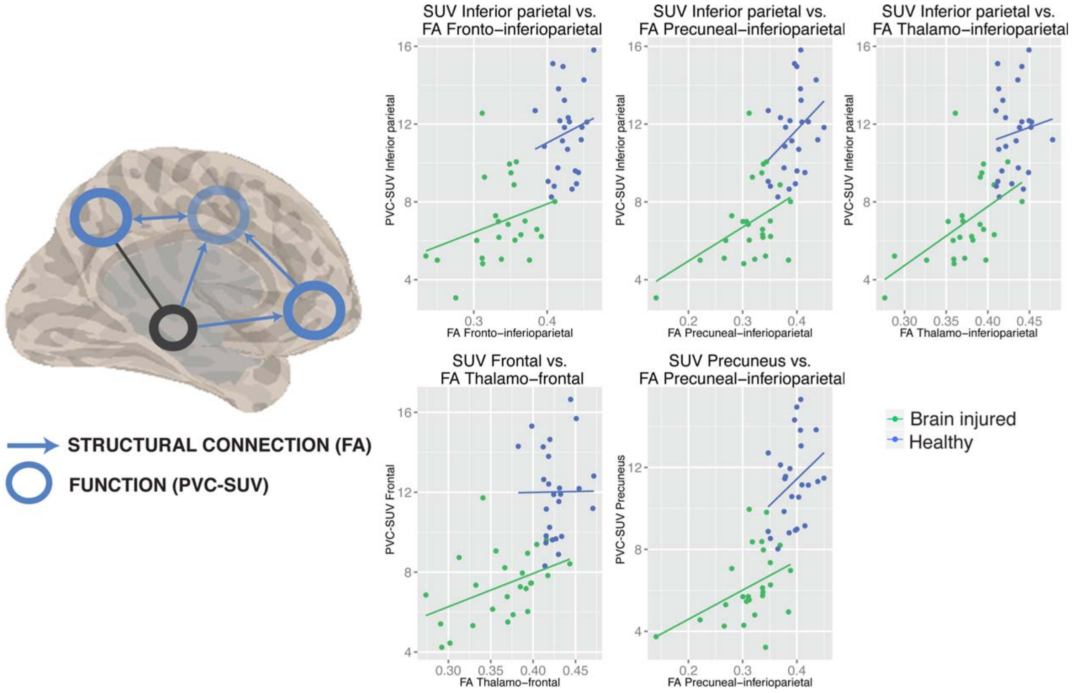

Figure 5.

Linear regression model of the function–structure relationship. Left side of the image shows a spatial representation of the function–structure relationships (*blue circles* for regions where the partial volume corrected - standardized uptake value (PVC-SUV) depended on FA, *blue arrows* for FA of tracts that drive SUV in adjacent regions). Right side of image shows five scatterplots of the linear regression models for healthy controls (*blue dots*), and patients (*green dots*), and significant main effect of FA (*lines*). Abbreviations: FA, fractional anisotropy; PVC-SUV, partial volume corrected-standardized uptake value. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

interioparietal FA ($P = 0.002$), and precuneus-inferioparietal FA ($P < 0.0001$). Similarly, SUV in the precuneus that depends on the precuneus-inferioparietal FA ($P = 0.002$). SUV of the frontal cortex is linearly related to thalamo-frontal FA ($P = 0.012$). Within the healthy population there is no evidence for a main effect of FA on SUV in any of the structure–function pairs (Table VI).

Whole brain regression analysis

To test if these results were limited to the DMN or reflective of general brain integrity, we performed a linear regression analysis to model how group and whole-brain structural integrity of white matter (FA) relate to whole brain grey matter metabolism (SUV). This additional analysis showed a main effect of group ($P = 0.0003$), but not of FA ($P = 0.17$). Instead, a small evidence for an interaction effect could be observed ($P = 0.03$) (Supporting Information Fig. 3). However, we did not find evidence for a structure–function relationship within each subgroup.

DISCUSSION

We here aimed to directly investigate, in severely brain injured patients, the relationship between functional brain activity and structural connectivity within the DMN in an objective and combined fashion using both FDG-PET and FA MRI. We show that a function–structure relationship is present in brain-damaged patients between functional metabolism of inferior-parietal, precuneus, and frontal regions and structural integrity of the frontal-inferiorparietal, precuneus-inferiorparietal, thalamo-interioparietal and thalamofrontal tracts. When focusing on EMCS versus DOC patients, we found a stronger relationship between structural integrity of thalamo-inferiorparietal tracts and thalamic metabolism in patients who have emerged from MCS as compared with DOC patients.

We first assessed function (PET metabolism) and structure (DWI-FA) independently, to replicate previous studies focusing on either measure separately. Indeed, marked impairments in SUV and FA were observed in patients.

TABLE II. Regression analysis SUV of the thalamus, FA, and group (healthy vs. brain injured)

SUV	FA	R^2	Beta	95% confidence interval		Sum Sq	Df	F	P-value	
Thalamus	Thalamo-frontal	0.722								
	FA		0.538	−1.029	2.105	0.026	1	0.309	0.581	
	Group		0.947	−0.819	2.713	4.823	1	56.763	0.000	***
	Interaction		−0.988	−5.216	3.240	0.019	1	0.221	0.640	
	Residuals					3.908	46			
Thalamus	**Thalamo-inferiorparietal**	0.721								
	FA		0.523	−1.358	2.404	0.036	1	0.425	0.518	
	Group		0.425	−1.623	2.474	4.338	1	50.929	0.000	***
	Interaction		0.255	−4.591	5.101	0.001	1	0.011	0.916	
	Residuals					3.833	45			
Thalamus	**Thalamo-precuneus**	0.731								
	FA		1.322	−0.882	3.526	0.083	1	1.007	0.321	
	Group		1.100	−0.641	2.841	4.200	1	50.900	0.000	***
	Interaction		−1.411	−5.623	2.801	0.038	1	0.456	0.503	
	Residuals					3.713	45			

Statistics and confidence interval of the regression models to predict SUV using FA of adjacent tracts and group (brain-injured patients or healthy control subjects). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.00$.

SUV in all DMN regions was lowered in brain-injured patients compared with healthy controls, with a 39%–42% reduction of metabolic rates in brain-injured patients in the cortical DMN regions and thalamus (Fig. 3). This is in accordance with previous findings on a global brain scale [Laureys et al., 1999a,; Rudolf et al., 1999; Stender et al., 2014a,b; Tommasino et al., 1995] and within the DMN specifically [Fridman et al., 2014; Nakashima et al., 2007; Thibaut and Bruno, 2012]. FA in all DMN tracts was diminished by about 13%–23% in brain-injured patients compared with healthy controls (Fig. 4), in line with previous reports [Fernández-Espejo et al., 2011, 2012; Gómez et al., 2012]. Our results support recent findings of diminished structural integrity of corticocortical and subcorticocortical DMN connections, which correlated with clinical severity in a group of eight patients [Lant et al., 2015].

The main aim of this study was to assess the function–structure relationship in the DMN and thalamus in healthy conscious subjects and coma survivors. First, as expected, we have replicated previous studies and shown that patients have significantly lower FA in all studied connections and SUV in all regions. Building on this, we showed that grey matter metabolic function can be partially explained by white matter anisotropy in several regions of

TABLE III. Regression analysis SUV of the frontal cortex, FA, and group (healthy vs. brain injured)

SUV	FA	R^2	Beta	95% confidence interval		Sum Sq	Df	F	P-value	
Frontal	Thalamo-frontal	0.644								
	FA		2.692	0.860	4.524	0.872	1	7.510	0.009	**
	Group		1.502	−0.563	3.566	2.232	1	19.222	0.000	***
	Interaction		−2.732	−7.674	2.211	0.144	1	1.238	0.272	
	Residuals					5.342	46			
Frontal	**Frontal-inferiorparietal**	0.607								
	FA		2.254	0.253	4.255	0.688	1	5.090	0.029	*
	Group		0.836	−1.417	3.088	1.165	1	8.624	0.005	**
	Interaction		−1.254	−6.696	4.187	0.029	1	0.216	0.645	
	Residuals					6.081	45			
Frontal	**Frontal-precuneus**	0.587								
	FA		1.635	−0.754	4.024	0.133	1	0.926	0.341	
	Group		1.574	−0.487	3.635	1.685	1	11.736	0.001	**
	Interaction		−2.955	−8.174	2.264	0.187	1	1.299	0.260	
	Residuals					6.603	46			

Statistics and confidence interval of the regression models to predict SUV using FA of adjacent tracts and group (brain-injured patients or healthy control subjects). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.00$

TABLE IV. Regression analysis SUV of the precuneus, FA, and group (healthy vs. brain injured)

SUV	FA	R^2	Beta	95% confidence interval		Sum Sq	Df	F	P-value	
Precuneus	Thalamo-precuneus	0.675								
	FA		4.249	1.348	7.150	0.863	1	6.037	0.018	*
	Group		2.318	0.026	4.609	3.351	1	23.434	0.000	***
	Interaction		−4.503	−10.047	1.041	0.383	1	2.676	0.109	
	Residuals					6.434	45			
Precuneus	**Frontal-precuneus**	0.622								
	FA		1.865	−0.841	4.570	0.196	1	1.063	0.308	
	Group		1.692	−0.642	4.026	2.527	1	13.725	0.001	***
	Interaction		−3.018	−8.928	2.893	0.194	1	1.056	0.309	
	Residuals					8.468	46			
Precuneus	**Precuneus-inferioparietal**	0.720								
	FA		2.618	1.245	3.990	2.465	1	16.571	0.000	***
	Group		0.469	−1.037	1.976	3.393	1	22.810	0.000	***
	Interaction		−0.148	−4.068	3.772	0.001	1	0.006	0.940	
	Residuals					6.843	46			

Statistics and confidence interval of the regression models to predict SUV using FA of adjacent tracts and group (brain-injured patients or healthy control subjects). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.00$.

the DMN within the patient cohort. More specifically, metabolism of the frontal cortex, precuneus, and inferior parietal cortex can be explained by fronto-inferioparietal, precuneal-inferiorparietal, and thalamo-inferioparietal as well as thalamo-frontal structural integrity (FA). These results are in line with the limited previous studies indicating there might be a link between structural integrity and glucose metabolism. For example, one study correlating metabolism with white matter bundles in the DMN in healthy subjects found that working memory is related to a structure–function correlation in the cingulum [Yakushev et al., 2013]. Further studies have shown that diffusion measures have been correlated to glucose uptake in patients with Alzheimer's disease and dementia [Bozoki et al., 2012; Kuczynski et al., 2010; Yakushev et al., 2011], children with occipital lesions [Jeong et al., 2015], normal aging [Inoue et al., 2008], and epilepsy [Chandra et al., 2006]. However, all of these studies use simple correlations instead of regressions measures, and thus do not take population-specific changes into account. This could result in false positive-correlations, driven by main effects of group on the (in) dependent variables. We provide proof that metabolic function is indeed directly related to structural integrity, surpassing existing correlational results.

TABLE V. Regression analysis SUV of the inferioparietal cortex FA, and group (healthy vs. brain injured)

SUV	FA	R^2	Beta	95% confidence interval		Sum Sq	Df	F	P-value	
Inferior parietal	Thalamo-inferioparietal	0.683								
	FA		5.278	2.989	7.567	2.562	1	20.321	0.000	***
	Group		1.824	−0.668	4.317	1.075	1	8.528	0.005	**
	Interaction		−3.697	−9.593	2.199	0.201	1	1.595	0.213	
	Residuals					5.673	45			
Inferior parietal	**frontal-inferioparietal**	0.585								
	FA		2.961	0.680	5.241	1.216	1	6.928	0.012	*
	Group		0.863	−1.705	3.431	0.953	1	5.426	0.024	*
	Interaction		−1.395	−7.597	4.808	0.036	1	0.205	0.653	
	Residuals					7.901	45			
Inferior parietal	**Precuneus-inferioparietal**	0.668								
	FA		3.283	1.656	4.910	2.477	1	18.739	0.000	***
	Group		0.502	−1.020	2.023	1.400	1	10.591	0.002	**
	Interaction		−0.552	−4.525	3.421	0.010	1	0.078	0.781	
	Residuals					5.949	45			

Statistics and confidence interval of the regression models to predict SUV using FA of adjacent tracts and group (brain-injured patients or healthy control subjects). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.00$.

TABLE VI. Statistics and confidence interval of the regression models to predict SUV of the thalamus using FA of adjacent tracts and group (DOC patients and patients who recovered from DOC)

SUV	FA	R^2	Beta	95% confidence interval		Sum Sq	Df	F	P-value	
Thalamus	Thalamo-frontal	0.200								
	FA		0.270	−1.459	1.999	0.044	1	0.516	0.480	
	Group		−3.965	−7.742	−0.188	0.003	1	0.038	0.848	
	Interaction		10.299	0.456	20.142	0.401	1	4.735	0.041	*
	Residuals					1.777	21			
Thalamus	**Thalamo-inferiorparietal**	0.333								
	FA		−0.282	−2.243	1.679	0.028	1	0.394	0.537	
	Group		−3.891	−6.512	−1.270	0.002	1	0.023	0.882	
	Interaction		9.986	3.252	16.720	0.685	1	9.567	0.006	**
	Residuals					1.431	20			
Thalamus	**Thalamo-precuneus**	0.296								
	FA		0.852	−1.356	3.060	0.118	1	1.607	0.219	
	Group		−6.133	−11.053	−1.212	0.000	1	0.000	0.999	
	Interaction		16.134	3.197	29.071	0.497	1	6.767	0.017	*
	Residuals					1.468	20			

[a] Statistics and confidence interval of the regression models to predict SUV using FA of adjacent tracts and group (brain-injured patients or patients who recovered the ability to functionally communicate or use objects in a functionl manner). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.00$.

Interestingly, we did not find a structure–function relationship at the global brain level, suggesting that our results do not solely reflect general brain integrity. Instead, the function–structure relationship of the DMN might be directly related to consciousness. This has been shown in single-modality studies, for example functional connectivity [Vanhaudenhuyse et al., 2010b), white matter structural integrity [Fernández-Espejo et al., 2011, 2012; Gomez et al., 2012], and metabolic function [Thibaut et al., 2012]. Here we show for the first time a direct function–structure relationship within this network.

As expected, healthy control subjects showed FA and SUV within normal range and therefore we are unable to make inferences about whether one drives the other. Next we investigated the function–structure relationship within our patient population, comparing EMCS with DOC patients (MCS and UWS). EMCS patients are able to use objects and/or functionally communicate, and thus by definition conscious. Apart from one region-connection pair, all observed main effects of FA seen in the healthy vs. brain injured analysis were also observed in the analysis between the two patient populations, indicating that there is a positive linear relation between functional and structural integrity of the DMN. Furthermore, in contrast to DOC patients, EMCS patients show a significantly stronger function–structure interaction between the function of the thalamus and the structural integrity of the thalamo-inferiorparietal tract. On the uni-modal level our results match previous research in post-comatose patients finding that structural cortico-thalamic connections are diminished [Lant et al., 2015] and thalamic metabolism is lowered [Fridman et al., 2014]. These findings can be explained by the mesocircuit theory, which proposes that large-scale dysfunction is due to a global decrease of excitatory neurotransmission which in turn alters cerebral activity. More specifically, the globus pallidus is disinhibited and overactive, inhibiting the thalamic excitatory output to the frontal cortex [Schiff, 2010]. By combining both functional metabolism and white matter structural information we here provide further evidence for the validity of this theory, supporting the hypothesis that thalamo-cortical connectivity plays an important role in emergence of consciousness [Schiff, 2010]. We limited ourselves to the DMN because of the large body of literature on this brain-network relating to consciousness. Therefore, future research should extend these findings to more specific sub-cortical regions, such as the globus pallidus or specific thalamic regions.

We do not find any difference between patients based on aetiology, even though several studies have shown that temporal dynamics of Wallerian degeneration vary given different aetiologies [Kumar et al., 2009; Luyt et al., 2012] and that traumatic brain injury, unlike anoxia, might selectively affect DMN white matter integrity [Bonnelle et al., 2011; Warner et al., 2010]. Multicentre collaborations should provide sufficiently large datasets to study these effects in the future.

Methodologically, several comments can be addressed when dealing with brain-injured patients, especially concerning normalization, SUV, and tractography procedures. We here chose to perform a within-subject ROI labelling rather than applying a common atlas after spatial normalization as this latter procedure might results in a lack of inter-subject anatomical correspondence in severely injured brains. As there is no consensus on the most reliable calculation of standard uptake value, we accounted for the partial-volume effect [Rousset et al., 2007]. Tractography based on constrained spherical deconvolution is optimal with b-values of 2,500–3,000 s/mm^2 [Tournier et al., 2013], but crossing fibres can still be more reliably modelled than with simple DTI-based models using our lower b-value of 1,000 s/mm^2 [e.g., see for effective application: Roine et al., 2015]. Future studies should strive to acquire diffusion-weighted images using isotropic voxels, as anisotropic voxel sizes produce datasets in which the fibre orientation estimates depend on the position of the subject in the scanner. Anisotropic voxel sizes were mitigated in this study by linear interpolation of the fibre orientation distributions during the fibre tracking step.

CONCLUSION

We here assessed the function–structure relationship within healthy, conscious subjects and severely brain damaged patients with varying levels of consciousness through direct combined investigation of function (FDG-PET), and structure (MRI-DWI). Levels of structural integrity (FA) and metabolic function (standardized metabolic rates) are significantly diminished in patients compared with controls. Furthermore, a significant positive function–structure relationship can be observed within most regions of the DMN. This relationship may be network-specific, as it does not appear at the whole-brain level. Finally, we show that EMCS compared with DOC show a significantly stronger thalamo-cortical function–structure relationship, which is in line with the mesocircuit hypothesis.

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Appendix E

Paper V

Resting state networks and consciousness: alterations of multiple resting state network connectivity in physiological, pharmacological, and pathological consciousness States

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Resting state networks and consciousness

Alterations of multiple resting state network connectivity in physiological, pharmacological, and pathological consciousness states

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In order to better understand the functional contribution of resting state activity to conscious cognition, we aimed to review increases and decreases in functional magnetic resonance imaging (fMRI) functional connectivity under physiological (sleep), pharmacological (anesthesia), and pathological altered states of consciousness, such as brain death, coma, vegetative state/unresponsive wakefulness syndrome, and minimally conscious state. The reviewed resting state networks were the DMN, left and right executive control, salience, sensorimotor, auditory, and visual networks. We highlight some methodological issues concerning resting state analyses in severely injured brains mainly in terms of hypothesis-driven seed-based correlation analysis and data-driven independent components analysis approaches. Finally, we attempt to contextualize our discussion within theoretical frameworks of conscious processes. We think that this "lesion" approach allows us to better determine the necessary conditions under which normal conscious cognition takes place. At the clinical level, we acknowledge the technical merits of the resting state paradigm. Indeed, fast and easy acquisitions are preferable to activation paradigms in clinical populations. Finally, we emphasize the need to validate the diagnostic and prognostic value of fMRI resting state measurements in non-communicating brain damaged patients.

Keywords: default mode network, resting state networks, consciousness, sleep, anesthesia, coma, hypnosis

INTRODUCTION

In the past decades, neuroimaging research has been focusing on studying brain function in "resting" conditions, when subjects receive no external stimulation. Functional magnetic resonance imaging (fMRI) resting state connectivity studies stress that the brain at rest is characterized by coherent fluctuations in the blood-oxygen-level-dependent (BOLD) signal. These BOLD fluctuations can be detected in the low frequency range (<0.1 Hz; Cordes et al., 2001), they are distinct from respiratory and cardiovascular signal contribution (De Luca et al., 2006) and organize the brain in large-scale cerebral networks (Damoiseaux et al., 2006). The most widely studied resting state network (RSN) is the default mode network (DMN), encompassing precuneus/posterior cingulate cortex (PCC), mesiofrontal/anterior cingulate cortex (ACC), and temporoparietal junction areas (**Figure 1**). This network of areas was initially identified in positron emission tomography (PET) studies as regions less active when performance on cognitive tasks was compared to resting control condition, such as eye fixation or eyes closed (Shulman et al., 1997; Mazoyer et al., 2001). Later, the DMN was also identified in fMRI and in terms of cognitive function, its activity has been linked to self-related and internal processes, such as stimulus-independent thoughts (McKiernan et al., 2006), mind-wandering (Mason et al., 2007), social cognition (Schilbach et al., 2008), introspection (Goldberg et al., 2006), monitoring of the "mental self" (Lou et al., 2004), and integration of cognitive processes (Greicius et al., 2003). Interestingly, areas of the DMN can be assigned to specific cognitive functions, for example the PCC seems to be important in autobiographical memory while the frontal areas may be important for self-reference (Whitfield-Gabrieli et al., 2011). In that respect, resting state acquisitions can, at least to a certain degree, be informative of cognitive function. Importantly for clinical studies, the resting state paradigm is particularly appealing because it does not require sophisticated experimental setup to administer external stimuli and surpasses the need for patients' contribution (e.g., language comprehension and/or production or motor responses; Soddu et al., 2011). Hence, resting state protocols are a suitable means to study clinical populations, in which communication cannot be established at the bedside, such as patients with disorders of consciousness [e.g., coma, "vegetative state (VS)"/unresponsive wakefulness syndrome (UWS), minimally conscious state (MCS)]. It has been suggested that resting state analyses can be used in a clinical setting to identify group differences, to obtain patient-specific diagnostic and prognostic information, to perform longitudinal studies and monitor

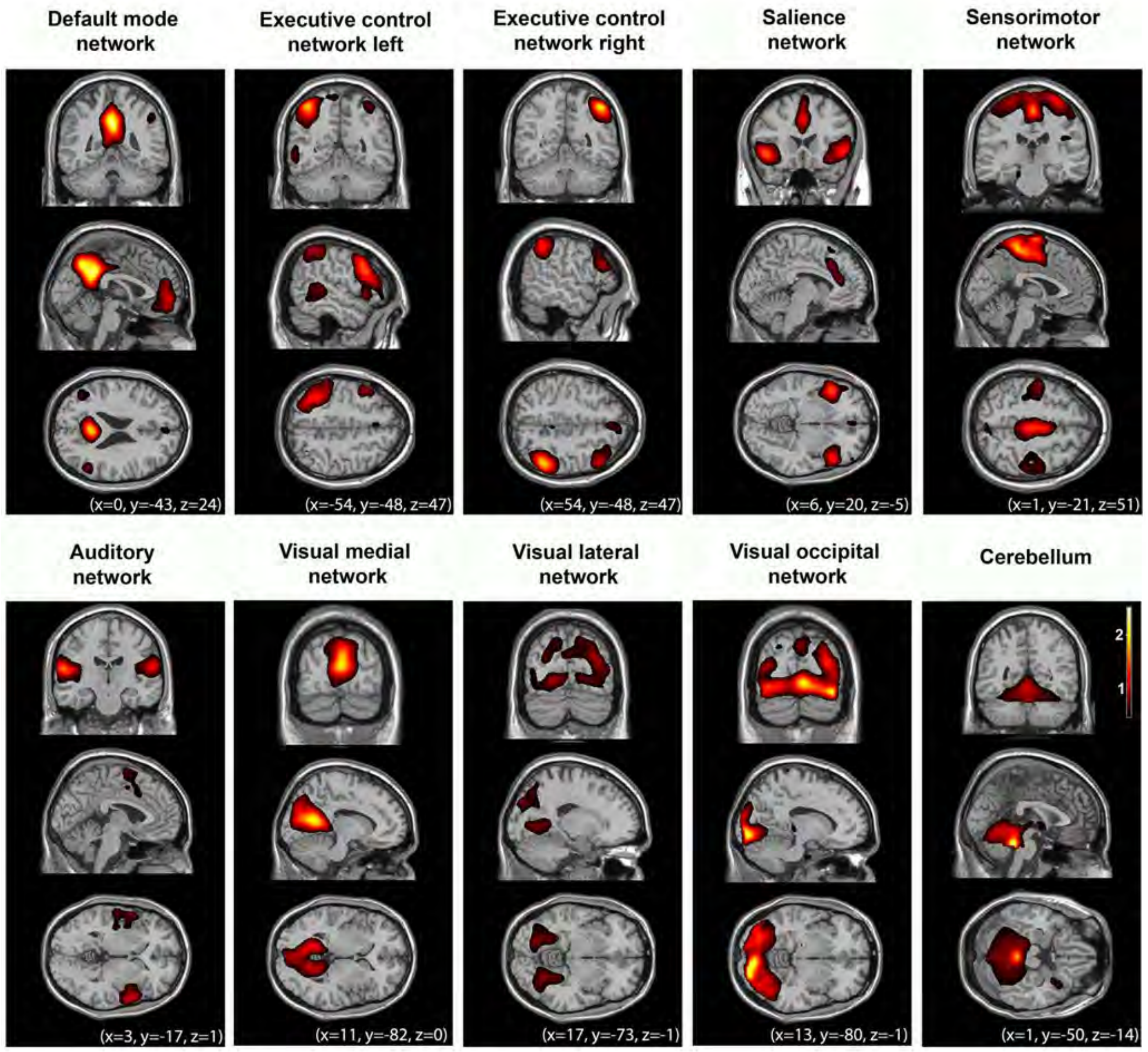

FIGURE 1 | Multiple cerebral networks can be identified with fMRI in healthy controls (*n* = 10) during normal wakeful resting state using independent component analysis. These networks reflect "higher-order" cognitive (i.e., default mode, left and right executive control, salience networks), and "lower-order" sensorimotor, and sensory (auditory, visual) function. For illustrative purposes, group-level spatial maps (*z* values) are rendered on a structural T1 magnetic resonance template and *x*, *y*, and *z* values indicate the Montreal Neurological Institute coordinates of the represented sections.

treatment effects, to cluster heterogeneous diseases such as schizophrenia or even to guide treatments, such as surgical interventions (Fox and Greicius, 2010).

With an aim to better determine the functional role of resting state activity in healthy conditions and to further comprehend its contribution to clinical states, the present review will adopt a "lesion" approach. Indeed, patients' neurological data can give us information about the functional role of the resting state activity to consciousness. We will review changes in functional connectivity in the DMN under physiological (sleep, hypnosis), pharmacological (sedation, anesthesia), and pathological (coma-related states) alteration of consciousness. The functional contribution of the anticorrelated activity between DMN and the "extrinsic" system to (un)conscious states will also be discussed. We will further focus on functional connectivity changes in multiple RSNs, such as the bilateral executive control, salience, sensorimotor, auditory, and visual networks (Beckmann et al., 2005; Damoiseaux et al., 2006; De Luca et al., 2006; Fox and Raichle, 2007; Smith et al., 2009). With regards to resting state assessments of severely brain-injured patients, we will highlight some methodological issues mainly in terms of hypothesis-driven seed-based correlation analysis and data-driven independent components analysis. Finally, we will attempt to contextualize our discussion within theoretical frameworks around conscious processes.

(UN)CONSCIOUS STATES AND RESTING STATE DEFAULT MODE NETWORK ACTIVITY

To date, there is no universal definition for consciousness covering all its essential characteristics (Zeman, 2001). Here, we define consciousness in an operational way based on clinical practice, stressing that consciousness can be reduced to two components, arousal and awareness (Posner et al., 2007). Arousal refers to the level of alertness and it is clinically evidenced by eyes opening. Awareness refers to the content of consciousness and it is clinically evidenced by command following or by observing non-reflex motor behavior, such as eye tracking and localized responses to pain (Posner et al., 2007). Sleep is the best example to describe the relationship between these two components: the drowsier we become as we move toward deep sleep, the less aware we get of our surroundings and ourselves (a notorious exception is the oneiric activity during rapid eye movement sleep during which we remain behaviorally unconscious; Hobson and Pace-Schott, 2002). Based on this definition, subjects in pathological and pharmacological coma (i.e., anesthesia) are not conscious because they are not awake (American Society of Anesthesiologists Task Force on Intraoperative Awareness, 2006). Similarly, under sedation (a drug-dose dependent impairment of consciousness) and hypnotic state (a suggestion-dependent alteration of conscious experience; The Executive Committee of the American Psychological Association – Division of Psychological Hypnosis, 1994) subjects report an altered state of awareness as they move toward lower wakefulness levels. A unique dissociation between arousal and awareness is observed in patients in a VS (also called UWS; Laureys et al., 2010) who recover wakefulness but their motor responses are merely reflexive and, hence, not indicative of conscious awareness (Laureys et al., 2005). Patients in VS/UWS should not be mistaken with patients in a MCS. Patients in MCS, although unable to functionally communicate with their environment, do show fluctuating remnants of willful behavior (Giacino et al., 2002). Based on the level of their purposeful behavioral repertoire, MCS patients were recently subcategorized as MCS+ (i.e., showing command following,) and MCS− (i.e., showing visual pursuit, localization of noxious stimulation, or non-contingent behaviors, such as appropriate smiling or crying to emotional stimuli; Bruno et al., 2011). This kind of clinical distinction highlights the importance of motor output to the evaluation of consciousness. Patients with a locked-in syndrome (LIS), however, have no means of producing speech, limb, or facial movements but still are awake and conscious (Posner et al., 2007). Evidently, by solely measuring motor responses, these patients can be mistaken for unconscious (Laureys et al., 2005). Similarly, consciousness in patients with aphasia can be underestimated if the clinician does not account for such deficit. As a consequence, valid motor- and language-independent assessment of residual brain function in non-communicating patients is of both clinical and ethical importance.

The resting state paradigm surpasses the requirement for motor output or language comprehension. To date, neuroimaging protocols investigating connectivity of the DMN during resting state are not conclusive as to its exact functional role. Nevertheless, resting state fMRI studies suggest that activity of this network is generally reduced as a function of the level of consciousness (**Table 1**). For example, it has been shown that with the advancement of sleep, connectivity between the frontal and posterior parts of the DMN decreases yet persists (Horovitz et al., 2009). Decreases in functional connectivity were also observed in PCC of the DMN under pharmacological unconsciousness with propofol (Boveroux et al., 2010; Schrouff et al., 2011) and sevoflurane (Martuzzi et al., 2010). Importantly for clinical populations, connectivity in the PCC was shown to be indistinguishable between controls and LIS patients, relatively preserved in MCS, significantly reduced in VS/UWS patients (Vanhaudenhuyse et al., 2010) and could not be identified in brain death (i.e., irreversible coma with absent brainstem reflexes; Boly et al., 2009). Similarly during a passive auditory task, DMN deactivations, which are thought to interrupt ongoing introspective processes, showed a reduction in MCS whereas VS/UWS patients did not show such task-induced deactivations (Crone et al., 2011). These studies suggest that DMN functional connectivity correlates, at least partially, with the level of ongoing conscious cognition. This is in agreement with functional connectivity studies on intermediate states of awareness. For example, in hypnotic state there is only relative (Demertzi et al., 2011b) or no connectivity decreases in the DMN (McGeown et al., 2009). Similarly, during moderate sedation, little (Greicius et al., 2008) or no changes (Stamatakis et al., 2010) in DMN connectivity have been observed. During light sleep there is no change (Horovitz et al., 2008; Larson-Prior et al., 2009). Nevertheless, in deep sleep brain activity shows increased modularity, which hinders the brain to integrate information and therefore might account for decreased consciousness during dreamless sleep (Boly et al., 2012).

Taken together, changes in the DMN functional connectivity in altered consciousness states could suggest modified self-related conscious mentation. Indeed, it has been suggested that in normal waking conditions, resting state activity in the posterior cingulate and frontal areas accounts for self-referential thoughts (Whitfield-Gabrieli et al., 2011). Therefore, it could be inferred that decreased connectivity in the DMN reflects restricted abilities for self-referential processing, like in patients with disorders of consciousness. One should keep in mind, though, that our limited understanding of the dynamic neural complexity underlying consciousness and its resistance to quantification in the absence of communication make it difficult to establish strong claims about self-consciousness in non-communicating subjects.

DMN FUNCTIONAL ANTICORRELATIONS

Since the early studies of resting state, it was suggested that the brain's baseline activity can be organized in two brain networks showing anticorrelated activity to each other: an "intrinsic" and an "extrinsic" network (Fox et al., 2005; Fransson, 2005; Golland et al., 2007; Tian et al., 2007). The "intrinsic" network coincides with the DMN and is involved in the same cognitive processes as the DMN. The "extrinsic" system encompasses lateral frontoparietal areas resembling the brain activations during goal-directed

Table 1 | FMRI studies showing alterations in resting state functional connectivity of multiple networks in physiological (sleep, hypnosis), pharmacological (sedation), and pathological states of unconsciousness.

		N	**Functional connectivity change**	**Method**	**Study**
DMN	Light sleep	14	Connectivity persists	Seed-based	Horovitz et al. (2008)
		10	Connectivity persists	Seed-based	Larson-Prior et al. (2009)
	Slow wave sleep	14	↑: PCC correlation with IPC. Correlation within nodes persistent ↓: Correlation PCC with MPFC became absent	Seed-based	Horovitz et al. (2009)
		25	↓: PCC, PHG, MPFC	ICA	Sämann et al. (2011)
	Light sedation	16	↑: PCC and areas outside of the DMN	Seed-based	Stamatakis et al. (2010)
		12	↓: General deceased connectivity, focal decreases PCC	ICA	Greicius et al. (2008)
	Anesthesia	20	↓: PCC/precuneus, MPFC, superior frontal sulci, parahippocampal gyrus, and bilateral TPJ	Seed-based and ICA	Boveroux et al. (2010)
		14	↑: PCC and STG ↓: PCC and adjacent areas	Seed-based	Martuzzi et al. (2010)
		18	↓: Reduction connectivity within the DMN and between the DMN and other networks	ICA	Schrouff et al. (2011)
	Hypnosis	18	↓ right middle and superior frontal gyrus	Seed-based	McGeown et al. (2009)
		12	↑: Middle frontal and bilateral angular gyri ↓: PCC and bilateral parahippocampal areas	ICA	Demertzi et al. (2011b)
	Comatose states	2	↓: Connectivity is absent in brain dead, decreased PCC, and thalamus connectivity Preserved cortico-cortical connectivity	ICA	Boly et al. (2009)
		11	↓: Connections between PCC and MPFC Locked-in patients showed near to normal connectivity	Seed-based and ICA	Soddu et al. (2012)
		14	↓: All areas showed less connectivity in disorders of consciousness, decrease of connectivity was negatively correlated with consciousness. PCC most significant decrease	ICA	Vanhaudenhuyse et al. (2010)
		13	Presence of DMN has prognostic value	ICA	Norton et al. (2012)
Executive control network	Light sleep	10	No difference	Seed-based	Larson-Prior et al. (2009)
	Slow wave sleep	25	Correlations within the network persist but decrease	ICA	Sämann et al. (2011)
	Light sedation	20	↓: Right: middle frontal and posterior parietal cortices. Left: residual in middle frontal, PCC, and temporo-occipital cortices	Seed-based and ICA	Boveroux et al. (2010)
Salience	Slow wave sleep	14	↑: Connectivity between insula and left ACC ↓: Decrease between connectivity in the insula and supplementary motor cortex and left middle frontal gyrus	Seed-based	Martuzzi et al. (2010)

(Continued)

Table 1 | Continued

		N	Functional connectivity change	Method	Study
	Hypnosis	8	↑: Increases in mid-insula, primary sensory, and orbitofrontal cortex	Seed-based	Derbyshire et al. (2004)
Sensorymotor network	Light sleep	10	No difference	Seed-based	Larson-Prior et al. (2009)
	Slow wave sleep	14	↑: Connectivity within the network	Seed-based	Martuzzi et al. (2010)
	Light sedation	12	↑: Within-network increases	ICA	Greicius et al. (2008)
Auditory	Slow wave sleep	14	No difference	Seed-based	Martuzzi et al. (2010)
	Light sedation	20	No difference	Seed-based and ICA	Boveroux et al. (2010)
Visual	Light sleep	10	No difference	Seed-based	Larson-Prior et al. (2009)
	Light sedation	14	↑: Primary visual area with the cuneus and lingual gyrus	Seed-based	Martuzzi et al. (2010)
	Anesthesia	20	No difference	Seed-based and ICA	Boveroux et al. (2010)

Upper arrow denotes increases in functional connectivity; lower arrow denotes decreases in functional connectivity. (DMN, default mode network; PCC, posterior cingulate cortex; PHG, parahippocampal gyrus; IPC, inferior parietal cortex; MPFC, medial prefrontal cortex; TPJ, temporoparietal junction; STG, superior temporal gyrus, ICA, independent component analysis).

behavior and it has been linked to cognitive processes of external sensory input, such as somatosensory (e.g., Boly et al., 2007), visual (e.g., Dehaene and Changeux, 2005), and auditory (e.g., Brunetti et al., 2008). Previous studies showed that these two systems are of a competing character in the sense that they can disturb or even interrupt each other (e.g., Tian et al., 2007). Such anticorrelated pattern is also illustrated in activation studies on motor performance (Fox et al., 2007), perceptual discrimination (Sapir et al., 2005), attentional lapses (Weissman et al., 2006), and somatosensory perception of stimuli close to somatosensory threshold (Boly et al., 2007). We recently determined the cognitive-behavioral counterpart of such "resting state" activity and showed that activity in the DMN corresponded to behavioral reports of "internal" awareness (i.e., self-related thoughts). Conversely, subjective ratings for "external" awareness (i.e., perception of the environment through the senses) correlated with the activity of an "extrinsic" system (encompassing lateral frontoparietal cortices; Vanhaudenhuyse et al., 2011). These findings depict that the anticorrelated pattern between DMN and the extrinsic system is of functional significance to conscious cognition. With an aim to further characterize the role of these two systems to subjective awareness, we sought to modulate their relationship by means of hypnosis. We found that, as compared to a control condition of autobiographical mental imagery, there was a hypnosis-related reduction in connectivity in the "extrinsic" system, reflecting a decreased sensory or perceptual awareness. Interestingly, this modulated activity was paralleled to subjective reports of increased sense of dissociation from the environment and reduced intensity of "external thoughts" (Demertzi et al., 2011b).

Taken together these data indicate that DMN and anticorrelated extrinsic system activity underlies (at least partially) conscious ongoing mentation. It should be mentioned that fMRI anticorrelations were previously subject to debate in the literature. It has been argued, for instance, that fMRI functional anticorrelations are nothing more than noise in the signal due to regression of the brain's global activity during data preprocessing (Anderson et al., 2010). Other data, however, suggest that the anticorrelations persist both with and without global signal regression, suggesting some underlying biological origins for this anticorrelated pattern (Fox et al., 2009; Chai et al., 2012). We would agree with the latter evidence which is supported by studies in unconscious conditions, such as anesthesia (Boveroux et al., 2010; **Figure 2**), sleep (Sämann et al., 2011), and in unresponsive patients (Boly et al., 2009) where these anticorrelations generally reduce or even disappear, accounting for their functional contribution to conscious cognition.

BEYOND THE DMN: RESTING STATE ACTIVITY IN MULTIPLE CEREBRAL NETWORKS

Importantly, the different functions of a brain region cannot be understood in isolation, meaning in terms of functional segregation, but only in conjunction with regions it interacts with, that is in terms of functional integration (Seghier et al., 2010). Therefore, we further focus our review on other "large-scale higher-order" (bilateral executive control and salience networks) and sensorimotor-sensory (auditory, visual) RSNs, which can be consistently identified in healthy conditions (**Figure 2**).

The executive control network during normal wakefulness encompasses bilateral middle, inferior and superior frontal cortices, bilateral inferior parietal lobes, ACC/supplementary motor area (SMA), and bilateral insular cortices (**Figure 1**). Resting state independent components analysis identified this network in a lateralized manner. The left executive control network is thought to be more involved in cognitive and "language" paradigms while the right executive control network relates to perceptual, somesthetic, and nociception processing (Smith et al., 2009; Laird et al., 2011). Activity in both these two networks is reduced during deep sleep (Sämann et al., 2011) and anesthesia (Boveroux et al., 2010) whereas light sleep does not seem to mediate functional connectivity in these networks (Larson-Prior et al., 2009; **Table 1**). Taken together, these results highlight the involvement of the executive control networks in the perception of the external world, in line with previous suggestion that activity of these areas is a necessary condition for conscious (i.e., reportable) visual perception (Dehaene et al., 2003).

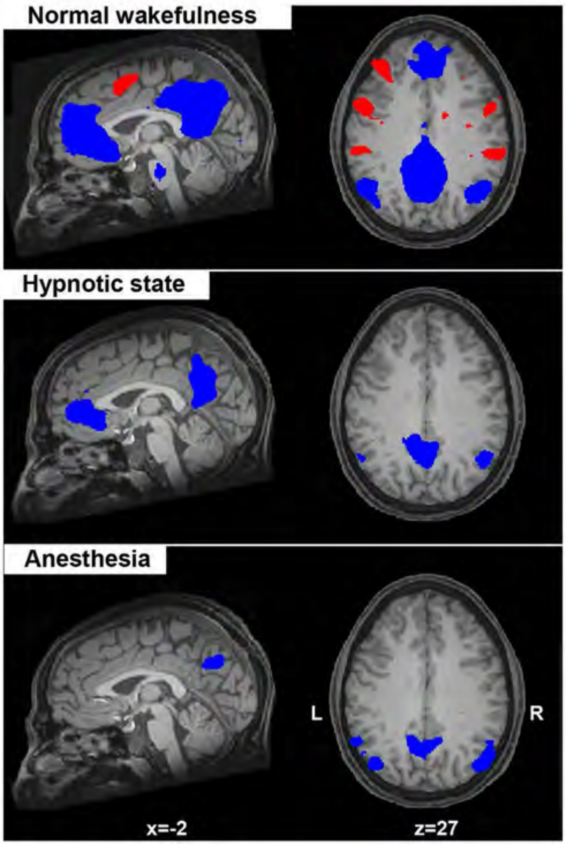

FIGURE 2 | Spontaneous fMRI BOLD activity in the default mode network (in blue; considered to reflect self-related mentation) anticorrelates with the activity of a lateral frontoparietal system (in red; considered to mediate conscious perception of the external world). Here, this anticorrelated activity is shown for normal wakefulness, hypnotic state, and during deep anesthesia. Of note is the absence of the activity in the "extrinsic" frontoparietal system in the two conditions of altered sense of awareness (hypnosis, anesthesia) which is considered as suggestive of a diminished "external" awareness (i.e., the perception of the environment through the senses). Statistical maps are thresholded at a false discovery error rate $p < 0.05$ and rendered on a structural T1 magnetic resonance image of a healthy subject (x and z values indicate Talairach coordinates of the represented sections).

The salience network encompasses fronto-insular and ACCs (**Figure 1**) with connections to subcortical and limbic structures. In normal conditions, this network is implicated in the orientation toward salient emotional stimuli (Seeley et al., 2007), conflict monitoring, information integration, and response selection (Cole and Schneider, 2007; Roberts and Hall, 2008). It has been proposed that the salience network enables the switch between internal attention (the default mode) and task-related states (Menon and Uddin, 2010). The salience network has also been linked to pain-related processes both during acute stimulus-induced pain (Tracey and Mantyh, 2007), during resting state while anticipating pain (Ploner et al., 2010; Wiech et al., 2010), and after hypnotic suggestions for creating pain experiences in the absence of a noxious stimulus (Derbyshire et al., 2004). Under light sevoflurane sedation, increased connectivity between the ACC and the insula was observed, although connectivity between the insula and the secondary somatosensory cortex was reduced (Martuzzi et al., 2010). Analysis of the salience network in comatose states could be beneficial for the study of pain and possible suffering in these patients in the absence of external stimulation. Indeed, such stimulations are not always feasible due to sophisticated setups or due to patients' clinical picture. Hence, salience network resting state analysis could shed light on the cerebral substrate that could account for patients' orientation to salient stimuli, including painful ones.

The sensorimotor network resembles the activations seen in motor tasks (Biswal et al., 1995). In normal wakefulness it encompasses the SMA/midcingulate cortex, bilateral primary motor cortex, and bilateral middle frontal gyri (Biswal et al., 1995; Greicius et al., 2008; **Figure 1**). During light sedation the sensorimotor network shows increases in functional connectivity (Greicius et al., 2008; Martuzzi et al., 2010). To date, the above networks have not been further investigated under other unconscious states.

The auditory network, important in audition, such as tone/pitch discrimination, music, and speech (Laird et al., 2011) in normal wakefulness, encompasses primary and secondary auditory cortices, including Heschl's gyrus, bilateral superior temporal gyri, and posterior insular cortex (**Figure 1**). During normal wakefulness, resting state independent component analysis (ICA) also identifies the visual network in three independent components (**Figure 1**). One network, the lateral visual network includes the middle temporal visual association area at the temporo-occipital junction and is most important in complex (emotional) stimuli (Laird et al., 2011). The other networks include medial and occipital visual networks, important in simple visual (e.g., a flickering checkerboard), and higher-order visual stimuli (e.g., orthography), respectively (Beckmann et al., 2005; Damoiseaux et al., 2006; Allen et al., 2011; Laird et al., 2011). No difference in connectivity was identified between both these primary auditory and visual sensory networks and light sleep (Larson-Prior et al., 2009), or between awake and sedation (Boveroux et al., 2010; Martuzzi et al., 2010). One study showed increased temporal synchrony in auditory and visual areas in light midazolam sedation (Kiviniemi et al., 2005). The visual cortex has been shown to possess higher amplitude of BOLD fluctuations when asleep (Fukunaga et al., 2006). This indicates that resting state activity continues in these areas during sleep, and thus transcends consciousness. Finally, reliably indicated as possessing functional connectivity is the cerebellum. This network is associated with action and somesthesis (Laird et al., 2011), but not yet thoroughly studied in altered states of consciousness.

ANALYZING RESTING STATE DATA FROM PATHOLOGICAL BRAINS: METHODOLOGICAL ISSUES

The clinical neuro-investigation of severely brain-injured patients with the resting state paradigm is technically easier compared to activation (Schiff et al., 2005) or "active" mental imagery protocols (e.g., Monti et al., 2010). This is because patients do not have to perform any task, and such data can have faster translation into clinical practice (Soddu et al., 2011). Depending on the adopted methodology, several issues need to be taken into account when analyzing resting state acquisitions from clinical populations. To date, two main approaches are employed; hypothesis-driven seed-voxel correlation analysis and data-driven ICA (see **Table 1** for the adopted approach by each reviewed study). Each method has its own advantages, yet their methodological difficulties, especially in non-collaborative patients, which merit to be acknowledged.

HYPOTHESIS-DRIVEN METHOD: SEED-BASED CORRELATION ANALYSIS

The seed-voxel approach uses extracted BOLD time course from a region of interest and determines the temporal correlation between this signal (the seed) and the time course from all other brain voxels (Fox et al., 2005). This creates a whole-brain voxel-wise functional connectivity map of covariance with the seed region. It is the most straightforward method to analyze functional connectivity of a particular brain region. The method gives direct answers to specific hypotheses about functional connectivity of that region. It is attractive and elegant for many researchers as the data can be interpreted relatively easily when a well-defined seed area is used. When applying this approach to the study of resting state activity in patients with disorders of consciousness, several controversial issues arise. A first general issue concerns regressing out the global activity from the BOLD signal, which might induce spurious anticorrelations (Fox et al., 2009; Murphy et al., 2009). However, in the case of brain death (a condition where the brain totally lacks neuronal activity and arterial blood flow), this type of regression is an important step to obtain the obvious zero connectivity in this condition (Boly et al., 2009). Alternatively, a non-zero BOLD signal measured in brain death can be taken to be artifactual, contaminated by head motion or heart beating (Soddu et al., 2011). Next, patients with severe brain injuries may suffer from structural deformations resulting from traumatic brain injury and focal hemorrhages. Additionally, patients with severe chronic brain injuries usually develop atrophy and secondary hydrocephalus (i.e., *ex vacuo* dilation of the ventricles). This implies that even if a statistical structural normalization procedure has been performed, the selection of a proper seed region can become difficult and will require visual inspection by an expert eye. This issue adds to the already intrinsic challenges of an *priori* selection of the seed region which, in principle, can lead to as many possible overlapping networks as the number of possible seeds (Cole et al., 2010). Using seed-based analysis, other noisy confounds might be influencing the data (e.g., head motion, vascular activity, scanner artifacts). To reduce such noise, the BOLD signal can be preprocessed by regressing out head motion curves as well as ventricular and white matter signal, and each of their first-order derivative terms (Fox et al., 2005). Finally, as for all group-level analyses, one has to take into account the between-subject variability, such as cortical folding or functional localization between individuals or groups (Cole et al., 2010) which can be extremely challenging in severely deformed brains.

DATA-DRIVEN METHOD: INDEPENDENT COMPONENT ANALYSIS

Data-driven methods are used to analyze whole-brain connectivity patterns without the need of *a priori* seed regions. ICA is the most widely used methodology with high level of consistency in results within subjects (van den Heuvel and Hulshoff Pol, 2010). ICA divides an entire dataset into different maximally statistical independent components and thus is able to isolate cortical connectivity maps from non-neural signals (Beckmann et al., 2005). Spontaneous activity is therefore automatically separated from noise, such as head motion or physiological confounds (e.g., cardiac pulsation, respiratory, and slow changes in the depth and rate of breathing; Beckmann and Smith, 2004). This method has the advantage that it can evaluate and compare the coherence of activity in multiple distributed voxels (Cole et al., 2010). The advantage is that it divides different RSNs into different components. However, ICA does not provide any classification or ordering of the independent components. It is therefore perceived as more difficult to understand due to the complex representation of the data. The most straightforward method for labeling the components is by visual inspection, but this lacks reproducibility and could be hard to perform in cases with a large component dimensionality. Alternatively, an automatic selection is preferable but the way to choose the right independent component remains a delicate issue. By merely performing a spatial similarity test with a predefined template has been shown not to be successful for choosing the right component (Soddu et al., 2012). Some automatic approaches for component selection have been proposed, based on template matching using the "goodness of fit" as an outcome index. However, these methods have to be interpreted with care especially in cases of deformed brains as in patients with a traumatic brain injury or comatose state. It was recently proposed that when selecting independent components in patients populations, spatial, temporal, and a "compromise" between spatial and temporal properties of the network of interest need to be met (Soddu et al., 2012). For example, a component can be erroneously selected as the RSN of interest if the selection is based on the spatial pattern ignoring the properties in the time domain (**Figure 3**, bottom right panel). Additionally, the determination of the proper dimensionality (i.e., the "right" number of estimated components) remains unclear. Extracting many components can result in the spatial segregation of the network of interest into multiple sub-networks (Smith et al., 2009). It was shown, for example, that the use of 75 components can reduce the DMN into four components and the sensorimotor network in six (Allen et al., 2011). When applying ICA in pathological brains it is probably more useful not to select a large quantity of components, because high component dimensionality can further reduce the chances of identifying a network due to decrease in spatial pattern and spectral properties (Tohka et al., 2008).

Other techniques to analyze resting state data exist such as methods that focus on the (fractional) amplitude of low frequency fluctuations [(f)ALFF; Zang et al., 2007; Zuo et al., 2010), or on the small world characteristics using correlations and graph analysis (Bullmore and Sporns, 2009; Zalesky et al., 2012].

CONCLUSIONS AND PERSPECTIVES

The default mode network is the most widely studied network in the resting state literature and has been linked to self-related processes. To date, fMRI resting state studies show that DMN connectivity is reduced under altered states of consciousness, such as sleep, sedation/anesthesia, hypnotic state, and clinical states

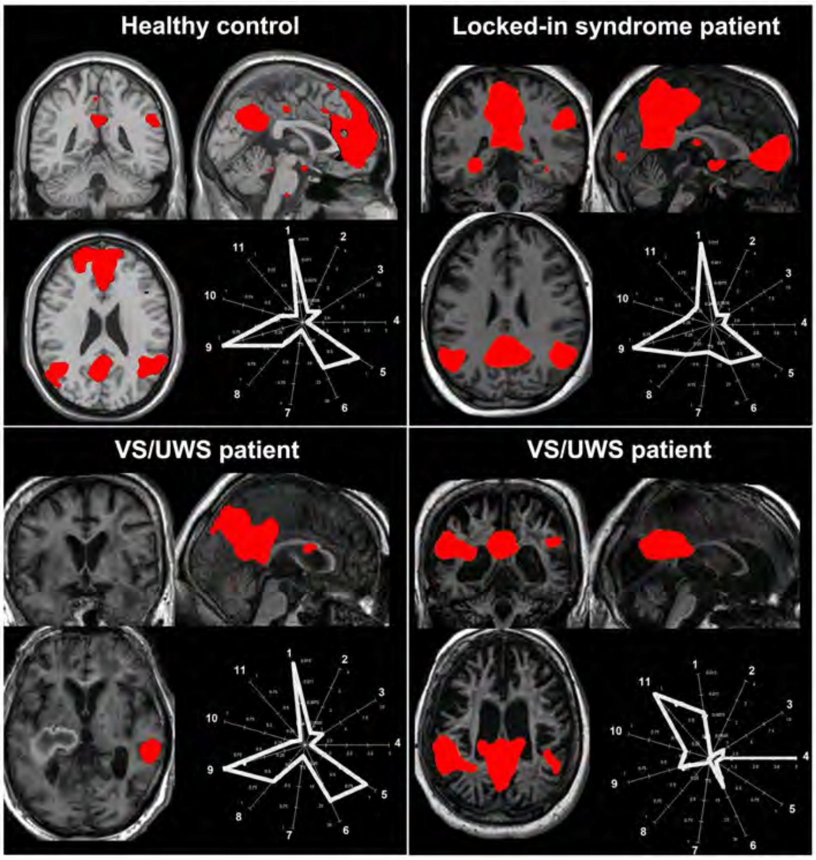

FIGURE 3 | The challenge of selecting the "right" independent component as the resting state network of interest in pathological conditions. The figure illustrates the spatial pattern (brain maps, *z* values 0.8–10) and spatial-temporal properties (fingerprints: a representation of the component in a multidimensional space of parameters; De Martino et al., 2007) of the default mode network in healthy consciousness states (healthy subject, patient with locked-in syndrome; upper row) and in two patients with vegetative state/unresponsive wakefulness syndrome (VS/UWS; lower row). For the healthy control, the locked-in syndrome and the VS/UWS patient in the lower left corner, the default mode network shows the characteristic properties in both the spatial and the temporal domain (i.e., the fingerprints pick in the 0.02–0.05 Hz frequency band labeled with the number 9) even if for the VS/UWS patient the spatial pattern is only partially preserved. Of note is that the second VS/UWS patient exhibits the spatial pattern of the default mode network but importantly the time course of this component is characterized by high frequency fluctuations, in the 0.1–0.25 Hz frequency band and high spatial entropy (labeled, respectively, with the number 11 and 4 in the fingerprint). Therefore, such activity cannot be considered of neuronal origin. As a consequence, if the component selection was merely based on a spatial similarity test (e.g., with a predefined template), then this component could be erroneously selected and further statistically analyzed. A "compromise" in the selection of the appropriate network of interest in the space and time domain is needed to will eventually exclude non-neuronal contributions [Fingerprint labels: (1) degree of clustering, (2) skewness, (3) kurtosis, (4) spatial entropy, (5) autocorrelation, (6) temporal entropy, power: (7) 0–0.008 Hz, (8) 0.008–0.02 Hz, (9) 0.02–0.05 Hz, (10) 0.05–0.1 Hz, (11) 0.1–0.25 Hz].

of disorders of consciousness (VS/UWS, MCS, coma, and brain death). Such connectivity alterations can be discussed in two non-mutually exclusive ways. On one hand, one can refer to these reductions in resting state connectivity during altered conscious states as reflecting reduced capacities for (conscious) cognitive processing (e.g., Vanhaudenhuyse et al., 2010). On the other hand, we can equally talk about persistent (albeit reduced) functional connectivity pattern in unconscious states, which transcends the level of consciousness, and which is considered as a physiologic baseline (e.g., Raichle et al., 2001). In any case, it seems that the purposes and questions of each study will eventually determine how such alterations can be further discussed and interpreted. Both the scientific and clinical implications for cognition seem to be the essence of resting state connectivity measurements.

At the scientific level, resting state analyses shed light on the necessary conditions needed for conscious awareness to take place. In other words, in the absence of external stimulation, resting state *functional* connectivity paradigms could quantify the minimal prerequisites under which cognitive processes can become "conscious." This could mean that in the presence of an adequate neural substrate (i.e., the RSN), one could infer preserved capacities for conscious cognition. Of course the absence of functional connectivity cannot be taken as a proof for incapacity for conscious awareness. Indeed, it has been suggested that functional connections are best recruited after external stimulation (Honey et al., 2009). In any case, the sufficiency of the RSNs integrity to consciousness remains to be further determined with studies measuring *effective* connectivity (Churchland, 2007).

In summary, we here reviewed studies in resting state fMRI connectivity of "higher-order" associative cerebral networks (default mode, right and left executive control, and salience) and "lower-level" sensory (auditory and visual) and sensorimotor networks under various altered states of consciousness. As previously proposed, in order for humans to be conscious of something, incoming information (via sensory networks) needs to be made globally available to multiple brain systems via long-range neurons associative networks (Dehaene and Changeux, 2011). Here, the reviewed studies suggest that resting state connectivity is preserved but altered in most RSNs under physiological and pharmacological states, impeding information integration. It should be noted here that it was not among our aims to exhaustively review all spectrum of altered states of consciousness. Much research has been conducted in states of altered sense of awareness, such as in neuropsychiatric disorders (e.g., dementias and schizophrenia; for a review see Buckner et al., 2008), meditation (Brewer et al., 2011; Josipovic et al., 2012), and drug-related states such as alcohol (Esposito et al., 2010), amphetamine (Roberts and Garavan, 2010), or psychedelic drugs (Carhart-Harris et al., 2012) which in general show changes in the connection between the posterior cingulate and frontal areas. Resting state investigations have also been attempted using other modalities, such as electroencephalography (e.g., Lehembre et al., 2012). Rather, we here focused on RSNs obtained using fMRI. We reviewed changes in functional connectivity as a function of various states of wakefulness. This aim lies within our ultimate clinical goal to better document, manage and predict residual brain functioning of patients with disorders of consciousness. As these patients are incapable of functional communication with their environment, they might be wrongly diagnosed as unconscious when locked-in (Laureys et al., 2005) or when suffering from aphasia (Majerus et al., 2009). The ethical implications of erroneous diagnostics are apparent, especially when pain (Demertzi et al., 2009, 2012) and end-of-life issues (Demertzi et al., 2011a) are discussed.

At the clinical level, the study of resting state activity in pathological states of consciousness can become demanding due to both clinical and methodological issues. For example, patients who show increased prescan motion activity will need to be anesthetized to reduce the noise during data acquisition. Apart from the clinical issue of applying anesthetics to these vulnerable patients, the effect of anesthesia will need to be accounted for in the acquired data. This is added to the methodological challenge of the spatial normalization of severely deformed brains (Shen et al., 2007). Additionally, identified resting state connectivity patterns need to be interpreted according to the studied population. In brain death, for instance, it was shown that resting state fMRI activity is absent in line with the clinical neurological criteria for the diagnosis of death (Boly et al., 2009). Therefore, in cases where resting state activity, in the DMN for example, is identified, such findings can be pertained to motion and other artifacts, not indicative of neuronal activity (Soddu et al., 2011). The characterization of the fMRI functional connectivity of other RSNs in comatose states remains to be further elucidated. It can be expected, though, that in such severely constrained situations, like in disorders of consciousness, the functional integrity of most RSNs is considerably restricted accounting for patients' limited capacities for conscious cognition.

Despite intrinsic limitations, resting state data are technically easier to obtain in patients' population, as compared to auditory (Schiff et al., 2005) or visual (Monti et al., 2012) activation protocols or "active" mental imagery protocols (Monti et al., 2010; Bardin et al., 2011). The challenge now is twofold: first, to unravel the relationship (i.e., correlations, anticorrelations) between and among the RSNs under various conscious conditions. The second challenge is to move from static functional connectivity measurements to the assessment of the temporal dynamics of such associations, meaning looking at changes in functional connectivity across time. Such imperatives are justified when considering the nature of intrinsic brain activity, which is ongoing and which characterizes most areas of the brain, beyond the DMN (Raichle and Snyder, 2007).

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Appendix F

Paper VI

Intrinsic functional connectivity differentiates minimally conscious from unresponsive patients

Demertzi A*, Antonopoulos G*, **Heine L**, Voss H U, Crone S J, Kronbichler M, Trinka E, Angeles C, Bahri M, Phillips C, Di-Perri C, Gomez F, Tshibanda L, Soddu A, Vanhaudenhuyse A, Charland-Verville V, Schiff N D, Whitfield-Gabrieli S*, Laureys S*.

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Intrinsic functional connectivity differentiates minimally conscious from unresponsive patients

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Despite advances in resting state functional magnetic resonance imaging investigations, clinicians remain with the challenge of how to implement this paradigm on an individualized basis. Here, we assessed the clinical relevance of resting state functional magnetic resonance imaging acquisitions in patients with disorders of consciousness by means of a systems-level approach. Three clinical centres collected data from 73 patients in minimally conscious state, vegetative state/unresponsive wakefulness syndrome and coma. The main analysis was performed on the data set coming from one centre (Liège) including 51 patients (26 minimally conscious state, 19 vegetative state/unresponsive wakefulness syndrome, six coma; 15 females; mean age 49 ± 18 years, range 11–87; 16 traumatic, 32 non-traumatic of which 13 anoxic, three mixed; 35 patients assessed >1 month post-insult) for whom the clinical diagnosis with the Coma Recovery Scale-Revised was congruent with positron emission tomography scanning. Group-level functional connectivity was investigated for the default mode, frontoparietal, salience, auditory, sensorimotor and visual networks using a multiple-seed correlation approach. Between-group inferential statistics and machine learning were used to identify each network's capacity to discriminate between patients in minimally conscious state and vegetative state/unresponsive wakefulness syndrome. Data collected from 22 patients scanned in two other centres (Salzburg: 10 minimally conscious state, five vegetative state/unresponsive wakefulness syndrome; New York: five minimally conscious state, one vegetative state/unresponsive wakefulness syndrome, one emerged from minimally conscious state) were used to validate the classification with the selected features. Coma Recovery Scale-Revised total scores correlated with key regions of each network reflecting their involvement in consciousness-related processes. All networks had a high discriminative capacity ($>80\%$) for separating patients in a minimally conscious state and vegetative state/unresponsive wakefulness syndrome. Among them, the auditory network was ranked the most highly. The regions of the auditory network which were more functionally connected in patients in minimally conscious state compared to vegetative state/unresponsive wakefulness syndrome encompassed bilateral auditory and visual cortices. Connectivity values in these three regions discriminated congruently 20 of 22 independently assessed patients. Our findings point to the significance of preserved abilities for multisensory integration and top–down processing in minimal consciousness seemingly supported by auditory-visual crossmodal connectivity, and promote the clinical utility of the resting paradigm for single-patient diagnostics.

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Keywords: consciousness; traumatic brain injury; resting state connectivity; sensory systems; anoxia

Abbreviations: CRS-R = Coma Recovery Scale-Revised; MCS = minimally conscious state; UWS = unresponsive wakefulness syndrome; VS = vegetative state

Introduction

As patients with acute or chronic disorders of consciousness are by definition unable to communicate, their diagnosis is particularly challenging. Patients in coma, for example, lay with eyes closed and do not respond to any external stimulation. When they open their eyes but remain unresponsive to external stimuli they are considered to be in a vegetative state (VS; Jennett and Plum, 1972) or, as most recently coined, unresponsive wakefulness syndrome (UWS; Laureys *et al.*, 2010). When patients exhibit signs of fluctuating yet reproducible remnants of non-reflex behaviour, they are considered to be in a minimally conscious state (MCS; Giacino *et al.*, 2002). To date, the diagnostic assessment of patients with disorders of consciousness is mainly based on the observation of motor and oro-motor behaviours at the bedside (Giacino *et al.*, 2014). The evaluation of non-reflex behaviour, however, is not straightforward as patients can fluctuate in terms of vigilance, may suffer from cognitive (e.g. aphasia, apraxia) and/or sensory impairments (e.g. blindness, deafness), from small or easily exhausted motor activity and pain. In these cases, absence of responsiveness does not necessarily correspond to absence of awareness (Sanders *et al.*, 2012). Alternatively, motor-independent technologies can aid the clinical differentiation between the two patient groups (Bruno *et al.*, 2010).

Up to now, accurate single-patient categorization in MCS and VS/UWS has been performed by means of transcranial magnetic stimulation in combination with EEG (Rosanova *et al.*, 2012; Casali *et al.*, 2013) and by combining different EEG measures (Sitt *et al.*, 2014). In terms of patient separation by means of functional MRI, activation (which utilise sensory stimulation; Schiff *et al.*, 2005; Coleman *et al.*, 2007; Di *et al.*, 2007) and active paradigms (which probe mental command following; Owen *et al.*, 2006; Monti *et al.*, 2010; Bardin *et al.*, 2012) have been used to detect convert awareness in these patients. An apparent limitation of the latter approaches is that patients may demonstrate motor and language deficits which incommode these assessments and heighten the risk of false-negative findings (Giacino *et al.*, 2014). The application of these paradigms can also be constrained due to each institution's technical facilities.

Alternatively, functional MRI acquisitions during resting state do not require sophisticated setup and surpass the need for subjects' active participation. Past resting state functional MRI-based assessment of patients has focused on the default mode network, which mainly encompasses anterior and posterior midline regions, and which has been involved in conscious and self-related cognitive processes (Raichle *et al.*, 2001; Buckner *et al.*, 2008). Such investigations have shown that default mode network functional connectivity decreases alongside the spectrum of consciousness, moving from healthy controls to patients in MCS, VS/UWS and coma (Boly *et al.*, 2009; Vanhaudenhuyse *et al.*, 2010; Norton *et al.*, 2012; Soddu *et al.*, 2012; Demertzi *et al.*, 2014; Huang *et al.*, 2014). In patients, the precuneus and posterior cingulate cortex of the default mode network have been also characterized by decreases in functional MRI resting state low frequency fluctuations and regional voxel homogeneity (which refers to the similarity of local brain activity across a region) (Tsai *et al.*, 2014). Reduced functional MRI functional connectivity has been further identified for interhemispheric homologous regions belonging to the extrinsic or task-positive network (implicated in the awareness of the environment; Vanhaudenhuyse *et al.*, 2011) in patients as compared to controls (Ovadia-Caro *et al.*, 2012). Reduced interhemispheric connectivity has been also indicated by means of partial correlations (Maki-Marttunen *et al.*, 2013). In terms of graph theory metrics, comatose patients were shown to preserve global network properties but cortical regions, which worked as hubs in healthy controls, became non-hubs in comatose brains and vice versa (Achard *et al.*, 2011, 2012). Similarly, chronic patients showed altered network properties in medial parietal and frontal regions as well as in the thalamus, and most of the affected regions in unresponsive patients belonged to the so-called 'rich-club' of highly interconnected central nodes (Crone *et al.*, 2014). More recently, functional MRI-based single-patient classification has been performed by considering as discriminating feature the neuronal properties of various intrinsic connectivity networks (Demertzi *et al.*, 2014). The discrimination between 'neuronal' and 'non-neuronal' was based on the spatial and temporal properties (fingerprints) of the identified networks that were extracted by means of independent component analysis (De Martino *et al.*, 2007). According to specific criteria (Kelly *et al.*, 2010), 'non-neuronal' components were those that showed activation/deactivation in peripheral brain areas, in the cerebrospinal fluid (CSF) and white matter, as well as those showing high frequency fluctuations (>0.1 Hz), spikes, presence of a sawtooth pattern and presence of thresholded voxels in the superior sagittal sinus. Conversely, 'neuronal' were those networks when at least 10% of the activations/deactivations were found in small to larger grey matter clusters localized to small regions of the brain. Based on this definition of neuronality, the 'neuronal' properties of the default mode and auditory network were able to separate single-patients from healthy controls with 85.3% accuracy. Nevertheless, the discrimination accuracy between patients in MCS and VS/UWS reached only a chance level (Demertzi *et al.*, 2014).

Taken together, these studies show that the so far resting state functional MRI-based differentiation of patients has been performed either at the group-level or concerned the classification between healthy and pathological groups. As a consequence, clinicians remain with the challenge of how to implement the resting state functional MRI paradigm on an individualized basis for the more challenging discrimination between the MCS and VS/UWS (Edlow *et al.*, 2013). Here, we aimed at promoting the MCS-VS/UWS single-patient differentiation by using resting state functional MRI measurements in this clinical population. To this end, we studied systems-level resting state functional MRI functional connectivity in traumatic and non-traumatic patients with acute and chronic disorders of consciousness with the aim to (i) estimate the contribution of each network to the level of consciousness as determined by behavioural assessment; (ii) rank the capacity of each network to differentiate between patients in MCS and VS/UWS; and (iii) automatically classify independently assessed patients.

Materials and methods

Subjects

Three data sets were used, including patients scanned in Liège [to address study aims (i) and (ii)], Salzburg and New York [to address study aim (iii)]. Inclusion criteria were patients in MCS, VS/UWS and coma following severe brain damage studied at least 2 days after the acute brain insult. Patients were excluded when there was contraindication for MRI (e.g. presence of ferromagnetic aneurysm clips, pacemakers), MRI acquisition under sedation or anaesthesia, and uncertain clinical diagnosis. Healthy volunteers were free of psychiatric or neurological history. The study was approved by the Ethics Committee of the Medical School of the University of Liège, the Ethics Committee of Salzburg, and the Institutional Review Board at Weill Cornell Medical College. Informed consent to participate in the study was obtained from the healthy subjects and from the legal surrogates of the patients.

Data acquisition

All data were acquired on 3 T Siemens TIM Trio MRI scanners (Siemens Medical Solutions). For the Liège data set, 300 multislice T_2*-weighted images were acquired with a gradient-echo echo-planar imaging sequence using axial slice orientation and covering the whole brain (32 slices; voxel size = $3 \times 3 \times 3$ mm^3; matrix size = 64×64; repetition time = 2000 ms; echo time = 30 ms; flip angle = 78°; field of view = 192×192 mm). For the Salzburg data set, 250 T2*-weighted images (36 slices with 3-mm thickness; repetition time = 2250 ms; echo time = 30 ms; flip angle = 70°; field of view = 192×192 mm). For the New York data set, 180 T_2*-weighted images were acquired (32 slices; voxel size = $3.75 \times 3.75 \times 4$ mm^3; matrix size = 64×64; repetition time = 2000 ms; echo time = 30 ms; flip angle = 90°; field of view = 240×240 mm).

Subject-level connectivity analysis

Data analysis is illustrated in Fig. 1.

Data preprocessing

Preprocessing and connectivity analyses were performed in the same way for all subjects across the three data sets. The three initial volumes were discarded to avoid T_1 saturation effects. For anatomical reference, a high-resolution T_1-weighted image was acquired for each subject (T_1-weighted 3D magnetization-prepared rapid gradient echo sequence). Data preprocessing was performed using Statistical Parametric Mapping 8 (SPM8; www.fil.ion.ucl.ac.uk/spm). Preprocessing steps included slice-time correction, realignment, segmentation of structural data, normalization into standard stereotactic Montreal Neurological Institute (MNI) space and spatial smoothing using a Gaussian kernel of 6 mm full-width at half-maximum. As functional connectivity is influenced by head motion in the scanner (Van Dijk *et al.*, 2012), we accounted for motion artifact detection and rejection using the artifact detection tool (ART; http://www.nitrc.org/projects/artifact_detect). Specifically, an image was defined as an outlier (artifact) image if the head displacement in *x*, *y*, or

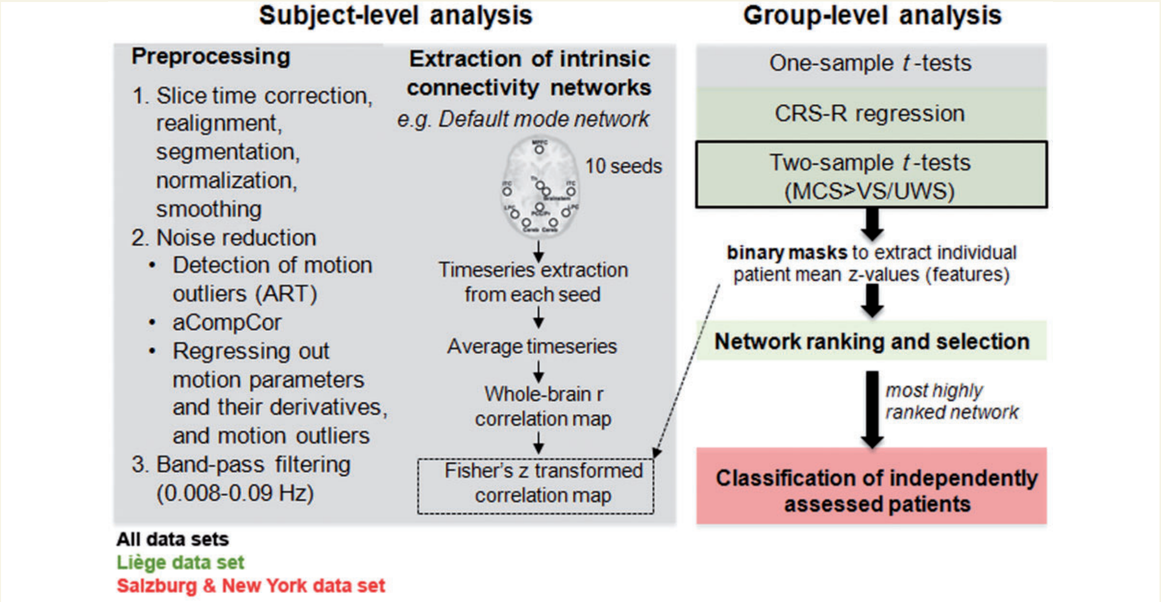

Figure 1 Analysis pipeline. Data analysis at the subject-level encompassed signal preprocessing and extraction of the intrinsic connectivity networks. Data analysis at the group-level encompassed estimation of functional connectivity in the networks of interest, estimated the contribution of each network to the level of consciousness by means of CRS-R total score regression analysis, and identified connectivity differences between the group of patients in MCS and VS/UWS for each network. Network ranking methodology was used to rank characteristic features (i.e., connectivity differences per network)to discriminate individual patients into the groups of MCS and VS/UWS. Two independent data sets of patients, assessed in Salzburg and New York, were used to further validate patient classification. Different colours indicate the three data sets and how these where used along the analysis pipeline.

z direction was > 0.5 mm from the previous frame, or if the rotational displacement was > 0.02 radians from the previous frame, or if the global mean intensity in the image was > 3 standard deviations (SD) from the mean image intensity for the entire resting scan. Outliers in the global mean signal intensity and motion were subsequently included as nuisance regressors (i.e. one regressor per outlier within the first-level general linear model). Therefore, the temporal structure of the data was not disrupted.

For noise reduction, previous methods subtracted the global signal across the brain (a controversial issue in resting state analyses; Murphy *et al*., 2009; Saad *et al*., 2012; Wong *et al*., 2012), and the mean signals from noise regions of interest (Greicius *et al*., 2003; Fox *et al*., 2005). Here, we used the anatomical component-based noise correction method (aCompCor; Behzadi *et al*., 2007) as implemented in CONN functional connectivity toolbox (http://www.nitrc.org/projects/conn/; Whitfield-Gabrieli and Nieto-Castanon, 2012). The aCompCor models the influence of noise as a voxel-specific linear combination of multiple empirically estimated noise sources by deriving principal components from noise regions of interest and by including them as nuisance parameters within the general linear models. Specifically, the anatomical image for each participant was segmented into white matter, grey matter, and CSF masks using SPM8. To minimize partial voluming with grey matter, the white matter and CSF masks were eroded by one voxel, which resulted in substantially smaller masks than the original segmentations (Chai *et al*., 2012). The eroded white matter and CSF masks were then used as noise regions of interest. Signals from the white matter and CSF noise regions of interest were extracted from the unsmoothed functional volumes to avoid additional risk of contaminating white matter and CSF signals with grey matter signals. A temporal band-pass filter of 0.008–0.09 Hz was applied on the time series to restrict the analysis to low frequency fluctuations, which characterize functional MRI blood oxygenation level-dependent resting state activity as classically performed in seed-correlation analysis (Greicius *et al*., 2003; Fox *et al*., 2005). Residual head motion parameters (three rotation and three translation parameters, plus another six parameters representing their first-order temporal derivatives) were regressed out.

Extraction of intrinsic connectivity networks

Functional connectivity adopted a seed-based correlation approach. Seed-correlation analysis uses extracted blood oxygenation level-dependent time series from a region of interest (the seed) and determines the temporal correlation between this signal and the time series from all other brain voxels. Evidently, the selection of the seed region is critical because, in principle, it can lead to as many overlapping networks as the number of possible selected seeds (Cole *et al*., 2010). Additionally, a network disruption can be expected due to patients' underlying neuropathology, as the chosen seed may no longer be included in the overall network. Using more seed regions, this issue can be overcome and therefore ensure proper network characterization in patients. Here, the seeds that were selected to replicate the networks were defined as 10-mm (for cortical areas) and 4-mm radius spheres (for subcortical structures) around peak coordinates taken from the literature (Supplementary material). For each network, time series from the voxels contained in each seed region were extracted and then averaged together. In that way, the resulting averaged time course was estimated by taking into account the time courses of more than one regions. The averaged time series were used to estimate whole-brain correlation r maps that were then converted to normally distributed Fisher's z transformed correlation maps to allow for group-level comparisons.

Group-level connectivity analysis

For the Liège data set, one-sample *t*-tests were ordered to estimate network-level functional connectivity for patients in MCS, VS/UWS and in coma; the data from healthy controls were used as a reference to ensure proper network characterization. An exploratory analysis looked for network-level connectivity changes as a function of patients' aetiology and chronicity. Two 2 × 2 factorial designs between aetiology (traumatic, non-traumatic)/ chronicity (acute, chronic) and the clinical entities (MCS, VS/UWS) were ordered. If an interaction effect was identified, these variables had to be entered as regressors in the general linear models.

To address the first aim of the study, i.e. to estimate the contribution of each network to the level of consciousness, patients' Coma Recovery Scale-Revised (CRS-R) total scores were used as regressors to determine the relationship between each network's functional connectivity and the level of consciousness. As a control, CRS-R total scores were used as regressors of functional connectivity for the cerebellum network (three regions of interest, Supplementary material), which is known to be minimally implicated in conscious-related processes (Tononi, 2008; Yu *et al*., 2015).

To address the second aim of the study, i.e. to determine the capacity of each network to differentiate between patients in MCS and VS/UWS, initially two-sample *t*-tests were ordered to identify the regions of each network showing higher functional connectivity in patients in MCS compared to VS/UWS (Liège data set). The resulting difference maps were saved as masks, which were used subsequently for the network ranking and selection step. All results were considered significant $P < 0.05$ corrected for multiple comparisons at false discovery rate (FWE; cluster-level).

Network ranking and selection

Using the REX Toolbox (http://www.nitrc.org/projects/rex/), the difference masks which were calculated in the previous step were used to extract mean connectivity values (average z-values across the whole mask) from the first-level contrast images estimated for each network. Therefore, one value per subject per network was created leading to a 6 × 1 vector per subject (i.e. 45 × 6 matrix). These vector values were considered as features in a feature ranking methodology (Saeys *et al*., 2007) as implemented in Matlab (http://www.mathworks.nl/help/bioinfo/ref/rankfeatures.html). The results of the feature (i.e. network) ranking were verified by means of single-feature linear support vector machine classifier (Burges, 1998). Supplementary material contains further details on the network ranking procedure and results.

To address the third aim of the study, i.e. to automatically classify independently assessed patients coming from two other clinical centres, we focused on the network which was ranked most highly during the network ranking procedure. For that network, a linear kernel support vector machine classifier (Burges, 1998) with regularization parameter $C = 1$ was used. This parameter was chosen based on its wide use in the machine learning procedure (Phillips *et al*., 2011). The features that were used for the training were individual mean connectivity values extracted from the first-level contrast images using the relevant network binary mask as described above. To avoid single feature classification, hence running the risk of overfitting, more features were included for the classifier's training. The number of features was based on the number of clusters showing higher connectivity in patients in MCS compared to VS/UWS as indicated by the contrast manager of the CONN toolbox during the connectivity analysis (FWE $P < 0.05$, cluster-level correction).

Classification of independently assessed patients

The final validation of the classifier was performed on a new set of connectivity values extracted from independently assessed patients in Salzburg ($n = 15$) and New York ($n = 7$). The data preprocessing, extraction of intrinsic connectivity network, and feature extraction followed an identical procedure as described above for the Liège data set. To test for robustness, we also evaluated whether the same classifier generalized to healthy controls subjects scanned in two centres (Liège, Salzburg; no healthy control data were available for the New York centre).

Results

Subjects

In Liège, between April 2008 and December 2012, 177 patients with disorders of consciousness underwent MRI scanning. Of these, 80 (45%) were excluded due to sedation or anaesthesia during scanning. Of the remaining 97 patients scanned in an awake state, five due to change of diagnosis within a week after scanning, 14 because they showed functional communication, 15 due to technical reasons or movement artifacts, and 12 due to incongruence between clinical diagnosis and fluorodeoxyglucose (FDG)-PET scanning (Stender *et al*., 2014). As regards the latter criterion, we decided to exclude patients showing widespread PET activation in midline and frontoparietal regions while the bedside diagnosis indicated the VS/UWS, in order to avoid confounds due to clinical ambiguity.

The included 51 patients were behaviourally diagnosed with the CRS-R (Giacino *et al*., 2004) as in MCS = 26, VS/UWS = 19 and coma = 6 (15 females; mean age 49 ± 18 years, range 11–87; 16 traumatic, 32 non-traumatic of which 13 were anoxic, three mixed; 35 patients were assessed in the chronic setting, i.e. >1 month post-insult). Data from an age-matched group of 21 healthy volunteers (eight females; mean age 45 ± 17 years; range 19–72) were used as a reference to the connectivity analyses and to validate the generalizability of the classifier without being included in the training. The data set from Salzburg included 10 MCS and five VS/UWS patients; the data set from New York included five MCS, one VS/UWS and one patient emerged from MCS. All patients' demographic and clinical characteristics are summarized in the Supplementary material.

For the Liège data set, the effects of the denoising procedure are summarized in the Supplementary material. Also, the number of motion outlier images did not differ among healthy controls (mean = 9 ± 8), patients in MCS (mean = 22 ± 17), VS/UWS (mean = 17 ± 12), coma (mean = 2 ± 2) (for all *t*-tests, $P < 0.05$). The exploratory analysis indicated a main effect for the clinical entity (i.e. MCS, VS/UWS) on the functional connectivity of each network. No interaction was identified between the clinical entity and aetiology (traumatic: MCS = 13, VS/UWS = 1; non-traumatic: MCS = 12 + 1 mixed; VS/UWS = 16 + 2 mixed) or chronicity (acute MCS = 5, VS/UWS = 6; chronic MCS = 21, VS/UWS = 13; average length of time since the injury was 902.3 days, minimum = 2 days, maximum = 9900).

Group-level connectivity analysis

For the default mode, frontoparietal, salience, auditory, sensorimotor and visual network, functional connectivity encompassed regions classically reported for healthy controls; all six networks showed reduced connectivity in patients in MCS, connectivity was hardly identified in patients in VS/UWS and was absent in comatose patients (Supplementary material).

CRS-R total scores correlated with functional connectivity in key regions of each network (Fig. 2). In contrast, when the CRS-R total scores were used as regressors of connectivity in the cerebellum, which is known for its minimal involvement in consciousness processes (Tononi, 2008), no areas showed connectivity with the behavioural scores. For illustrative purposes, the cerebellar network in healthy controls is presented in the Supplementary material.

The regions that showed higher functional connectivity in patients in MCS compared to VS/UWS for each network are summarized in Fig. 3. To minimize the possibility that differences in functional connectivity reflected differences in brain anatomy, we performed a two-sample *t*-test voxel-based morphometry on the normalized grey matter and white matter segmented masks (smoothed at 6 mm full-width at half-maximum). No differences in grey matter volume between patients in MCS and VS/UWS were identified at FWE $P < 0.05$ either at the whole-brain or at the cluster-level. Similarly, the analysis of white matter volumes identified no differences between the two groups, even at a liberal threshold $P < 0.001$ (whole brain level) uncorrected for multiple comparisons. The average grey matter and white matter volumes in the two patient groups are reported in the Supplementary material.

Network ranking and selection

All networks were found to discriminate between patients in MCS and VS/UWS with an acceptable accuracy (Supplementary material). Among them, the auditory network was the most highly ranked system to separate patients in MCS from those in VS/UWS.

Validation with independent data set

Functional connectivity of the auditory network was further used to classify independently assessed patients. The classification was performed on the connectivity strength in bilateral auditory and visual cortices (Fig. 3). This three-feature vector was preferred to a single-feature classification (i.e. the average connectivity across all areas of the auditory network mask) to avoid over-fitting of the classifier. Based on these three clusters' connectivity strength (*z*-values), 20 of 22 patients independently assessed in Salzburg and New York were discriminated congruently (Fig. 4 and Supplementary material), namely the CRS-R diagnosis matched the classification outcome. As in Phillips *et al.* (2011), for each feature we calculated its weighted vector 'w', which determines the orientation of the decision surface, indicative of which feature drives the classification (Bishop, 2006). For the right auditory cortex it was w = −1.7890, for the left auditory cortex w = −0.4002 and for the occipital cortex w = −0.7362. The patient who was misclassified as being in MCS had a CRS-R total score of 5 on the day of scanning (indicating the VS/UWS; Patient 11 of centre two, Supplementary material) and she evolved to MCS 38 days later (Auditory Function: 1, Visual Function: 3, Motor Function: 2, Oromotor/Verbal Function: 2, Communication: 0, Arousal: 2). The patient who was misclassified as being in VS/UWS had a CRS-R total score of 9 on the day of scanning (indicating the MCS; Patient 13 of centre two, Supplementary material) based on the presence of localization to noxious stimulation but this behaviour could not be elicited in neither previous (AF: 1, VF: 0, MF: 0, O/VF: 1, COM: 0, AR: 2) or subsequent evaluations (AF: 2, VF: 1, MF: 2, O/VF: 1, COM: 0, AR: 2). To test robustness, we evaluated whether the same classifier generalized to healthy control subjects scanned in Liège and Salzburg ($n = 39$; no healthy control data were available for the New York centre). The majority of healthy controls (37 of 39; 95%) were classified as MCS (Supplementary material).

Discussion

We here aimed at determining the clinical utility of the resting state functional MRI paradigm in patients with disorders of consciousness by employing a systems-level

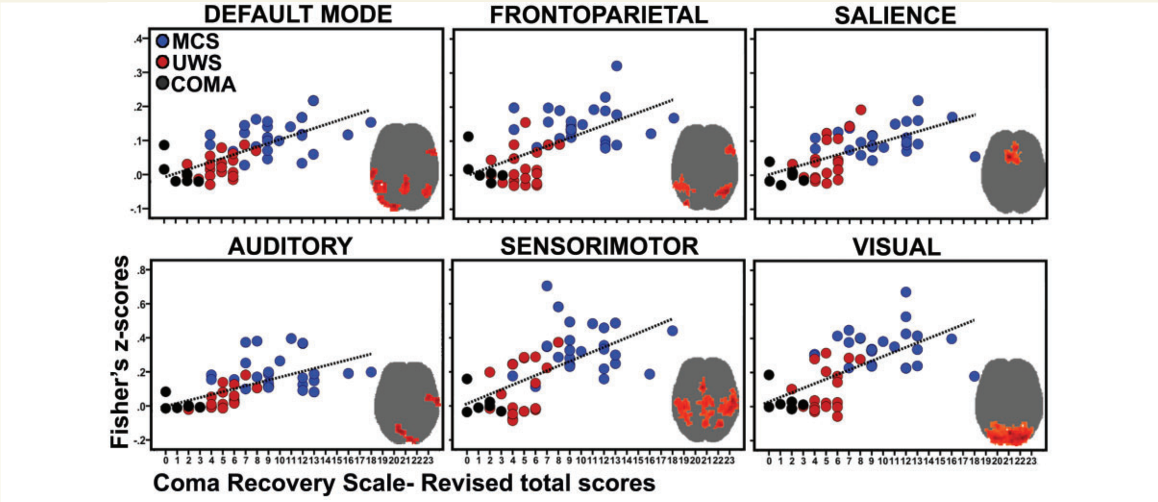

Figure 2 **The intrinsic connectivity networks are involved in consciousness-related processing.** Functional connectivity of all studied networks (areas in red) correlate with the level of consciousness as determined by behavioural assessment with the Coma Recovery Scale-Revised (total scores) in patients in MCS, VS/UWS and coma. Statistical maps are thresholded at FWE $P < 0.05$ (cluster-level) and are rendere on a glass brain template (transverse view).

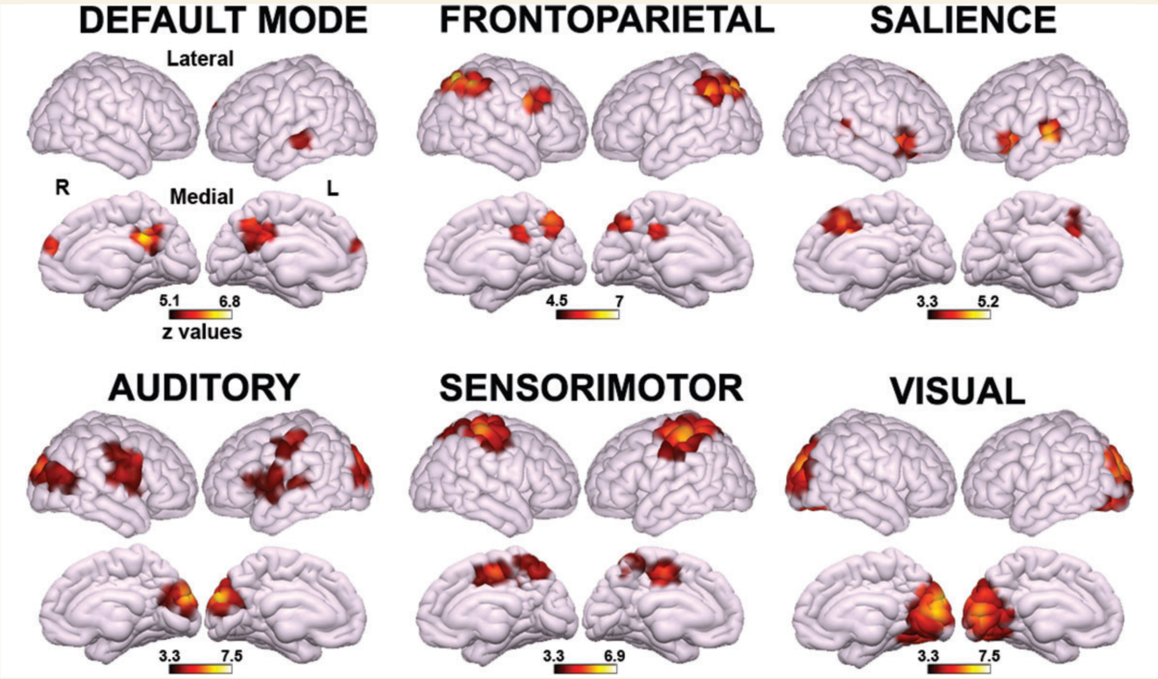

Figure 3 **Regions showing higher functional connectivity in patients in MCS compared to patients in VS/UWS for each network.** Statistical maps are thresholded at FWE $P < 0.05$ (cluster-level) and are rendered on 3D surface plot template (*top* = lateral view; *bottom* = medial view).

approach. Resting state functional MRI connectivity of the default mode, frontoparietal, salience, auditory, sensorimotor and visual networks were first shown to correlate with behavioural CRS-R assessment scores, highlighting their contribution to the level of consciousness. Previous studies on the default mode network, linked to autobiographical memory, mind-wandering, and unconstrained cognition (Buckner *et al.*, 2008), also showed

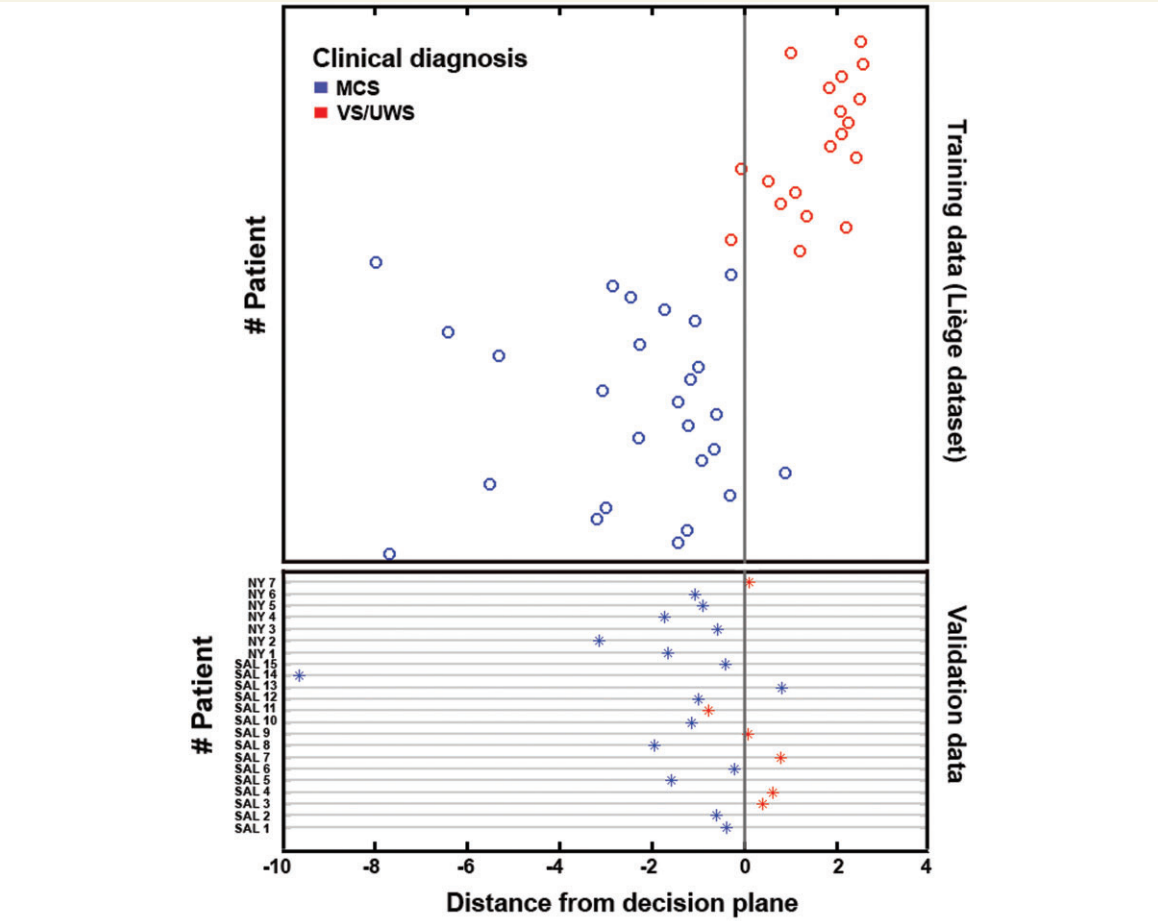

Figure 4 The auditory-visual crossmodal functional connectivity discriminates single patients in MCS from patients in VS/UWS. The 3D space indicating connectivity between left auditory, right auditory and occipital cortex (Supplementary material) has been compressed into two dimensions to represent the distance of each patient (in circles) from the decision plane (arbitrary values). The *upper* panel plots the data of patients (in circles) who were used for the classifier's training (Liège data set, $n = 45$). The *lower* panel summarizes the classifier's decision on the validation data set including patients (in asterisks) independently assessed in Salzburg ($n = 15$) and New York ($n = 7$). Based on the crossmodal interaction, 20 of the 22 independently assessed patients were classified congruently, namely the behavioural diagnosis matched the classification outcome.

consciousness-level dependent reductions in connectivity under physiological (Horovitz *et al.*, 2009; Samann *et al.*, 2011) and pharmacological unconsciousness (Greicius *et al.*, 2008; Boveroux *et al.*, 2010; Stamatakis *et al.*, 2010; Amico *et al.*, 2014). Similarly, the frontoparietal network, which has been linked to perceptual and somesthetic processing (Smith *et al.*, 2009; Laird *et al.*, 2011) and is considered critical for conscious reportable perception (Dehaene *et al.*, 2003), showed reductions in functional connectivity during sleep (Larson-Prior *et al.*, 2009; Samann *et al.*, 2011; Boly *et al.*, 2012) and anaesthesia (Boveroux *et al.*, 2010). The salience network, which has been involved in conflict monitoring, information integration, response selection, interoceptive processes (Seeley *et al.*, 2007; Smith *et al.*, 2009; Ploner *et al.*, 2010; Wiech *et al.*, 2010) and the emotional counterpart of pain (Seeley *et al.*, 2007; Shackman *et al.*, 2011), also showed modulations in connectivity under propofol anaesthesia (Guldenmund *et al.*, 2013). Here, the positive correlation between CRS-R scores and the salience network anterior cingulate cortex could account for the preserved capacities of some patients to orient their attentional resources towards environmental salient stimuli, such as noxious stimulation, corroborating previous PET data (Boly *et al.*, 2008). With regards to sensory networks, little changes have been reported under physiological and pharmacological unconsciousness (Heine *et al.*, 2012). Nevertheless, propofol-induced disconnections have been shown between the default mode network and motor cortex, reticular activating system and the thalamus

(Stamatakis *et al.*, 2010). In particular, the thalamus is of critical importance to consciousness (Dehaene and Changeux, 2005; Tononi, 2008). In our analysis the significance of the thalamus was controlled by involving it among the regions of interest in the three large-scale networks, namely the default mode network, frontoparietal and salience. The direct comparison between patients in MCS and VS/UWS did not identify any differences in network-level thalamic connectivity. However, a recent study with patients with disorders of consciousness using a target-detection task showed that respondents had a greater connectivity between the anterior thalamus and prefrontal cortex. These findings suggest that thalamo-frontal circuits are important for cognitive top–down processing (Monti *et al.*, 2015). Interestingly, when the cerebellum was used as a control network, CRS-R total scores did not correlate with any regions of this network in patients. Such findings confirm previous suggestions that the cerebellum has minimal implication in conscious-related processing (Tononi, 2008; Yu *et al.*, 2015). Taken together, the positive correlation between clinical scores and each network's functional connectivity highlight that the here studied networks are an appropriate means to study, at least to a certain degree, residual cognitive function in this patient cohort.

Importantly for clinical practice, we further aimed at determining the capacity of each network to differentiate between patients in MCS and VS/UWS. In terms of functional MRI-based differentiation of patients, to date differences in functional connectivity have been observed only at the group-level for the default mode (Boly *et al.*, 2009; Vanhaudenhuyse *et al.*, 2010; Norton *et al.*, 2012; Soddu *et al.*, 2012; Demertzi *et al.*, 2014), the frontoparietal and the auditory networks (Demertzi *et al.*, 2014). Here, we replicated these findings and further showed group differences in functional connectivity for the salience, sensorimotor and visual networks. Moving towards single-patient network-based differentiation, we found that all networks were able to differentiate patients with an acceptable accuracy ($>86\%$). Such high rate of accuracy can be partly attributed to the fact that the network ranking was based on features extracted from the same population for which between-group differences were already known. To avoid a double-dipping effect, we aimed at validating the most highly ranked network in two independently assessed patient data sets (Salzburg and New York) and across healthy controls. To that end, we opted for single-patient classification based on the connectivity strength of the auditory network. Based on this network's connectivity, 20 of the 22 new patients were classified congruently, i.e. the clinical diagnosis matched the classification outcome. Of note is that the classifier positioned the independently assessed patients closer to the decision plane compared to patients included in the training set. This could be explained by the abovementioned favouring of the Liège training data set during the network ranking procedure, which might have led to a stricter classification of the validation set. Although the intrinsic connectivity networks have been shown to be robust independent of different scanning parameters (Van Dijk *et al.*, 2010), the different parameters employed in each of the three centres might also have influenced the classifier's estimation. Alternatively, the use of a relevance vector machine classifier (Phillips *et al.*, 2011), which returns probabilities of a patient belonging to a clinical condition instead of using a binary decision, could be a more sensitive way to classify patients less strictly.

The classification results further highlight the challenges posed by behavioural examination (Majerus *et al.*, 2005) which in many cases underestimates patients' level of consciousness (Schnakers *et al.*, 2009). Here, the validation of the auditory network's classifier worked congruently for the majority of the included patients (20/22). Interestingly, the patient who was misclassified as MCS had a profile of VS/UWS on the day of scan but evolved to MCS 38 days later. The other patient was misclassified as VS/UWS but had a clinical profile of MCS on the day of scanning based on the presence of localization to noxious stimulation (note that this behaviour could not be elicited in any other evaluations). The validation of the classifier's outcome to the clinical evaluation was used as a starting point in our analysis. Therefore, a well-defined diagnostic baseline was critical for the subsequent patient classification. To that end, repeated clinical examinations with the CRS-R (average number of assessments $n = 6$ per patient) were performed. The clinical diagnosis was further confirmed with FDG-PET imaging, which has been shown to have high sensitivity in identifying patients in MCS (Stender *et al.*, 2014). Therefore, patients with an ambiguous profile on clinical assessment and neuroimaging data were not included in the analysis. Similarly, patients who received sedatives to minimize motion in the scanner (Soddu *et al.*, 2011) were further excluded. The reason to exclude sedated patients was because of our limited understanding of the potential effect of anaesthetics on network connectivity (Heine *et al.*, 2012). We here recognize the importance of increasing the classification power for patients scanned after receiving anaesthetics, given that many patients undergo anaesthesia not only to restrict scanner motion but also for neuroprotective reasons (Schifilliti *et al.*, 2010). Future investigations which will aim to disentangle between the variances of anaesthetics and pathology in functional connectivity measures are certainly essential. Finally, even though patients were scanned in an 'awake' state, the monitoring of patients' state of vigilance during data acquisition was not feasible because of technical difficulties. Hence, one cannot exclude the possibility that patients could have fallen asleep during scanning, which could subsequently influence the assessment of functional connectivity.

One explanation of why the auditory network was identified as the system with the highest discriminative capacity could concern its underlying functional neuroanatomy. Apart from temporal cortices, the auditory network further encompasses regions in occipital cortex, pre- and postcentral areas, insula and anterior cingulate cortex (Damoiseaux *et al.*, 2006; Smith *et al.*, 2009; Laird *et al.*, 2011; Maudoux *et al.*, 2012; Demertzi *et al.*, 2014). The direct comparison between patients in MCS and VS/UWS restricted the identified areas to bilateral auditory and visual cortices. This pattern of auditory-visual functional connectivity has been previously described in normal conscious subjects during rest as well (Eckert *et al.*, 2008) and is in line with functional MRI results in consciousness research. For example, preserved functional MRI activity in temporal and occipital areas has been shown for healthy subjects during mental counting of auditory temporal irregularities; interestingly, this activation was identified only in those subjects who were attentive and aware of the auditory violations (Bekinschtein *et al.*, 2009). At a functional level, the auditory-visual functional connectivity, also referred to as crossmodal interaction, is considered relevant for multisensory integration (Clavagnier *et al.*, 2004). Multisensory integration has been suggested as a facilitator for top–down influences of higher-order regions to create predictions of forthcoming sensory events (Engel *et al.*, 2001). Such top–down connectivity was recently found with an EEG oddball paradigm that differentiated patients in MCS from VS/UWS (Boly *et al.*, 2011). Interestingly, decreased crossmodal auditory-visual interaction has been reported in healthy subjects with preserved structural connections but under pharmacologically-induced anaesthesia (Boveroux *et al.*, 2010). In that study, recovery of consciousness paralleled the restoration of the crossmodal connectivity suggesting a critical role of this connectivity pattern to consciousness level-dependent states.

In our results, the crossmodal interaction was more preserved in patients in MCS compared to unresponsive patients. The reduction in functional connectivity between the auditory-visual cortices in VS/UWS could be partly attributed to disrupted anatomical connections, often encountered in post-comatose patients (Perlbarg *et al.*, 2009; Fernandez-Espejo *et al.*, 2010, 2011; Stevens *et al.*, 2014; van der Eerden *et al.*, 2014). The tight link between functional and structural connectivity was recently shown in primates during propofol-induced unconsciousness with regards to resting state functional MRI dynamic fluctuations. In this study, functional connectivity was fluctuating less frequently among distinct consciousness states, it was mostly linked to the state characterizing unconsciousness and this pattern was mostly explained by the underlying structural connectivity (Barttfeld *et al.*, 2015). Here, the negative differences between the two patient groups on voxel-based morphometry of grey and whiter matter segments is suggestive that the changes in functional connectivity cannot be fully attributed to the underlying anatomical abnormalities. We recognize that analyses with diffusion-weighted imaging and its relation to functional data would allow for more confident statements about residual functional connectivity in our clinical sample.

In conclusion, we here identified that systems-level resting state functional MRI showed consciousness-dependent breakdown not only for the default mode network but also for the frontoparietal, salience, auditory, sensorimotor and visual networks. Functional connectivity between auditory and visual cortices was the most sensitive feature to accurately discriminate single patients into the categories of MCS and VS/UWS. Our findings point to the significance of multisensory integration and top–down processes in consciousness seemingly supported by crossmodal connectivity. In the future, efforts need to be made to promote the feasibility of such a complex approach in the clinical setting and promote the clinical utility of the resting paradigm for single-patient diagnostics.

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Supplementary material

Supplementary material is available at *Brain* online.

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Appendix G

Paper VII

Behavioral Responsiveness in Patients with Disorders of Consciousness

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Chapter 3
Behavioral Responsiveness in Patients with Disorders of Consciousness

Lizette Heine, Steven Laureys, and Caroline Schnakers

Abstract Until now, the main way to assess whether a patient is conscious or not is to observe what he can do spontaneously or in response to stimulation. Although there is a growing body of research on the subject, detecting oriented/voluntary responses is still extremely challenging. Motor, verbal, and cognitive impairments; fluctuations of vigilance; and medications with impact on the central nervous system are among the factors complicating the diagnosis. Establishing a proper diagnosis is nevertheless of high clinical relevance when considering patients' prognosis and treatment. In this review, we will characterize the behavioral patterns of the various levels of consciousness, we will explain how challenging it is to detect signs of consciousness, and which tools currently exist to help in the assessment of those signs. Secondarily, we will present preliminary data investigating the interest of various sensory modalities in determining the diagnosis of patients with severe brain injury.

Keywords Vegetative state • Minimally conscious state • Consciousness • Assessment • Diagnosis • Sensory stimulation

3.1 Introduction

Some patients surviving extensive brain damage only regain limited levels of consciousness. Until now, the main way to assess whether a patient is conscious or not is to observe what he/she can do spontaneously or in response to stimulation. Although there is a growing body of research on the subject, detecting oriented/voluntary

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responses is still extremely challenging. Motor, verbal, and cognitive impairments; fluctuations of vigilance; and medications with impact on the central nervous system are among the factors complicating the diagnosis. Establishing a proper diagnosis is nevertheless of high clinical relevance. Conscious and unconscious patients have different outcomes. Patients in a minimally conscious state have a better long-term prognosis compared to those in a vegetative state [1]. Twelve months after brain injury, about half of the patients in minimally conscious state improve and show a good functional outcome, whereas only a very small percentage (3 %) of patients in vegetative state do so [2]. The diagnosis also has an impact on the patients' daily care and therapeutic choices when it comes to the administration of pharmacological interventions such as pain medication or new non-pharmacological interventions such as neurostimulation (deep brain stimulation or transcranial direct current stimulation) [3, 4]. Finally, regarding end-of-life decisions, previous legal cases in several countries have established the right of the medical team to withdraw artificial nutrition and hydration in patients diagnosed as being in a vegetative state [5]. In such context, a correct diagnosis is therefore crucial. In this review, we will characterize the behavioral pattern of the various levels of consciousness, we will explain how challenging it is to detect signs of consciousness, and which tools currently exist to help in the assessment of those signs. Secondarily, we will also present preliminary data investigating the interest of various sensory modalities in determining the diagnosis of patients with severe brain injury.

3.2 Behavioral Pattern in Disorders of Consciousness

When the patient is in a coma, there is no arousal and no consciousness. During this transient condition, patients' eyes are continuously closed (even following stimulation), autonomic functions are reduced, and respiratory assistance is needed [6] (Table 3.1). Most patients recover from a coma within hours to weeks after injury. However, some patients can recover arousal (i.e., open their eyes spontaneously or in response to stimulation) without being conscious (no oriented/voluntary responses). These patients are in a state called "vegetative state" (VS) [7] (Table 3.1). In this state, breathing occurs without assistance since autonomic functions (e.g., cardiovascular regulation, thermoregulation) are preserved. The patients may also moan, demonstrate smiling, crying, or grimacing even though inappropriate and appearing out of context [7, 8]. This state can be either transient or persistent (when above a month post-injury). After a year for traumatic etiologies and 3 months for nontraumatic etiologies, the VS can be considered as permanent. These patients have, in that case, less than 5 % of chances to recover. Only then, the ethical and legal issues around withdrawal of hydration and nutrition may be discussed [9]. Note that, given the negative connotation of the term "vegetative state," The European Task Force on Disorders of Consciousness has recently proposed to use the more neutral and descriptive term "unresponsive wakefulness syndrome" (VS/UWS) [10].

Table 3.1 Summary of the behavioral features for coma, VS/UWS, MCS–, MCS+, and emergence from MCS

Level of consciousness	Behavioral features
Coma	No arousal/eye opening
	Impaired spontaneous breathing/brainstem reflexes
	No oriented or purposeful behaviors
	No groans, vocalizations, or verbalizations
	No language comprehension/response to command
Vegetative state/unresponsive wakefulness syndrome	Arousal/spontaneous or stimulus-induced eye opening
	Preserved spontaneous breathing/brainstem reflexes
	No oriented or purposeful behaviors
	Groans and/or vocalizations but no verbalizations
	No language comprehension/response to command
Minimally conscious state	Fluctuation of vigilance (MCS–/+)
	Preserved spontaneous breathing/brainstem reflexes
	MCS–: object localization-reaching-manipulation and/or sustained visual fixation and/or visual pursuit and/or automatic motor behavior and/or localization to pain
	MCS+: command following and/or object recognition and/or intelligible verbalization and/or intentional communication
	Emergence: functional communication and/or functional object use on at least two consecutive assessments

Consciousness recovery consists of regaining fluctuating but reproducible nonreflexive-oriented and/or voluntary behaviors. Such state is called the “minimally conscious state” (MCS) [11] (Table 3.1). Behaviors that suggest consciousness are, for example, command following, visual pursuit, object localization, or contingent responses to emotional stimuli. MCS has recently been divided into two categories, MCS+ (plus) and MCS- (minus), based on the complexity of behavioral responses. Patients in an MCS- show nonreflexive-oriented responses such as visual pursuit or localization to noxious stimuli, while MCS+ refers to patients showing nonreflexive voluntary responses such as command following, intelligible verbalization, and/or nonfunctional communication [12, 13]. When patients demonstrate reliable “functional communication” (i.e., accurate yes-no responses to situational orientation questions) or “functional object use” (i.e., appropriate use of different common objects) on two consecutive assessments, the patient is considered to have emerged from the MCS (EMCS) [11] (Table 3.1). After emerging from MCS, these patients are not considered as being in a disorder of consciousness anymore. However, they often remain confused, disoriented, and sometimes agitated. The term “acute confusional state” (ACS) has recently been used to describe these patients [14].

3.3 Misdiagnosis

Differentiating MCS from VS/UWS can be challenging since voluntary and reflexive behaviors can be difficult to distinguish and subtle signs of consciousness may be missed. The development of diagnostic criteria for MCS [11] would reasonably be expected to reduce the incidence of misdiagnosis relative to the rates reported before these criteria were established [15, 16]. However, recent studies found that around 40 % of patients believed to be in VS/UWS were still misdiagnosed [17, 18] (Fig. 3.1).

The high rate of misdiagnosis likely reflects different sources of variance. Variance in diagnostic accuracy may result from biases contributed by the examiner, the environment, and/or the patient. First, examiner errors may arise when the range of behaviors sampled is too narrow, response-time windows are over- or under-inclusive, criteria for judging purposeful responses are poorly defined, and examinations are conducted too infrequently to capture the full range of behavioral fluctuation. The use of standardized rating scales offers some protection from these errors, although failure to adhere to specific administration and scoring guidelines may jeopardize diagnostic accuracy. Second, the environment in which the patient is evaluated may bias assessment findings. Paralytic and sedative medications, restricted range of movement stemming from restraints and immobilization techniques, poor positioning, and excessive ambient noise/heat/light can decrease or

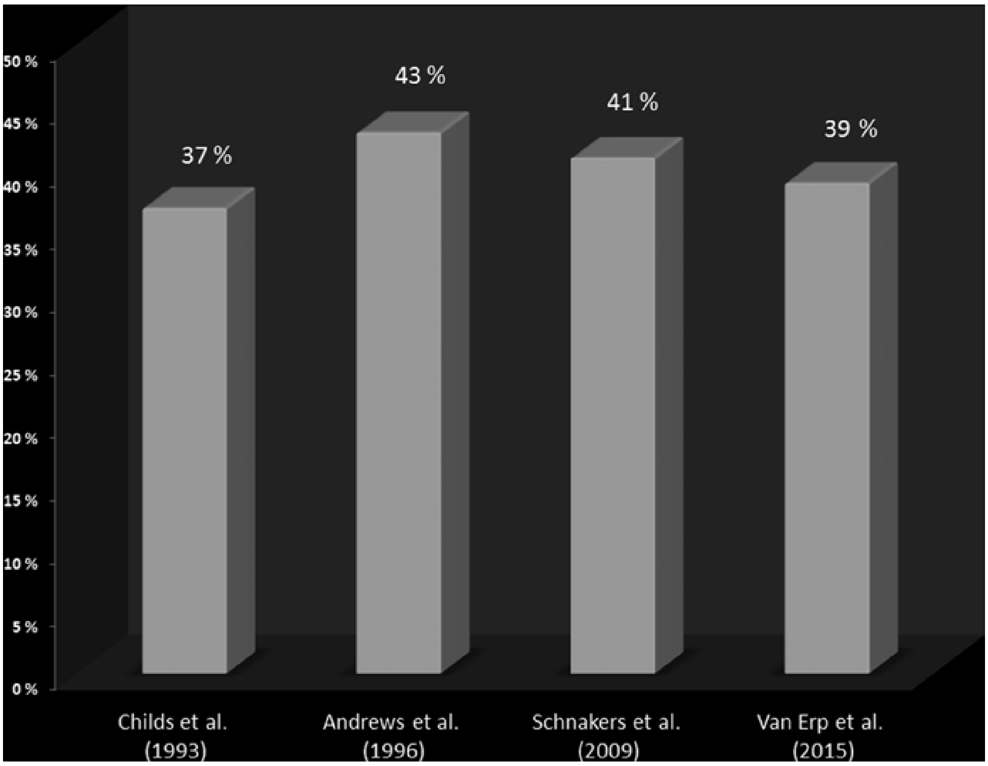

Fig. 3.1 Previous findings on misdiagnosis rate in patients with severe brain injury

distort voluntary behavioral responses. The last source of variance concerns the patient. Fluctuations in arousal level, fatigue, subclinical seizure activity, occult illness, pain, cortical sensory deficits (e.g., cortical blindness/deafness), motor impairment (e.g., generalized hypotonus, spasticity, or paralysis), or cognitive deficits (e.g., aphasia, apraxia, agnosia) constitute a bias to the behavioral assessment and therefore decrease the probability to observe signs of consciousness.

Some sources of error can be avoided, but this is not always possible or within the examiner's control. It is, however, particularly crucial to optimize the way consciousness assessments are performed as clinical management, from treatment of pain to end-of-life decision-making, often depends on behavioral observations. For this reason, the use of standardized and sensitive behavioral scales can substantially help clinicians to detect subtle signs of consciousness.

3.4 Clinical Assessment of Disorders of Consciousness

Behavioral assessment is based on two main components: wakefulness and awareness. Wakefulness refers to the patient's level of arousal and is assessed by observing eye opening. Awareness is related to subjective experiences and can be subdivided into awareness of the external world (i.e., perception of the environment or "consciousness") and awareness of the internal world (i.e., stimulus-independent thoughts such as mental imagery and inner speech or "self-awareness"). Raters assessing patients with severe brain injury will mainly assess consciousness of the environment, since self-awareness is difficult to evaluate when only based on bedside observations and not on patients' report. The assessment of consciousness can be done through repeated examinations revealing reproducible, oriented, or voluntary behavioral responses to various stimuli (the most common being auditory, verbal, and motor stimuli). The first scale widely used and known for assessing severely brain-injured patients recovering from coma is the Glasgow coma scale (GCS) [19]. This scale is short and can easily be incorporated into routine clinical care. Despite its widespread use, the GCS has been criticized for fluctuant inter-rater reliability and problems of scoring in patients with ocular trauma, tracheostomy, or ventilatory support [20]. The Full Outline of UnResponsiveness (FOUR) has been developed to replace the GCS for assessing severely brain-injured patients in intensive care [21]. The scale includes four subscales assessing motor and ocular responses, brainstem reflexes, and breathing. The total score ranges from 0 to 16. Unlike the GCS, the FOUR does not assess verbal functions to accommodate the high number of intubated patients in intensive care. It also assesses brainstem reflexes and breathing and, therefore, helps to better monitor comatose and VS/UWS patients. The FOUR also tracks emergence from VS/UWS since it includes the assessment of early signs of consciousness such as visual pursuit. The scale is globally more sensitive than the GCS for diagnosing MCS but like the GCS is not adapted to a rehabilitation setting.

Since the 1970s, a high number of scales have been validated for being used in subacute and chronic patients with severe brain injury (Table 3.2). Recently, the

Table 3.2 Behavioral responses assessed by scales developed for patients with disorders of consciousness

Name of the scale (Reference)	Response to command	Contingent emotional response	Object localization/ manipulation	Intelligible verbalizations	Oriented response to sensory stimulation				
					V	N	T	O	G
Coma Recovery Scale-Revised [24]	*		*	*	*	*			
Western Neuro Sensory Stimulation Profile [25]	*	*	*	*	*		*	*	
Sensory Modality Assessment & Rehabilitation Technique [34]	*	*	*	*	*		*	*	*
Wessex Head Injury Matrix [23]	*	*	*	*	*				
Disorder of Consciousness Scale [27]	*			*	*		*	*	
Sensory Stimulation Assessment Measure [35]	*		*	*	*		*	*	*
Glasgow Coma Scale [19]	*			*		*			
Reaction Level Scale [36]	*			*	*	*			
Innsbruck Coma Scale [37]						*			
Glasgow-Liège Scale [38]	*			*		*			
Full Outline of UnResponsiveness [21]	*				*	*			
Coma/Near-Coma Scale [39]	*			*	*		*	*	
Comprehensive of Level of Consciousness Scale [40]	*			*	*				

V visual, *N* nociceptive, *T* tactile, *O* olfactory, *G* gustatory, *bold* scale recommended with minor reservations by the ACRM, *italic* scales recommended with moderate reservations by the ACRM

American Congress of Rehabilitation Medicine (ACRM) has conducted a systematic evidence-based review of the available scales to provide recommendations for use according to validity, reliability, outcome prediction, and diagnostic sensitivity [22]. Among the scales evaluated, the Wessex Head Injury Matrix (WHIM) has been recommended with moderate reservations. The WHIM was developed to capture changes in patients in VS/UWS through emergence from post-traumatic amnesia [23]. This tool is particularly sensitive to detect changes in patients in MCS not captured by other scales such as the GCS. The WHIM has been structured according to the sequence of recovery observed in 88 patients recovering from traumatic brain injury. The scale assesses arousal level and concentration, visual pursuit, communication, cognition (i.e., memory and spatiotemporal orientation), and social behaviors. The WHIM score represents the rank of the most complex behavior observed. Despite a good validity, its reliability is still unproven, and, even though superior to the GCS, its diagnostic sensitivity is lower than other standardized scales such as the Coma Recovery Scale-Revised (CRS-R) [24]. In fact, according to the ACRM, the CRS-R is the most reliable tool for differentiating disorders of consciousness and received the strongest recommendation with minor reservations [22]. This scale was developed in 1991 and revised in 2004. Its primary purpose is to differentiate VS/UWS from MCS and MCS from EMCS. It measures auditory, visual, motor, and verbal functions as well as communication and arousal. Each of these subscales is hierarchically structured; the lowest scores reflect reflexive behaviors, while the highest scores indicate cognitively mediated behaviors. This scale has clear definitions for both the administration and the scoring of each item. The CRS-R can be administered reliably by trained examiners and produces reasonably stable scores over repeated assessments. Validity analyses have shown that the CRS-R is capable of discriminating patients in MCS from those in VS/UWS better than the GCS, the FOUR, and the WHIM [24].

Other scales such as the Western Neuro Sensory Stimulation Profile (WNSSP) [25], the Sensory Modality Assessment Technique (SMART) [26], and the Disorders of Consciousness Scale (DOCS) [27] have acceptable standardized administration and scoring procedures and have also been recommended with moderate reservations by the ACRM. On the contrary to the CRS-R whose main purpose is the diagnosis, the WNSSP, the SMART, and the DOCS are rather used when applying a sensory stimulation treatment to patients with severe brain injury. Sensory stimulation programs usually consist in presenting different types of environmental stimuli to the patient in order to optimize her/his consciousness level. These programs are supposed to constitute enriched environments which are supposed to enhance synaptic reinnervation, improve brain plasticity, and therefore accelerate the recovery from coma. However, even though numerous studies investigated the interest of these sensory stimulation programs, none of these studies has proven the efficacy of such treatment since the findings did not allow to differentiate spontaneous recovery from recovery due to treatment. Despite this, scales such as the WNSSP, the SMART, or the DOCS could still be interesting in a diagnostic context since they include the assessment of more sensory modalities than the CRS-R (i.e., tactile, olfactory, and gustatory modalities). The interest of those modalities for detecting signs of consciousness has nevertheless never been evaluated.

3.5 Can More Sensory Modalities Increase Diagnostic Sensitivity?

It has previously been shown that some sensory modalities are more sensitive to detect consciousness than others. In studies investigating misdiagnosis, oriented eye movements (i.e., visual pursuit and fixation) have been reported as the responses the most frequently missed during behavioral assessments [28, 17, 18]. In parallel, the visual modality of the CRS-R has been shown as the subscale allowing the highest detection of MCS as compared to the auditory, motor, or verbal modalities [29, 30]. Oriented visual responses are particularly interesting to detect since it is one of the first signs of consciousness appearing during patients' recovery and as it is associated with good outcome [31, 32, 2]. Until now, no study has investigated the interest of other sensory modalities (such as tactile, olfactory, and gustatory) when assessing consciousness, even though several scales recommended by the ACRM include such modalities (Table 3.2).

In a preliminary study, we therefore decided to investigate the interest of tactile, olfactory, and gustatory modalities in the assessment of consciousness. We assessed 38 patients (46 ± 16 years old, 17 traumatic, 21 chronic) diagnosed as being in a VS/UWS ($n = 15$) or in a MCS ($n = 23$) by using the CRS-R. Tactile, olfactory, and gustatory stimuli used in the WNSSP, the SMART, and the DOCS have been administered in each patient in a randomized order. Tactile stimuli included tap on the shoulder, nasal swab, feather (applied on arms, fingers, and face), air into the neck, hair touching, vibration on the arm, scrub (i.e., kitchen scouring pad applied over the arm), and firm hand pressure on the arm. Each of these stimuli was applied for 10 s on both sides of the body on three consecutive trials. Olfactory stimuli included vinegar, syrup, and ammonia which were held under the patients' nose for 10 s (patient's mouth closed) on three consecutive trials. In case of tracheotomy, the entrance of the cannula was covered. Gustatory stimuli included vinegar and syrup. A stick soaked of this flavor was introduced into the patient's mouth for 10 s on three consecutive trials. Several recommendations had to be followed such as applying the treatment while the patients were in a wakeful state with eyes open in a setting with minimal ambient noise and respecting a 30 min rest before each session (i.e., absence of nursing care). Oriented responses (e.g., eyes/head toward or away from the stimulus, hand toward or pushes away the stimulus, congruent facial expression, mouth opening, or tongue pumping) were considered as present when it was clear and reproducible, meaning it was observed at least two times to exclude reflexive behaviors. The oriented responses obtained using those tactile, olfactory, and gustatory stimulations have then been compared to the diagnosis obtained using the CRS-R. Patients' outcome has also been collected at 1 year after assessment ($n = 27$), using the Glasgow Outcome Scale (GOS) [33].

According to our results (Fig. 3.2), a minority of patients diagnosed as being in a VS/UWS by using the CRS-R showed oriented olfactory or gustatory responses (7 % and 14 %, respectively). The patient for whom we had outcome data (one missing data) did not recover consciousness a year after assessment. Additionally, oriented

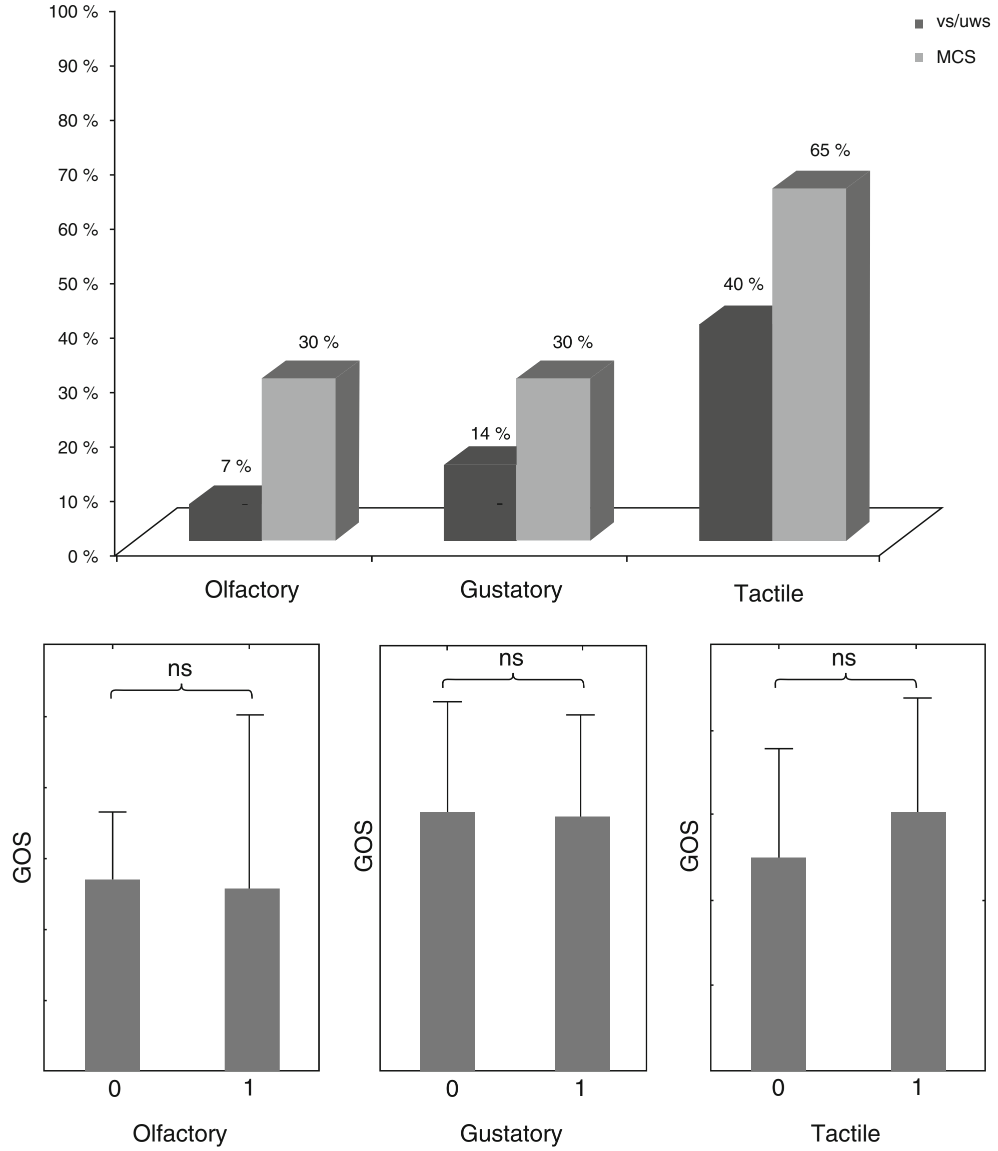

Fig. 3.2 Percentage of oriented responses in VS/UWS and MCS patients (panel **a**) and outcome at 1 year (averaged Glasgow Outcome Score – GOS, with 95 % confidence intervals) according to the absence (*0*) or presence (*1*) of oriented responses (panel **b**) ("ns" indicates difference is nonsignificant ($p > 0.05$))

olfactory or gustatory responses were absent in a majority of patients diagnosed as being in a MCS by using the CRS-R (70 %) and in a majority of patients who showed oriented eye movements (61 %). Using tactile stimuli, a higher percentage of patients diagnosed as being in a VS/UWS showed oriented responses (40 %). Oriented tactile responses were present in a majority of patients diagnosed as being in an MCS by using the CRS-R (65 %) and in a majority of patients who showed oriented eye movements (83 %). When considering the stimulus leading to the most frequent oriented responses, the nasal swab helped to detect 80 % of the oriented tactile responses. However, only one of the VS/UWS patients showing oriented tactile responses recovered consciousness a year after assessment (17 %). The patient (50 years old, 50 days after nontraumatic injury) was able to localize a tactile stimulus using her hand. Repeated CRS-R assessments, at that time, showed only reflexive behaviors (i.e., auditory startle, blinking to threat, flexion to noxious stimulation, oral reflexive movements, and arousal with stimulation). Two years after our assessment, the CRS-R indicated an EMCS. Finally, to test whether the outcome measured by the GOS differs according to the presence or absence of an oriented response, U Mann-Whitney tests were performed. There was no statistical difference for olfactory ($U=51.5$; $p=0.61$), gustatory ($U=49$; $p=0.5$), and tactile ($U=76.5$; $p=0.51$) modalities.

Considering our data, oriented olfactory and gustatory responses do not seem to be linked to consciousness since they are not observed in the majority of significant proportion of conscious patients and since they are not associated with consciousness recovery. Oriented tactile responses seem to be observed in most conscious patients but are not clearly related to consciousness recovery and could be false positives. This preliminary study hence seems to indicate that adding sensory modalities such as olfactory, gustatory, or tactile modalities to the CRS-R does not constitute a further help for decreasing the level of misdiagnosis in patients with disorders of consciousness.

3.6 Conclusion

Establishing a proper diagnosis is very important in the care of patients with severe brain injury. However, clinical assessment is difficult and can often lead to a misdiagnosis of the level of consciousness. The use of sensitive standardized tools is therefore crucial when establishing the diagnosis. The CRS-R is currently the most reliable and valid scale available and constitutes a substantial help in the differentiation of conscious vs. unconscious patients. Finally, even though our findings need to be replicated in a bigger sample, using gustatory, olfactory, or tactile stimuli that are included in several behavioral scales for the assessment of disorders of consciousness do not seem to be of further help when detecting consciousness in patients with severe brain injury.

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Appendix H

Paper VIII

Exploration of Functional Connectivity During Preferred Music Stimulation in Patients with Disorders of Consciousness

Heine L*, Castro M*, Martial C, Tillmann B, Laureys S, Perrin F.

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Exploration of Functional Connectivity During Preferred Music Stimulation in Patients with Disorders of Consciousness

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Preferred music is a highly emotional and salient stimulus, which has previously been shown to increase the probability of auditory cognitive event-related responses in patients with disorders of consciousness (DOC). To further investigate whether and how music modifies the functional connectivity of the brain in DOC, five patients were assessed with both a classical functional connectivity scan (control condition), and a scan while they were exposed to their preferred music (music condition). Seed-based functional connectivity (left or right primary auditory cortex), and mean network connectivity of three networks linked to conscious sound perception were assessed. The auditory network showed stronger functional connectivity with the left precentral gyrus and the left dorsolateral prefrontal cortex during music as compared to the control condition. Furthermore, functional connectivity of the external network was enhanced during the music condition in the temporo-parietal junction. Although caution should be taken due to small sample size, these results suggest that preferred music exposure might have effects on patients auditory network (implied in rhythm and music perception) and on cerebral regions linked to autobiographical memory.

Keywords: music, disorders of consciousness, fMRI, functional connectivity, auditory network, external network

INTRODUCTION

Patients with disorders of consciousness (DOC) are a patient population that is very difficult to assess. Following coma, these patients can be in an unresponsive wakefulness syndrome (UWS) where behavior is reflexive, and awareness of the self and surrounding is absent (The Multi-Society Task Force of Pvs, 1994; Laureys et al., 2010), or in a minimally conscious state (MCS) where behaviors indicating awareness are limited, fluctuating but reproducible (Giacino et al., 2002). Various interferences, both physical and cognitive impairments, or medical complications can affect the diagnosis based on clinical assessments of consciousness (Schnakers et al., 2009). This is one of the issues underlying the current misdiagnosis rate of 40% (Schnakers et al., 2009; van Erp et al., 2015). Consequently, numerous research is investigating the neural and cerebral responses of these patients, with the aim to provide unbiased and objective measures complementing bedside evaluation and helping diagnosis (Laureys and Schiff, 2011; Stender et al., 2014).

Previous research has also proposed to increase the sensitivity of clinical tests by using personally relevant stimuli (Perrin et al., 2015). For example, several behavioral studies have shown that a higher number of responses could be observed following self-referential stimuli, like the use of a mirror or the patient's own name, as compared to neutral stimuli (Vanhaudenhuyse et al., 2008; Cheng et al., 2013; Di et al., 2014). Neurophysiological studies have indicated that salient and emotional stimuli increase the probability of observing a cerebral response in patients with DOC. For example, the probability to observe a P300 event-related response (i.e., a brain response reflecting stimulus processing) is enhanced when the deviant stimulus is not a tone stimulus but the patient's own name (Perrin et al., 2006; Cavinato et al., 2011). Very recently, it has also been shown that preferred music (i.e., an autobiographical and emotional stimulus) has an effect on cognitive processes of patients with DOC. Indeed, observing a P300 to one's own name was increased in patients with DOC after having been exposed to their preferred music compared to a control condition (i.e., acoustically similar noise; Castro et al., 2015). This result is in agreement with a study showing increased behavioral responses after preferred music (Verger et al., 2014), and several single-case studies with DOC patients suggesting effects of music on a behavioral level (Magee, 2005; Magee et al., 2014).

Resting state functional MRI allows investigation of several distinct, reproducible and dynamic brain networks (Beckmann et al., 2005; Damoiseaux et al., 2006; De Luca et al., 2006; Laird et al., 2011), without the need for patients' cooperation (Soddu et al., 2011). The auditory network is one of the reliably observed networks, even though not yet extensively studied. This network encompasses primary auditory cortices including Heschls gyri, superior temporal gyri, insula, cingulate, post- and pre-central gyri, and supramarginal gyrus (Beckmann et al., 2005; Smith et al., 2009; Laird et al., 2011). The auditory network can be observed in 81% of healthy subjects, 46% in MCS, and is limited to 21% of UWS patients (Demertzi et al., 2014). In fact, it has strong power to discriminate MCS and UWS patients, making automatic classification possible (Demertzi et al., 2015). Another network that is also related to auditory processing (Brunetti et al., 2008) is the external network. This network is also related to external orientation, goal-directed behaviors, and cognitive processing of somatosensory (Boly et al., 2007), and visual (Dehaene and Changeux, 2005) input. The external network is often named the 'dorsal attention network,' or 'task positive' network (Greicius et al., 2003; Vanhaudenhuyse et al., 2010a). It has been shown to be anticorrelated with an internal/default mode network (Greicius et al., 2003; Vanhaudenhuyse et al., 2010a), implicated in self-awareness and stimulus-independent thoughts in healthy controls (Raichle et al., 2001; Greicius et al., 2009). Interestingly, auditory, external and internal/default mode networks include cortical regions that have been shown to be modulated by emotional sounds. Indeed, as compared to noise, meaningful sounds (infant cries or the patient's own name) are associated to a widespread activation of the auditory cortex and medial cortical structures in DOC patients (Laureys et al., 2004). Thus, the effect of music as reported in Castro et al. (2015) is probably also associated to functional connectivity changes of these regions.

We here aim to explore whether the effect of music in severely brain-damaged patients with DOC is related to functional connectivity changes. Functional MRI scans were acquired while participants were exposed to their preferred music as well as a control condition when they were exposed to the repetitive noise from the scanner (also present in the music condition). Using a functional connectivity parcellation (Gordon et al., 2014), we assessed functional connectivity using seed regions in both primary auditory cortices. We also analyzed network connectivity of the auditory network, the external network, and default mode network. We expect to observe changes, and more specifically increases, in functional connectivity in the auditory and attentional systems in patients with DOC during the music stimulation (vs. the control condition).

MATERIALS AND METHODS

Participants

Eight healthy participants (four female; mean age = 26 years, *SD* = 3), and seven patients (four MCS; three UWS) were scanned between March 2014 and April 2015 for this study. Patients were excluded for this study when any contraindication for MRI was present (e.g., presence of ferromagnetic aneurysm clips, pacemakers), or when patients needed sedation. Chronic patients with DOC were hospitalized for 1 week of assessment at the coma science group, University hospital of Liege, Belgium. Multiple behavioral assessments in the form of the CRS-R were completed, including one the morning before the (f)MRI acquisition. One patient showed drain artifacts on the T1 and functional MRI scan covering more then 40% of the brain, and in one patient the segmentation could not be reliably performed due to the lesion extent. Our patient population consisted thus of five patients (three MCS, two UWS; mean age = 50 years, *SD* = 10; **Table 1**). The ethics committee of the medical school of the University of Liège approved the study.

Music Stimulation and Procedure

Five musical excerpts were selected for each participant from a questionnaire on musical preference completed by family members or loved ones (for the patients) or the participant him/her self (for the healthy participants). These musical excerpts had a mean duration of 2 min and were all dynamic, musically coherent, and representative of the whole musical piece. The five excerpts were combined to create a musical stimulus of a duration of 10 min and 10 s, which overlaps with the duration of the functional scan. Fading in and fading out (around 2 s) was added to avoid rough transitions between the excerpts.

The functional scan was acquired twice during one MRI scanning session. Once with the participants' preferred music (i.e., music condition), and once when participants were exposed to the repetitive noise from the scanner (i.e., control condition). This control condition is the same as used for the investigation of a classical resting state. The order of the conditions was randomized between participants, and the two functional scans

TABLE 1 | Diagnostics of the five patients with disorders of consciousness (DOC).

		DOC1	**DOC2**	**DOC3**	**DOC4**	**DOC5**
Sex		Male	Female	Female	Male	Male
Age (years)		40	50	39	61	58
Time since injury (months)		12	6	26	13	25
Etiology		Trauma	Anoxic	Trauma	Anoxic	Anoxic
Diagnosis		UWS	UWS	MCS -	EMCS	MCS +
CRS-R score	A.	1	1	2	4	3
	V.	0	0	3	5	0
	M.	2	1	2	6	1
	O.	0	1	1	3	1
	C.	0	0	0	2	0
	Ar.	1	2	2	3	2
	Total	4	5	10	23	7
Structural MRI		Subcortical diffuse axonal injury, moderate enlargement of the ventricles, and atrophy of midbrain and sulci	Cortical and subcortical atrophy with severe post-anoxic leukoencephalopathy	Right lenticular lesion, diffuse axonal injury, and enlargement of the third ventricles	Extensive defects in region of the posterior cerebral artery, thalamus, and enlargement of right lateral ventricle	Global hemosiderosis and ischemic damage, white matter intensities (frontal + temporal), and enlargement of the ventricles
Neuroimaging (PET)		Indicated MCS	Consistent with an UWS	Consistent with MCS	Consistent with EMCS	Consistent with MCS

CRS-R, coma recovery scale revised; A., auditory function; V., visual function; M., motor function; O., oromotor/verbal function; C., communication; Ar., arousal; UWS, unresponsive wakefulness syndrome; MCS, minimally conscious state; EMCS, emergence from minimally conscious state.

were always separated by a delay of 10 min to reduce any potential order effects. Instructions and musical stimuli were delivered through MR compatible Siemens headphones. Participants were instructed to keep their eyes closed, stay awake, avoid any structured thoughts, and listen attentively to the music.

MRI Acquisition and Analysis

Two sets of 300 T2*-weighted images were acquired using a 3T Siemens TIM Trio MRI scanner (Siemens Medical Solutions, Erlangen, Germany) with a gradient-echo echo-planar imaging sequence using axial slice orientation and covering the whole brain (32 slices; voxel size = 3 mm × 3 mm × 3 mm; matrix size = 64 × 64 × 32; repetition time = 2000 ms; echo time = 30 ms; flip angle = 78°; field of view = 192 mm × 192 mm). The 10 initial volumes were discarded to avoid T1 saturation effects. Data preprocessing was performed using Statistical Parametric Mapping 8 (SPM8[1]). Preprocessing steps included realignment and adjustment for movement-related effects, slice time correction, co-registration of functional onto structural data, segmentation of structural data, spatial normalization of all data to standard stereotactic Montreal Neurological Institute (MNI) space using the normalization parameters which had resulted from the segmentation step. Normalized functional data were then smoothed using a Gaussian kernel with an isotropic 8 mm of full-width half-maximum.

Motion correction was applied using an automatic artifact detection tool for global mean and motion outliers[2]. Outliers in the global mean signal intensity and motion were identified and included in the subsequent statistical analysis as nuisance parameters (i.e., one regressor per outlier within the first-level general linear models). Specifically, an image was defined as an outlier (artifact) image if the head displacement in x, y, or z direction was greater than 0.5 mm from the previous frame, or if the rotational displacement was greater than 0.02 radians from the previous frame, or if the global mean intensity in the image was greater than 3 SD from the mean image intensity for the entire resting session. For our group of patients, the number of motion outlier images did not differ significantly between music and noise sessions (two-sided paired *t*-test; $p = 0.16$, music condition $m = 16$, $SD = 18$; control condition $m = 3$, $SD = 4$). Healthy participants did not show any movement-affected outlier scans.

Analyses of functional connectivity were performed using the connectivity toolbox "conn," version 15D[3] (Whitfield-Gabrieli and Nieto-Castanon, 2012). As recently recommended (Behzadi et al., 2007; Murphy et al., 2009; Saad et al., 2012; Wong et al., 2012), we used a regression of nuisance effects before bandpass filtering (RegBP; Hallquist et al., 2013). The data were despiked, and white matter (WM) and cerebrospinal fluid (CSF) components were regressed out as nuisance variables according to the aCompCor method. We then applied a linear detrending term. The residual BOLD time series went through a bandpass filter between 0.008 and 0.09 Hz to reduce the effect of low frequency drifts and high-frequency noise. All described steps are part of the standard procedure in the "conn" toolbox (Behzadi et al., 2007; Whitfield-Gabrieli and Nieto-Castanon, 2012). The residual head motion parameters (three rotation and three translation parameters, plus another six parameters representing their first-order temporal derivatives) were regressed out.

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[1] www.fil.ion.ucl.ac.uk/spm

[2] http://www.nitrc.org/projects/artifactdetect/

[3] http://www.nitrc.org/projects/conn

One pitfall of the analysis of resting state functional connectivity using seeds is the selection of seeds. The seed placement bias could lead to different and overlapping networks depending on the amount and placement of seeds (Cole et al., 2010). This bias can be reduced through the use of parcellations instead of spheres based on coordinates from the literature. We used a functional connectivity parcellation atlas based on a selection of parcels out of 330 parcels containing highly homogenous signal patterns (Gordon et al., 2014). This parcellation allowed us to perform two different analyses.

We first assessed functional connectivity on a seed based level. Two parcels were taken from the atlas of Gordon et al. (2014), localized at the structurally defined Heschl's gyrus (left and right). These two seeds were chosen for their importance in auditory processing. With these seeds group analysis was performed to assess functional connectivity within both conditions as well as differences between the preferred music and control condition. Furthermore, first level beta maps were extracted (i.e., fisher transformed correlation values) for each participant and used to create individual figures for our *a priori* regions during both conditions (supplementary material). Data of healthy subjects were not directly compared to patients due to age differences, thus the difference between the music and control condition within one patient could not be compared to the range of differences within controls. Therefore, no within-subject statistical analysis was performed.

Although studies in healthy subjects show that single seeds can reveal whole networks, this is not necessarily the case in brain-damaged patients. Network disruption can be expected due to underlying neuropathology excluding regions from overall networks. To assess overall network characterization it is advised to use multiple seeds/regions (Demertzi et al., 2015). All parcels belonging to the auditory network, external network, and default mode network according to Gordon et al. (2014) were assessed for our group of patients in each condition. For all networks, time courses of the parcels were averaged and correlated to the whole brain (Halko et al., 2014; Demertzi et al., 2015). Thus, this averaged time series was used to estimate whole-brain correlation r maps, which were then converted to normally distributed Fisher's z transformed correlation maps to allow for subsequent group-level analysis on the mean network connectivity (comparing music vs. control conditions). For all analyses on the group level (seed based and network based functional connectivity analysis) one sample t-tests were used for estimation of functional connectivity in each condition, and two-sample paired t-tests were used for between condition comparisons. The results were reported as significant when they exceeded a height threshold of uncorrected $p = 0.001$ with a family wise error corrected extent threshold of $p = 0.05$ at the cluster level. For clusters that showed significant stronger functional connectivity during the music condition contrast estimates (beta values) were extracted (Supplementary Figure S2). We did not compare the healthy group to our patient group due to differences in age, and the possible effects this might have on network integrity, as well as the possible differences in reaction to preferred music in terms of memory or emotion.

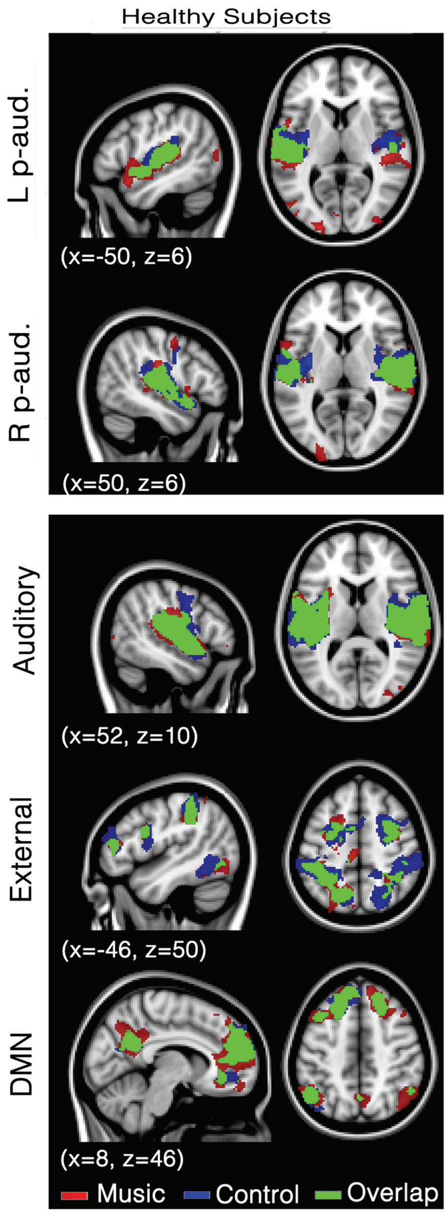

FIGURE 1 | Functional connectivity in healthy subjects during the music condition and the control condition. Maps indicate healthy subjects' ($N = 8$) functional connectivity during favorite music exposure (Red) and the control condition (Blue), and regions where functional connectivity was present in both conditions (Green). The top two panels show seed-based analyses, the lower three panels show mean network connectivity. Note that there is no significant difference between music and control condition. Results were analyzed in a network-based manner and thresholded with a family wise error corrected extended cluster level of $p < 0.05$. Standardized MNI T1 2x2x2 template was used to render results. (x,y,z) value indicates MNI coordinates of represented sections.

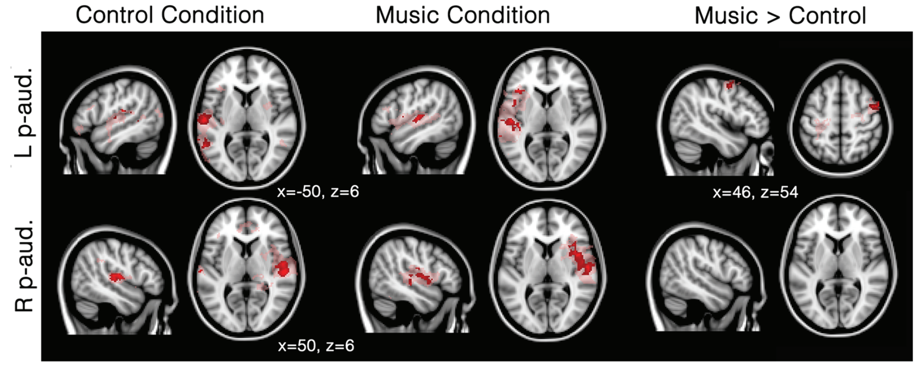

FIGURE 2 | Functional connectivity in patients during the music condition and the control condition using primary auditory seeds. Red/pink maps indicate patients' ($N = 5$) functional connectivity during the control condition (left) and favorite music exposure (middle) for both the left and right primary auditory cortex (L p-aud., and R p-aud.; respectively). Right maps show the regions that show significantly more functional connectivity during music condition compared to the control condition. Results were analyzed in a network-based manner and thresholded with a family wise error corrected extended cluster level of $p < 0.05$ (in red). For visualization a lowered threshold is indicated in pink (0.01 uncorrected height with family wise error corrected extended cluster level of $p < 0.05$). Standardized MNI T1 2x2x2 template was used to render results. (x,y,z) value indicates MNI coordinates of represented sections.

RESULTS

In healthy participants, seed-based analyses of both left and right primary auditory areas showed functional connectivity in areas considered as being part of the auditory network during both music and control conditions. Indeed, functional connectivity with seeds in both primary auditory cortices was observed in bilateral temporal gyri (encompassing Heschl's gyrus, opercular gyrus, insula, planum polare, and superior temporal areas), anterior cingulate, pre- and post-central areas and the occipital pole (**Figure 1**; Supplementary Table S1) in both conditions. No significant difference was observed between the two conditions. Similarly, the auditory network showed activation in bilateral temporal gyri (encompassing Heschl's gyrus, opercular, insula, planum polare, and superior temporal areas). This temporal cluster extended from inferior frontal, to precentral and angular areas. The auditory network also included the anterior cingulate, pre- and post-central areas and the occipital fusiform gyrus and cortex (**Figure 1**; Supplementary Table S2). The external network encompassed regions of bilateral inferior parietal sulcus and lobule, dorsolateral prefrontal, supramarginal, frontal eye field, lateral occipital and precentral, as well as cerebellar and insular areas. The default mode network showed functional connectivity with the precuneus, frontal pole and superior frontal gyrus, angular and lateral occipital gyrus, and middle temporal gyrus. For these three networks, the music condition did not significantly differ from the control condition.

In patients, seed-based analyses of patients showed that functional connectivity was mainly restricted to the areas surrounding each of the two seeds (i.e., left and right primary auditory cortex) for both the music and the control conditions; however, several other clusters of functional connectivity were also observed (**Figure 2**; **Table 2**). The left primary auditory seed showed functional connectivity with the middle temporal gyrus during the control condition, and the left frontal operculum, superior temporal gyrus and cerebellum during the music condition. The right primary auditory seed showed several smaller clusters in the temporal area as well as the supramarginal area during the control condition, and one large cluster of activation in the temporal cortex during the music condition. When the music condition was directly compared to the control condition, the left primary auditory seed showed more functional connectivity in the right precentral gyrus during music. No difference was observed with the right primary auditory seed for this direct comparison. Single subject first level beta values (i.e., Fisher's z transformed correlation values) were used to create individual patient figures for the two primary auditory seed activations during both conditions (Supplementary Figure S1). Correlation values during music and control conditions were mainly restricted to the areas surrounding each of the seeds, but in general, more voxels seemed to be strongly correlated in the music condition than in to the control condition (correlations higher than 0.8 were assessed and shown in the Supplementary Material).

Patients showed a severely limited auditory network of functional connectivity during both conditions (**Figure 3**; **Table 3A**). During the control condition, activation was only seen in bilateral temporal areas. During the music condition, the auditory network consisted of bilateral temporal gyri (only including left Heschl gyrus), as well as small clusters in the right inferior frontal gyrus and the left supramarginal gyrus; these were areas also included in the temporal cluster for the healthy subjects. When the music condition was compared to the control condition, the auditory network showed significantly more functional connectivity with the left precentral gyrus and a region on the junction of the middle frontal gyrus and frontal pole: the left dorsolateral prefrontal cortex.

The external network in patients was restricted to the inferior parietal sulcus and lobule, dorsolateral, middle frontal, and supra marginal areas during both control and music conditions.

TABLE 2 | Results of the seed-based analyses in the patients.

	MNI coordinates (x,y,z)			Cluster size	Cluster p-FWE	p-unc peak	Region	
Left primary auditory cortex								
Music	−40	24	6	223	0	0.000003	Left	Frontal operculum
	−40	−26	6	200	0	0.000023	Left	Heschl/planum temporale
	−68	−36	14	52	0.025011	0.000007	Left	Superior temporal gyrus
	0	−48	−8	50	0.030795	0.000067		Cerebellum
Control	−60	−20	6	403	0	0	Left	Heschl/planum temporale
	−68	−46	4	163	0	0.000003	Left	Middle temporal gyrus
Music > Control	46	0	54	113	0.000007	0.000002	Right	Precentral gyrus
Right primary auditory cortex								
Music	40	26	10	886	0	0.000003	Right	Temporal cortex: insula/central opercular/planum temporale/ Heschl/frontal operculum
Control	44	−16	10	379	0	0.000001	Right	Heschl gyrus/central opercular
	−68	−10	−2	85	0.00046	0.000047	Left	Superior temporal gyrus
	−64	−18	6	50	0.017807	0.000005	Left	Planum temporale
	28	−32	32	47	0.02515	0.000053	Right	Supramarginal gyrus

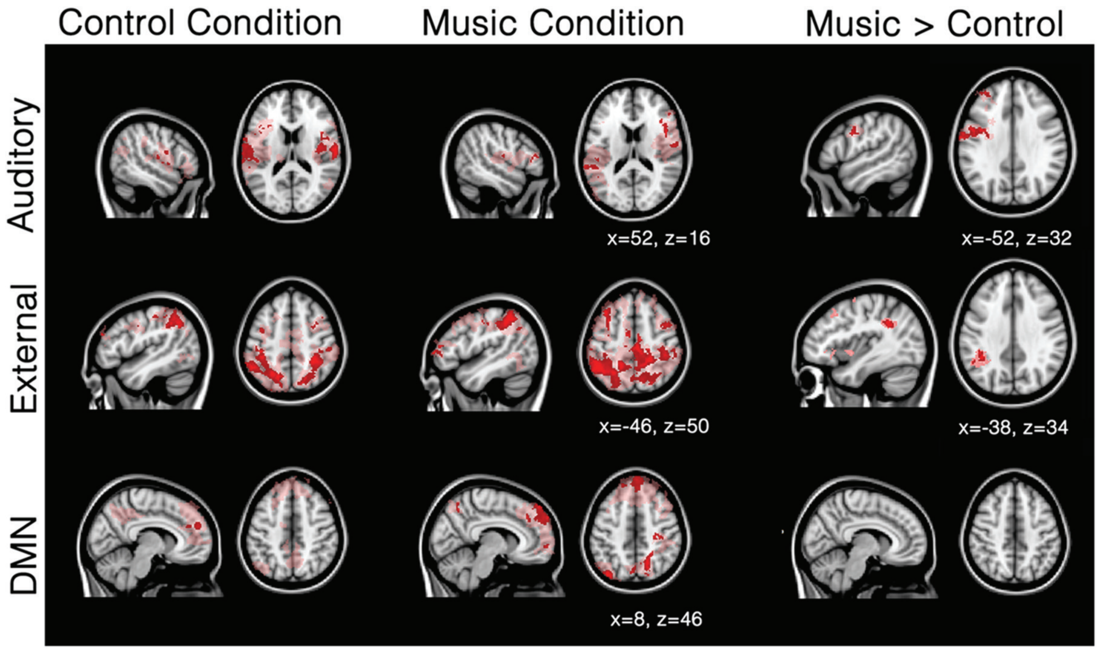

FIGURE 3 | Mean network connectivity in patients during the music condition and the control condition. Red/pink maps indicate patients' ($N = 5$) functional connectivity during the control condition (left) and favorite music exposure (middle) for the auditory network, external network, and default mode network (DMN). Right maps show the regions that show significantly more functional connectivity during music condition compared to the control condition. Results were analyzed in a network-based manner and thresholded with a family wise error corrected extended cluster level of $p < 0.05$ (in red). For visualization a lowered threshold is indicated in pink (0.01 uncorrected height with family wise error corrected extended cluster level of $p < 0.05$). Standardized MNI T1 2x2x2 template was used to render results. (x,y,z) value indicates MNI coordinates of represented sections.

(**Figure 3**; **Table 3B**). Compared to the control condition, music showed more functional connectivity with the supramarginal/ angular gyrus, also referred to as the temporoparietal junction.

The default-mode network in patients seemed disconnected in patients (**Figure 3**; **Table 3C**). The control condition only showed functional connectivity in the frontal pole/paracingulate gyrus. The music condition showed further functional connectivity with the precuneus, post-central gyrus, lateral occipital pole, and middle temporal gyrus. However, no difference could be found between the two conditions.

TABLE 3A | Results of network-based analysis in patients: auditory network.

	MNI coordinates (x,y,z)			Cluster size	Cluster p-FWE	p-unc peak	Region	
Auditory network								
Music	−66	−40	14	161	0.000004	0.000034	Left	Supramarginal gyrus
	40	20	18	109	0.000174	0.000002	Right	Inferior frontal gyrus
	60	−4	12	97	0.000466	0.000032	Right	Temporal, central opercular
	−50	−30	20	48	0.042949	0.000507	Left	Parietal operculum/Heschl
Control	−50	−40	10	1152	0	0.000004	Left	Temporal cortex: planum temporale/central opercular/ superior temporal
	28	6	2	997	0	0	Right	Temporal, central opercular/insula
	−36	20	12	208	0	0.000011	Left	Frontal operculum
	28	−26	26	46	0.046101	0.000094	Right	Parietal operculum
Music > Control	−66	−8	36	319	0	0.000001	Left	Precentral gyrus
	−28	42	30	44	0.028322	0.000019	Left	DLPFC

TABLE 3B | Results of network-based analysis in patients: external network.

	MNI coordinates (x,y,z)			Cluster size	Cluster p-FWE	p-unc peak	Region	
External network								
Music	58	−32	44	4974	0	0	Bilateral	Inferior parietal sulcus/inferior parietal lobule
	−36	24	52	424	0	0.000005	Left	DLPFC
	−52	32	16	122	0.000111	0.000008	Left	Middle frontal gyrus (small part FEF)
	−14	−10	64	116	0.000174	0.000013	Left	SMA
	48	12	56	100	0.000594	0.000021	Right	Middle frontal gyrus (small part FEF)
	30	34	−8	69	0.007915	0.000044	Right	DLPFC
	−38	−54	−12	59	0.019658	0.000013	Left	Lateral occipital/MT
	−56	−58	4	50	0.046263	0.000163	Left	Lateral occipital/MT
Control	−24	−62	48	2072	0	0.000001	Left	Inferior parietal sulcus/inferior parietal lobule
	12	−74	54	1026	0	0.000004	Right	Inferior parietal sulcus/inferior parietal lobule
	−32	14	24	403	0	0.000003	Left	SMA extending to small part FEF
	−42	48	24	104	0.000223	0.000012	Left	DLPFC
	34	8	52	82	0.001473	0.000065	Right	Middle frontal gyrus (small part FEF)
Music > Control	−42	−50	30	103	0.000078	0.000003	Left	Supramarginal/angular gyrus

DISCUSSION

In the present study, we aimed at assessing the potential effect of music on the brain's functional connectivity in patients with DOC. We compared patients' intrinsic brain activation while being exposed to their preferred music and during a control condition. For this purpose, seed-based functional connectivity as well as network-level functional connectivity was assessed. Seed-based functional connectivity analyses of primary auditory cortices showed significant differences in functional connectivity between music and control conditions for the patients. Network-level analyses showed that patients' functional connectivity is increased when being exposed to their preferred music in the auditory and external network (in comparison to the control condition).

In healthy participants, the network of functional connectivity based on both primary auditory regions encompasses large parts of the auditory cortex, superior temporal gyri, insula, cingulate cortex, central areas (pre and post), supramarginal gyrus, and occipital areas (**Figure 1**), in both the music condition and the control condition. These are, as expected, part of the auditory network (Beckmann et al., 2005; Damoiseaux et al., 2006; De Luca et al., 2006; Smith and Tindell, 2009; Laird et al., 2011; Demertzi et al., 2014). To assess network integrity, mean network connectivity was assessed in the auditory network, external network, and default mode network, i.e., networks that are

TABLE 3C | Results of network-based analysis in patients: default mode network.

	MNI coordinates (x,y,z)			Cluster size	Cluster p-FWE	p-unc peak	Region	
Default mode network								
Music	−26	32	34	1247	0	0.000001	Bilateral	Middle frontal gyrus/frontal pole/paracingulate gyrus
	12	−66	62	233	0	0.000004	Right	Precuneus/lateral occipital
	−38	−76	48	150	0.000014	0.000001	Left	Lateral occipital
	−30	52	2	110	0.000264	0.000124	Left	Frontal pole
	−58	−24	−12	81	0.002724	0.000004	Left	Middle temporal gyrus
	8	60	−4	56	0.025536	0.000149	Right	Frontal pole
	28	−24	46	53	0.034	0.000094	Right	Post-central gyrus
Control	−10	48	18	679	0	0.000034	Left	Frontal pole/paracingulate gyrus

respectively linked to auditory processing, external orientation, and internal thoughts.

Network-based second level analysis of functional connectivity showed that the auditory network was clearly replicated in our healthy subjects during both the music and control conditions. This network has consistently been observed in previous resting state studies investigating not only healthy participants but also DOC patients (Demertzi et al., 2014). In healthy participants it encompassed bilateral temporal gyri (including Heschl's gyrus, opercular, insula, planum polare, and superior temporal areas), extending to inferior frontal, precentral and angular areas, as well as clusters in anterior cingulate, pre- and post-central areas and the occipital fusiform gyrus (Beckmann et al., 2005; Damoiseaux et al., 2006; De Luca et al., 2006; Smith and Tindell, 2009; Laird et al., 2011; Demertzi et al., 2014). The external network has also been observed in healthy participants. It encompassed, as consistently observed in previous studies (Fox et al., 2005; Vanhaudenhuyse et al., 2010a), regions of bilateral inferior parietal sulcus and lobule, dorsolateral prefrontal, supramarginal gyrus, the frontal eye field, lateral occipital and precentral, as well as cerebellar and insular areas. The default-mode network showed functional connectivity in regions consistently observed in healthy participants and patient populations (Buckner et al., 2008). Most importantly, music did not show any increases in functional connectivity compared to the control condition for the seed-based and all three network-level analyses. This result is consistent with Castro et al. (2015) who observed that music (in comparison to noise) did not modify the event-related responses in healthy participants (while this was the case for the DOC patients). This observation suggests that the effects of music observed in previous research are possibly not present in healthy subjects (or that the cerebral responses could not be enhanced because they were already at ceiling). This finding could be due to the nature of our experimental material. Indeed Wilkins et al. (2014) have shown functional connectivity differences (in the default mode network and between auditory brain areas and the hippocampus) between two music materials that strongly differ in terms of emotion, i.e., preferred and disliked music (in healthy participants). It is thus possible, that our control condition, which can be considered as rather neutral, was not disliked enough to warrant significant differences in functional connectivity with the preferred music condition.

Seed-based analysis indicated that patients showed strongly limited functional correlations with the primary auditory cortices: activation was only observed around the seed areas and no long distance connectivity emerged within the auditory network. This finding is in line with previous research showing a linear decrease in functional connectivity ranging from healthy participants to unresponsive patients (Vanhaudenhuyse et al., 2010b; Thibaut et al., 2012; Demertzi et al., 2014). In fact, many studies have shown that functional connectivity still exists in DOC patients, and other forms of decreased levels of consciousness (Heine et al., 2012). Low-level activations in primary auditory cortices, without top–down feedback have also been observed in unresponsive patients (Laureys et al., 2000; Boly et al., 2011). In fact, patients seem to have a general disconnection between brain regions, notably missing long range connectivity (Casali et al., 2013). Our results are congruent with this observation as we observe mainly functional connectivity in the hemisphere of the seed. Furthermore, significant differences in the right precentral gyrus are observed during the preferred music condition compared to the control condition (**Figure 2**). This finding is in agreement with a previous study investigating DOC patients and reporting activation in the right superior temporal gyrus during three 10-s blocks of musical stimulation based on a famous song (Okumura et al., 2014).

First-level connectivity maps of each patient suggest larger areas of correlation near the seed during the music condition than during the control condition (Supplementary Figure S1). This difference seems to be present for all subjects, even the subjects clinically diagnosed as UWS (DOC1 and 2). This finding fits with the neuroimaging results observed in DOC1: diagnostic assessment based on PET metabolism suggested MCS (e.g., Stender et al., 2014). However, the second patient who was diagnosed as UWS (DOC2) both clinically and using neuroimaging, also showed more voxels correlated to the seed, indicating that the effect of music as reported here (if replicable in future studies with extended patient samples) might be present for all DOC. It is important to note that stronger correlating voxels were observed during the music condition (as compared to the control condition) in all patients for at least one seed. Also, no clear correlation with etiology, or time since injury can be seen due to the limited sample.

The three network analyses further revealed significant differences in the auditory network and external network, but not the default mode network, during the music condition. Patients showed a severely limited auditory network of functional connectivity during both conditions (**Figure 3**). During the control condition, activation was only seen in bilateral temporal areas. During the music condition, the auditory network was restricted to bilateral temporal gyri (only left including Heschl's gyrus) and small clusters in the right inferior frontal gyrus and the left supramarginal gyrus, areas included in the temporal cluster for the healthy subjects. The right inferior frontal gyrus is implicated in auditory memory as well as the processing of musical syntactic-like structures (Maess et al., 2001; Janata et al., 2002; Koelsch et al., 2002, 2005; Tillmann et al., 2003, 2006; Koelsch and Siebel, 2005; Albouy et al., 2013). When music was compared to the control condition, patients' auditory network showed significantly more functional connectivity with the left precentral gyrus (Note that the seed-based analysis also revealed significant increased functional enhancement in the right precentral gyrus during music; see **Figure 2**) and the left frontal pole. The precentral cluster overlaps with regions of the auditory network in healthy subjects. The lateral prefrontal cortex has also been linked to autobiographical memory (Svoboda et al., 2006; Cabeza and St Jacques, 2007), and has also been implicated in rhythm perception (Zatorre et al., 2007). The finding of increased functional connectivity in music compared to the control condition suggests that music has an effect on the auditory-related network in DOC patients, in whom short-term functional plasticity might appear following the lesions.

In patients, the external network observed during the control condition was restricted to clusters of functional connectivity in inferior parietal sulcus and lobule, dorsolateral, middle frontal, and supramarginal areas. In the music condition, the external network showed besides these regions also connectivity with the region MT and parts of the frontal eye field. When directly compared to the control condition, the music condition showed more functional connectivity with the supramarginal/angular gyrus. This cluster overlaps with the supramarginal regions activated during spatial orienting in healthy subjects (Corbetta and Shulman, 2002). Interestingly, this region overlaps with disconnected areas in UWS patients (Laureys et al., 2000). Laureys et al. (2000) proposed that a lack of integration between primary regions (that activate after simple auditory stimulations in UWS), and higher order regions like the temporoparietal junction and superior temporal gyri (activated in MCS after simple auditory stimuli; Boly and Faymonville, 2004) makes conscious processing unlikely (Laureys et al., 2000; Boly and Faymonville, 2004). Put differently, unconsciousness might be related to a disruption in feedback processing to the auditory regions (Boly et al., 2011).

CONCLUSION

The effect of music on functional cerebral connectivity is reminiscent of previous findings which have shown effects of music in brain-damaged patients (Soto and Funes, 2009; Särkämö and Soto, 2012; Verger et al., 2014; Castro et al., 2015). For example, a recent EEG study investigating DOC patients has shown that the patients' cerebral responses following the presentation of one's own name were increased after having been exposed to their preferred music (Castro et al., 2015). A "Mood and Arousal hypothesis," attributes the beneficial effects of music on cognition to an increase in mood and arousal (Chabris, 1999; Nantais and Schellenberg, 1999). Within this hypothesis, the effects of music in DOC patients might be due to an overall cortical arousal in the cerebral structures that have been reported to be involved in emotional and mood states. A second hypothesis attributes the effect of music to autobiographical priming (Castro et al., 2015). Interestingly, in the present study, an increased functional connectivity during the music condition (vs. the control condition) was shown in cortical structures linked to music perception, autobiographical memory and consciousness for DOC patients. These results need to be confirmed in an extended group of patients, and future studies should also disentangle the general effect of music (because of its acoustic and structural features) from its autobiographical effects (because of its emotional and meaningful contents in relation to the patients' personal memory).

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpsyg.2015.01704

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix I

Paper IX

Effects of preference and sensory modality on behavioral reactions in patients with disorders of consciousness

Heine L, Tillmann B, Hauet M, Juliat A, Dubois A, Laureys S, Kandel M, Plailly J, Luauté J, Perrin F.

Under review

Effects of preference and sensory modality on behavioral reaction in patients with disorders of consciousness

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Abstract

Reliable evaluation of patients with unresponsive wakefulness syndrome (UWS) or in a minimally conscious state (MCS) remains a major challenge. It has been suggested that the expression of residual cerebral function could be improved by allowing patients to listen to their favourite music. However, music's potential effect on behavioral responsiveness, as well as the effect of preferred stimuli in other sensory modalities (such as olfaction), remain poorly understood. The aim of the present study was to investigate the effect of sensory modality (auditory versus olfactory) and preference (preferred versus neutral) of the test stimuli on the subsequent performance of patients on the coma recovery scale-revised (CRS-R). We studied four items from the CRS-R (visual pursuit using a mirror, auditory localization of the own name, and two movements to command) in 13 patients (7 MCS; 6 UWS). The results showed that auditory stimuli triggered higher responsiveness compared to olfactory stimuli, and that preferred stimuli were followed by higher scores as compared to neutral ones. This suggests that preferential auditory stimuli at the bedside contributes to the expression of residual function, and could improve diagnostic assessment.

Introduction

Severe brain injury can lead to disorders of consciousness (DOC) comprising the unresponsive wakefulness syndrome (UWS), and minimally conscious state (MCS)[1]. Currently, the most reliable diagnosis of these challenging conditions can be achieved through the use of the Coma Recovery Scale-Revised (CRS-R)[2]. However, signs of consciousness such as meaningful affective behaviors can be hard to distinguish at the bedside [3,4]. It has been recently suggested that diagnostic assessment could be improved through the use of autobiographical and emotional context, such as preferred music [5,6]. For example, several studies have suggested that music improves patients interaction [7], emotional responses and cognitive capacity [8–10], though many of these are single cases or lack proper control conditions. Neuroimaging in patients with DOC has demonstrated more widespread functional connectivity in the auditory and external networks during preferred music compared to a noise control condition [11]. Furthermore, in an event-related potential study the presence of a discriminative response to patients' own name was also present following a period of preferred music, but not following a noise condition [6].

This research, however, did not disentangle the acoustic properties of music from a possible effect of preference. In addition, the effects of preferred stimuli (such as music) as a testing context on behavioural responses in DOC patients are not well established [10].

The aim of the present study was to test the potential beneficial effects of sensory modality (auditory, olfactory) and preference (preferred vs. neutral) on performance on items of the CRS-R. We chose to compare preferred music and odors because, in healthy subjects, these stimulation can influence behavior [12,13] and enhance episodic memory [14]. In brain injured patients it can improve behavioral vigilance performance [15]. Although they have not been extensively studied in DOC, olfactory stimuli are widely used in assessment scales like the WNSSP, SMART, DOCS [16–18].

Methods

Thirteen patients were assessed (6 UWS: 3 women, 52 ± 11 years, and 7 MCS: 1 woman, 37 ± 10 years; Table 1). Patients were recruited at the revalidation center of hospital of Lyon between November 2014 and August 2015. Patients were required to have had a DOC for longer than one month, be in stable condition, and demonstrate the presence of evoked potentials in response to auditory stimuli. The study was approved by the Lyon Ethics

Committee (CPP Sud-Est II, N°2014-A01062-45). Representatives of all patients provided written informed consent.

Four stimuli were presented in a 2x2 factorial design: two levels for modality (auditory or olfactory) and two levels for stimulus characteristic (preferred or neutral). Preferred stimuli were chosen and created for each patient on the basis of questionnaires filled out by a close relative (open choice for preferred music and forced-choice from a list of 51 pre-defined food flavorings).

Six neutral sound excerpts (i.e. continuous music-like noise stimuli) were created as control stimuli. These were constructed by selecting well-known songs from the genres of classical, rap, rock, reggae, French variety, and pop music, randomizing the overall phase spectrum, and deleting the slowest temporal envelope. Thus, they consisted of a spectral approximation of music, but did not share other acoustic characteristics (e.g., pitch, rhythm, envelope, or timbre).

Six neutral olfactory stimuli consisted of mixtures of monomolecular chemicals evaluated to be distinct, unfamiliar, unidentifiable through pretest (i.e., Citronellol, Rose oxide, Methyl octine carbonate, Ethyl acetyl acetate, Linalyl acetate, Cis-3-Hexenyl salicylate) [19]. The same neutral sound and odor stimuli were used for all patients.

Several items of the CRS-R were selected because they differentiate UWS from MCS: visual pursuit (using a mirror) and two different motor responses to command, chosen based on the patient’s capability. Localization to sound (to the patient’s own name), a non-differentiating item present in both patient populations, was chosen as the fourth item. Each patient was also assessed with the entire CRS-R twice, one prior to and once following the study, both within 48 hours (table 1).

For each of our 13 patients, four testing sessions were performed (separated by 3 to 7 days; Figure 1). Each session consisted of four trials, each trial including a 5-minute presentation of one of the four stimuli followed by one of the four CRS-R items. Each of the 16 stimulation-item combinations was presented once to each patient. The order of the stimuli and items was randomized both within and between patients. Each patient was thus seen six times (4 session, plus two complete CRS-R assessments) and total duration of the protocol was 3-4 weeks per subject.

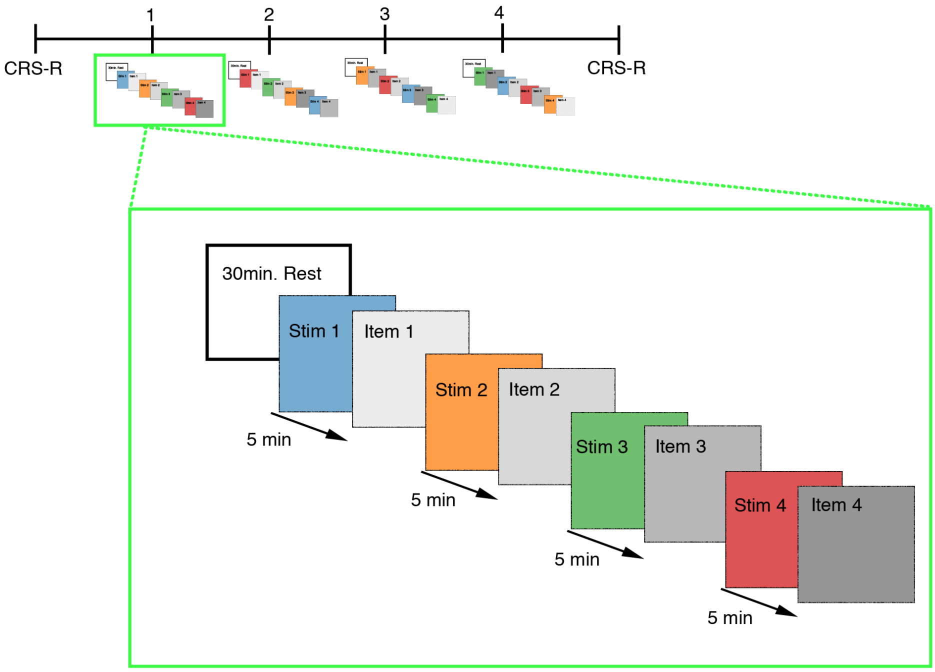

Figure 1. Schematic representation of the behavioral protocol. For each patient, four testing sessions were performed (separated by 3 to 7 days). Each session consisted of 4 trials, each including a 5-minute presentation of one of the four stimuli followed by one of the four CRS-R items. Each of the 16 stimulation-item combinations were presented once to each patient. The order of stimuli and items were randomized both within and between patients.

All sessions were filmed for subsequent scoring. For each item, the videos were scored blindly, i.e. without knowledge of the preceding stimulation, by three experimenters. Two measures were assessed from the recorded videos. “Item responses” were scored when correct reactions to the CRS-R item were observed. These were standardized by translating them into percentages of the maximum score (i.e., 8 for visual pursuit, and 4 for localization to sound and response to command, respectively). “Spontaneous signs” were scored in order to acknowledge clinical signs of awareness that occurred either during or after the stimulation. A score of 2 was given when signs of consciousness were present [20] (i.e. voluntary participation, orientation, emotional reactions, intelligible speech, or automatic movements only observed during stimulation and/or assessment). A score of 1 was given when reactions were present that were indistinguishable from unintentional behaviors or reflexes [21] (i.e. short orientation, partial participation, not the requested behavior, agitation/grimaces. A score of 0 was given when no reactions were observed. When blinded scores were not agreed upon between assessors, scores were averaged for subsequent statistical analysis. Notes were taken during each session by the experimenter present. These were however not used for statistical testing of the items responses, but were used for inter-rater reliability testing and aided assessment of spontaneous signs of consciousness.

All statistical analyses were performed using non-parametric tests, and reported when significant ($p < 0.05$). We first tested the effect of preference and sensory modality of the context on item responses and spontaneous signs. Inter-rater reliability, and the long term effect (difference between CRS-R scores before and after the protocol) were also assessed. Analysis was performed at the group level as previous studies have suggested that the beneficial effects of preferred music are independent of diagnosis [11,22].

Results

Item responses (Figure 2A,B)

For the scored item's responses, Friedman's ANOVA showed a difference between conditions ($\chi^2(3) = 10.48$, p=0.02), and Wilcoxon signed rank tests showed a main effect of modality. Considering preferred and neutral stimuli together, a higher proportion of responses was observed following the auditory stimuli than the olfactory stimuli (18.5% and 10% respectively; p=0.01; r=-0.35). A higher percentage of responses was also observed following the preferred sound than following either the preferred odor (20% and 12%, respectively; p=0.04; r=-0.41), or neutral odor (8%, p=0.01; r=-0.50; all Wilcoxon signed rank tests).

Spontaneous signs (Figure 2C,D)

Friedman's ANOVA was significant ($\chi^2(3) = 13.73$, p<0.01), and Wilcoxon tests showed a main effect of modality, as well as of preference. Scores were significantly higher after auditory stimuli (average score of 0.83) than after olfactory stimuli (score of 0.56, p p<0.01; r=-0.39), and following preferred stimuli (score of 0.8) compared to neutral stimuli (score of 0.58, p= p<0.01; r=-0.38). Spontaneous signs of consciousness were more frequent following the preferred sound (music, score of 0.98) than after the neutral sound (score of 0.67, p=0.02; r=-0.44), preferred odor (score of 0.62, p=0.01; r=-0.48), and neutral odor (score of 0.5, p p<0.01; r=-0.53).

It is worth noting that two patients diagnosed as UWS according to the pre- and post-study CRS-R assessments showed reactions following preferred stimuli. One patient showed tears during one specific song on both occasions it was played, and was purposefully uncooperative (i.e. eyes firmly closed with head-averting) during assessment. The other patient showed behavioral responses to the same response to command item only after preferred music and preferred odor stimuli.

Inter-rater reliability and long term effects

Inter-rater reliability showed high agreement between assessors (92.8%; mean bivariate Pearson correlation r=0.96). The agreement between the blind ratings (video scoring) and the scores assigned during the experiment (by experimenter present at the time) was lower, yet significant (74.5%, r=0.90). In fact, 15 percent of all items were scored higher during the experiment compared to the blinded scores, and five percent was scored lower then the blinded scores.

No significant differences were observed between the complete CRS-R administered before and after the four session protocol.

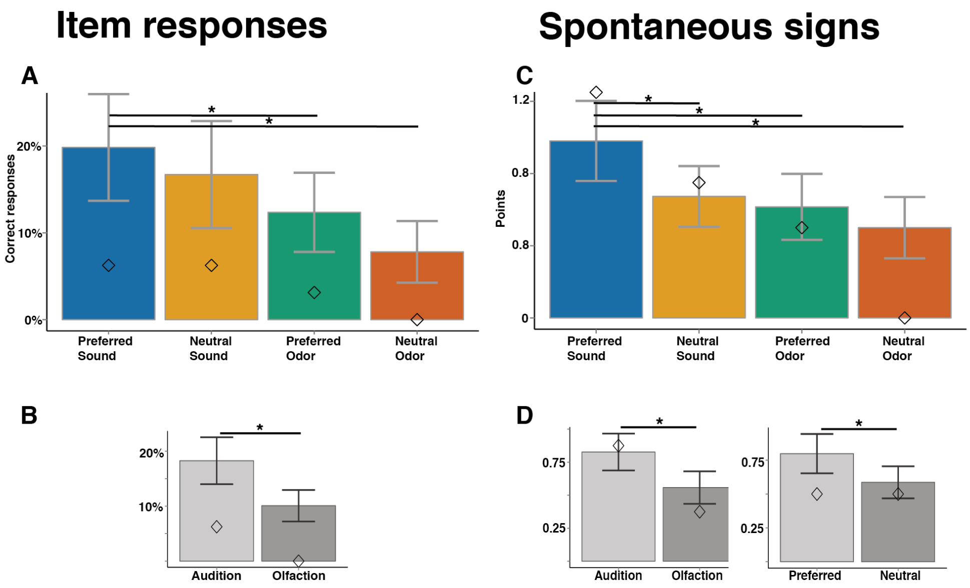

Figure 2. Scores for item responses and spontaneous signs. A. Mean standardized scores for CRS-R item responses after each stimulation. B. Significant main effects of modality (auditory > olfactory) for item responses. C. Mean scores for spontaneous signs of consciousness for each stimulation-item combinations. D. Significant main effects of modality (auditory > olfactory), and preference (preferred > neutral) for scores of spontaneous signs of consciousness. Lines and stars show significant differences between stimulations. Error bars represent standard error; diamonds indicate median.

Discussion

Here we assessed the effects of preferred auditory and olfactory stimuli on the behavioral responsiveness of patients with disorders of consciousness. Statistical analysis was performed on scores of four items of the CRS-R and on scores reflecting the frequency of spontaneous signs of consciousness.

Both scores showed that auditory stimuli triggered higher responsiveness compared to olfactory stimuli. Scores for spontaneous signs also showed a main effect of preference, with better scores for preferred stimuli. In addition, spontaneous signs were more frequent after preferred music than all other stimuli.

These findings establish a hierarchy among the different types of stimuli, placing preferred music at the top. This in in line with several studies reporting improved cognitive function after preferred music in these patients [7,9,11,22,23]. Systematic use of, for instance, the patient's preferred music during assessment might thus be advised. Indeed, two UWS patients showed emotional and behavioral reactions to autobiographical/emotional stimulations (one of the criteria for the diagnosis of MCS [20]). These behaviors were not observed during routine CRS-R assessments. This suggests that diagnostic assessment might be improved through the elicitation of meaningful (affective) behaviors due to the testing context. Correct diagnosis is crucial as it determines treatment and end-of-life decisions.

These results suggest that auditory stimuli (and in particular, preferred music) are better than olfactory stimuli at enhancing cognition or arousal in these patients. However, this interpretation must be considered with caution for several reasons. First, preferred auditory stimuli contained various changes (i.e. tone, rhythm, intensity), whereas olfactory stimuli had the same intensity and changed more gradually over time. Second, the preferred auditory stimuli were sampled with free choice, while the preferred olfactory stimuli were selected from a limited set. Thus, the personal salience, probably due to the reminiscence power of autobiographical memory might be higher for the musical stimuli. Finally, although olfactory dysfunction (13.5% in TBI after 1 year [24]), and dyfunctional olfactory neuronal processing [25] is present in a minority of DOC patients, we cannot exclude that there might be reduced olfactory abilities in our patient population. We detected a slight difference between preferred and neutral olfactory stimuli, however, suggesting that our patients were not anosmic.

Although this was not the aim of the study, it should be noted that the different types of stimuli did not modify the level of awareness in the long-term. Indeed, no difference was observed between the complete CRS-R before and after the several week-long protocol. Moreover, although interesting, it is not possible to compare the scores of each item tested during the experimental protocol to the specific item of the complete CRS-R before and after the protocol. Indeed, the full CRS-R assessment includes arousal protocols using deep-pressure stimulations, tactile as well as verbal stimulations to awaken the patient, none of which were present in the four experimental tests. Thus, future research should explore the potential cumulative effect of the arousal protocol and of stimulation with preferred music, as well as the difference between the effects of deep pressure and preferred stimuli on patients' responsiveness.

In conclusion, our data showed an effect of auditory stimulation and preference (i.e., preferred music) on patients' behavior, which can add relevant information for the evaluation of DOC patients. This indicates that improving the testing context could aid the expression of residual function in patients with disorders of consciousness, thereby improving diagnostic sensitivity.

Acknowledgments

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Author Contributions

PF conceived the study, and PF, HL, BT, PL, JL designed it. Acquisition and analysis of data was performed by HL, HM, JA, DA, KM. All authors participated in drafting of the manuscript and figures.

Conflicts of interest

There are no conflicts of interest.

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Table 1. Demographics

Patient	Sex	Age	Time since injury (months)	Etiology	Diagnosis (CRS-R)	CRS-R	A	V	M	O	C	Ar	Total
1	M	54	15	Encephalitis	MCS	Before	3	3	0	1	0	1	8
						After	1**	**	1	0	0	1	3
2	M	28	6	TBI	MCS	Before	4	5	4	1	0	1	15
						After	3	4	4	0	0	1	12
3	M	37	7	TBI	MCS	Before	0	3	3	0	0	2	8
						After	3	3	2	0	0	2	10
4	F	23	6	TBI	MCS	Before	3	3	5	1	0	2	14
						After	2	4	4	1	0	2	13
5	M	38	6	TBI	MCS	Before	3	3	3	0	0	2	11
						After	3	4	2	0	0	2	11
6	M	42	14	TBI	MCS	Before	1	1	0	0	0	1	3
						After	1	1	2	0	0	2	6
7	F	54	23	Anoxic (hemorrhage)	UWS	Before	0	0	0	2	0	1	3
						After	0	0	2	2	0	2	6
8	M	53	90	TBI	UWS	Before	1	0	0	0	0	1	2
						After	1	0	2	1	0	2	6
9	F	53	15	Anoxic (cardiac arrest)	UWS	Before	0	0	2	1	0	1	4
						After	0	0	2	1	0	1	4
10	F	63	45	Anoxic (hemorrhage)	UWS	Before	0	0	2	1	0	1	4
						After	0	0	2	1	0	1	4
11	M	58	36	Anoxic (cardiac arrest)	UWS	Before	0	0	2	1	0	2	5
						After	0	0	2	1	0	1	4
12	M	30	81	TBI	UWS	Before	0	0	2	1	0	2	5
						After	0	0	2	0	0	1	3
13	H	34	50	TBI	MCS	Before	2	3	1	0	0	1	7
						After	0	3	1	0	0	1	5

Abbreviations: M, Male; TBI, Traumatic brain injury; MCS, minimally conscious state; UWS, unresponsive wakefulness syndrome; **, uncooperative: purposeful eye closure.

Appendix J

Paper X

Functional connectivity in visual, somatosensory, and language areas in congenital blindness

Heine L, Bahri A M, Cavaliere C, Soddu A, Reislev N, Laureys S, Ptito M, Kupers R.

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Prevalence of increases in functional connectivity in visual, somatosensory and language areas in congenital blindness

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There is ample evidence that congenitally blind individuals rely more strongly on non-visual information compared to sighted controls when interacting with the outside world. Although brain imaging studies indicate that congenitally blind individuals recruit occipital areas when performing various non-visual and cognitive tasks, it remains unclear through which pathways this is accomplished. To address this question, we compared resting state functional connectivity in a group of congenital blind and matched sighted control subjects. We used a seed-based analysis with *a priori* specified regions-of-interest (ROIs) within visual, somato-sensory, auditory and language areas. Between-group comparisons revealed increased functional connectivity *within* both the ventral and the dorsal visual streams in blind participants, whereas connectivity *between* the two streams was reduced. In addition, our data revealed stronger functional connectivity in blind participants between the visual ROIs and areas implicated in language and tactile (Braille) processing such as the inferior frontal gyrus (Broca's area), thalamus, supramarginal gyrus and cerebellum. The observed group differences underscore the extent of the cross-modal reorganization in the brain and the supra-modal function of the occipital cortex in congenitally blind individuals.

Keywords: congenitally blind, functional connectivity, seed-based analysis, vision

Introduction

The loss of vision from birth causes a myriad of compensatory plastic changes. At the behavioral level, congenitally blind subjects outperform their sighted counterparts in a wide range of non-visual sensory discrimination tasks (Kupers and Ptito, 2014 for a recent review). For example, congenitally blind individuals show improved performance in tactile acuity at the finger tips (Wong et al., 2011) and perform better in pitch discrimination (Wan et al., 2010), syllable recognition (Gougoux et al., 2009) and sound localization (Fieger et al., 2006). Recent behavioral studies also indicate superior abilities in discrimination, identification and awareness of odors (Rosenbluth et al., 2000; Cuevas et al., 2009; Beaulieu-Lefebvre et al., 2011).

Compensatory plasticity is dependent on cross-modal reorganization of the brain in which the occipital cortex becomes recruited by various non-visual inputs (Kupers and Ptito, 2014). Brain imaging studies have highlighted the pivotal role of the visual cortex in the ability of the blind to perform non-visual tasks (Kupers et al., 2011b). Indeed, PET and fMRI studies have reported that congenitally blind individuals recruit their occipital cortex in tasks involving sound and tactile localization (Gougoux et al., 2005; Voss et al., 2008), tactile and auditory motion detection (Poirier et al., 2006; Ptito et al., 2009; Matteau et al., 2010; Sani et al., 2010), spatial navigation (Kupers et al., 2010), odor perception (Kupers et al., 2011a), language (Burton et al., 2003; Bedny et al., 2008, 2011; Striem-Amit et al., 2012) and memory processing (Raz et al., 2005).

Recent neuro-imaging studies also helped to illuminate the question how congenital blindness affects the structural organization of the brain, and through which pathways non-visual information reaches the occipital cortex. Structural brain imaging studies seem to concur that there are significant reductions in gray matter throughout the whole extent of the visual system. These include the optic chiasm, the lateral geniculate nucleus, the posterior pulvinar, and striate and extra-striate visual areas (Pan et al., 2007; Ptito et al., 2008b; Cecchetti et al., 2015). Regions of the ventral visual stream such as the inferior temporal gyrus and the lateral orbital cortex, as well as regions connected to the dorsal visual stream like the hippocampus also show volumetric reductions (Fortin et al., 2008; Jiang et al., 2009). In addition, cortical thickness is increased in the cuneus (Jiang et al., 2009; Kupers et al., 2011a), which is likely due to a reduction in cortical pruning during the early maturation process as a result of lack of visual input, and which may be indicative of alterations in connectivity. White matter changes in the visual pathways include atrophy of the optic tracts and the optic chiasm, reductions of the optic radiations, the splenium of the corpus callosum (Shimony et al., 2006; Pan et al., 2007; Ptito et al., 2008b; Tomaiuolo et al., 2014) and microstructural changes within the ventral visual pathways (Ptito et al., 2008a).

Recent studies have also tried to elucidate functional changes in the blind brain. Brain activation studies (Ptito et al., 2005; Klinge et al., 2010; Sani et al., 2010; Collignon et al., 2013; Ioannides et al., 2013; Kupers and Ptito, 2014) and transcranial magnetic stimulation (TMS) studies (Wittenberg et al., 2004; Kupers et al., 2006) have found evidence for increased functional connectivity of the occipital cortex with auditory and somatosensory areas. Several of the available resting state studies reported stronger connections of the occipital cortex with somatosensory (Watkins et al., 2012) and language areas (Liu et al., 2007; Bedny et al., 2011; Butt et al., 2013; Wang et al., 2013). Other studies, however, concluded that the occipital cortex of the blind has a general reduced connectivity with somatosensory/auditory regions (Yu et al., 2008; Burton et al., 2014), or even larger parts of the brain (Liu et al., 2007; Qin et al., 2014). Some of these differences may be due to small or inhomogeneous study populations, including both congenital and early blind subjects or subjects with and without residual light perception (Butt et al., 2013; Qin et al., 2013; Wang et al., 2013), or to the fact that the resting state scan was acquired after an active functional scanning paradigm (Bedny et al., 2011). To circumvent these issues, we analyzed resting state functional magnetic resonance imaging (rsfMRI) data of a homogeneous group of congenitally blind individuals lacking any residual light perception, using *a priori* defined regions of interest (ROIs) in areas with known roles in visual, somatosensory, auditory and language processing. No task-related functional brain scans were acquired before or after the resting state scans. Using state-of-the-art methods for analyzing rsfMRI data, we mapped out increases as well as decreases in functional connectivity in the congenitally blind brain.

Materials and Methods

Participants

We included 12 congenitally blind (CB; 5 females, 7 males; age: 42 ± 14 year) and 20 healthy sighted controls (SC; 12 females, 8 males; 42±14 year). Blind and sighted subjects were matched for age, gender, education, and handedness. All our congenitally blind subjects were born blind and had no history of light perception; **Table 1** lists their demographics. All participants gave informed consent and the ethics committee of the University of Copenhagen and Frederiksberg had approved the study protocol.

fMRI Data Acquisition and Analysis

MRI was conducted on a 3T scanner (Siemens Verio) equipped with a standard 32-channel head coil. Functional images were acquired with an EPI sequence (280 volumes, $TR = 2.15$ s, $TE = 26$ ms, flip angle = 78°, $FOV = 192\,\text{mm}^2$, 64 × 64 matrix, 43 axial slices of 4 mm). Scan duration was 15 min. Head motion was restricted by placement of comfortable padding around the participant's head. The three initial volumes were discarded

TABLE 1 | Demographics congenitally blind participants.

Subject	Age	Sex	Braille (WPM)	Cause of blindness
CB1	59	M	148	ROP
CB2	50	M	75	ROP
CB3	37	F	104	ROP
CB4	63	F	124	ROP, glaucoma
CB5	37	M	100	Unknown eye pathology
CB6	44	M	158	Retinoblastoma
CB7	51	M	75	ROP
CB8	29	F	91	ROP
CB9	28	F	115	ROP
CB10	59	M	130	ROP
CB11	25	F	118	ROP
CB12	27	M	94	ROP

ROP, retinopathy of prematurity; WPM, words per minute.

to avoid T1 saturation effects. For anatomical reference, a high-resolution T1-weighted image was acquired for each subject (T1-weighted 3D magnetization-prepared rapid gradient echo sequence "3D MP-RAGE"; $TR = 1.54\,\mathrm{s}$, $TE = 3.9\,\mathrm{ms}$, $FOV = 256 \times 256\,\mathrm{mm}$, 256×256 matrix, 92 slices of 1 mm thickness). Data preprocessing was performed using Statistical Parametric Mapping toolbox (SPM8, Welcome Department of Cognitive Neurology, London, UK) with MATLAB 7.12 (Mathworks Inc., Sherboorn, MA). Preprocessing steps included realignment and adjustment for movement-related effects, slice time correction, co-registration of functional onto structural data, segmentation of structural data, spatial normalization of all data to standard stereotactic Montreal Neurological Institute (MNI) space using the normalization parameters resulted from the segmentation step. Normalized functional data were then smoothed using a Gaussian kernel with an isotropic 8 mm of full-width half-maximum.

Motion correction was applied using an automatic artifact detection tool for global mean and motion outliers (http://www.nitrc.org/projects/artifact_detect/). The groups did not differ significantly in the number of movement artifacted time points ($p = 0.08$). More specifically, one sighted and one congenital blind subject showed movement. Outliers in the global mean signal intensity and motion were identified and included in the subsequent statistical analysis as nuisance parameters (i.e., one regressor per outlier within the first-level general linear models).

Analysis of functional connectivity was done using the connectivity toolbox "Conn," version 13o (http://www.nitrc.org/projects/conn; Whitfield-Gabrieli and Nieto-Castanon, 2012). An explicit gray matter mask was used. As recently recommended (Behzadi et al., 2007; Murphy et al., 2009; Saad et al., 2012; Wong et al., 2012), we used a regression of nuisance effects before bandpass filtering (RegBP; Hallquist et al., 2013). The data were despiked, and white matter (WM) and cerebrospinal fluid (CSF) components were regressed out as nuisance variables. Noise was regressed out according to the aCompCor method, where the influence of noise is modeled as a voxel-specific linear combination of multiple empirically estimated noise sources by deriving principal components from noise ROIs and by including them as nuisance parameters within the general linear models. This method protects against confounding correlations as produced by other methods, like global signal regression (Murphy et al., 2009; Chai et al., 2012; Saad et al., 2012; Wong et al., 2012). We then applied a linear detrending term. All described steps are part of the standard procedure in the "Conn" toolbox (Behzadi et al., 2007; Whitfield-Gabrieli and Nieto-Castanon, 2012). The residual BOLD time series went through a bandpass filter between 0.008 and 0.1 Hz to reduce the effect of low frequency drifts and high-frequency noise.

Regions of interest (ROIs) were taken from the literature (Geyer et al., 1999, 2000; Amunts et al., 2000; Binkofski et al., 2000; Rademacher et al., 2001; Rottschy et al., 2007; Scheperjans et al., 2008; Caspers et al., 2010, 2013; Kolster et al., 2010; Kujovic et al., 2013); they were defined as 6-mm radius spheres in both hemispheres. We included 15 seeds to assess functional connectivity (**Table 2**, **Figure 1**). These seeds were selected within the occipital cortex (i.e., V1, V2, hOC3V, hOC3D, hOC4V, hOC4D, MT/V5, and fusiform gyrus), parietal cortex (S1, lateral BA5, anterior BA7, posterior BA7 and BA40), auditory cortex (A1) and Broca's area.

TABLE 2 | Regions of interest (ROIs).

	Left hemisphere			Right hemisphere			Literature reference
	X	***Y***	***Z***	***X***	***Y***	***Z***	
VISUAL AREAS							
V1 (BA17)	−10	−77	3	20	−73	2	Amunts et al., 2000
V2 (BA18)	−13	−75	6	23	−71	6	Amunts et al., 2000
hOC3d	−15	−97	23	17	−95	24	Kujovic et al., 2013
hOC3v	−20	−88	−3	26	−84	−4	Rottschy et al., 2007
hOC4d	−17	−95	29	19	−94	29	Kujovic et al., 2013
hOC4v	−29	−84	−7	34	−80	−8	Rottschy et al., 2007
hMT (V5)	−48	−75	8	46	−78	6	Kolster et al., 2010
Fusiform gyrus	−30	−76	−9	33	−73	11	Caspers et al., 2013
SOMATOSENSORY AREAS							
S1 (BA3b)	−37	−28	55	37	−28	55	Geyer et al., 1999, 2000
BA5	−16	−51	73	13	−55	73	Scheperjans et al., 2008
BA7a	−19	−65	64	20	−65	64	Scheperjans et al., 2008
BA7pc	−34	−53	61	30	−52	61	Scheperjans et al., 2008
BA40 (PF)	−58	−43	39	62	−39	35	Caspers et al., 2008
LANGUAGE AREAS							
Broca's area	−42	26	17				Binkofski et al., 2000
AUDITORY AREAS							
A1 (BA41)	−42	−21	7	56	−13	8	Rademacher et al., 2001

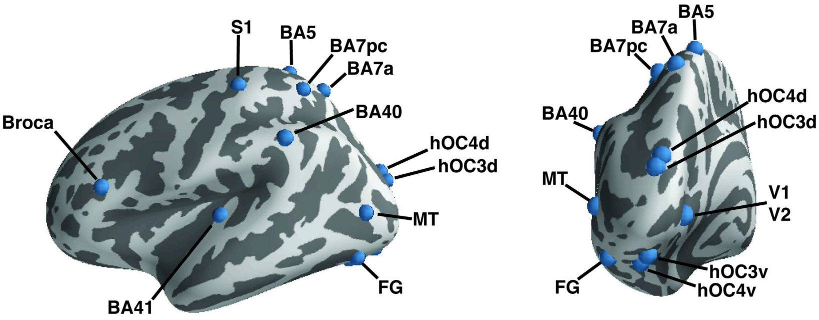

FIGURE 1 | A priori defined regions of interest. Regions of interest are shown on the left hemisphere of an inflated brain using PySurfer. Dark areas represent sulci, light gray areas gyri.

The fMRI time series from the left and right ROI seeds were averaged and Pearson correlations were calculated between their mean time course and the time course of all other voxels in the brain. Fisher-transformed correlation maps were generated using a general linear model (GLM) to allow for second-level between-group analyses. In all analyses, results were only reported as significant if they survived a height threshold of uncorrected $p < 0.001$ with an extent threshold of FWE-corrected $p < 0.05$ at the cluster level. Significant clusters from the second-level analysis were further examined using SPM. In order to eliminate results derived from a decrease in anti-correlations in blind compared to sighted controls, we used the anti-correlated voxels of the within SC group results as an exclusive mask for the CB > SC comparison. The opposite comparison (i.e., SC > CB) used the anti-correlated mask from the congenitally blind within group analysis. Maps were resliced to the MNI-152 1 mm dimensions using freesurfer and displayed on the FSaverage inflated brain using PySurfer (https://github.com/nipy/PySurfer/).

Results

Increased Functional Connectivity in the Blind

Within-group functional connectivity maps can be found in the Supplementary Material (**Figures S1**, **S2**). We found significant group differences in functional connectivity for five out of the eight visual seeds, including hOC3d, hOC3v, hOC4v, fusiform gyrus and hMT+ (**Table 3** and **Figure 2**). One of the most striking results was the increased connectivity between the occipital seeds and Broca's area in the left inferior frontal cortex. More specifically, CB showed an increase in connectivity between hMT+, hOC3d, hOC3v, hOC4v and the fusiform gyrus with the inferior and middle frontal areas, overlapping with BA44 and BA45. The fusiform gyrus and hOC3d also showed increased functional connectivity with the contralateral homolog of Broca's area in the right inferior frontal cortex. Next, connectivity of ventral stream areas hOC3v and fusiform gyrus with the inferior temporal cortex (BA20) was stronger in blind participants. Blind participants also had stronger connectivity patterns between hOC4v and the thalamus, and between hOC3d and hOC4v and the cerebellum. Finally, CB showed increased connectivity between the fusiform gyrus and the inferior parietal cortex and sulcus, which is dorsal to, but not overlapping with Wernicke's area (**Figure 2**). No group differences in connectivity were observed for V1, V2, and hOC4d.

Blind individuals also showed increases in functional connectivity for three somatosensory seeds. First, connectivity was increased among somatosensory areas. More specifically, SI and BA7pc showed a stronger connectivity with BA40, and the middle cingulate cortex (left and right respectively). In addition, SI had increased functional connectivity with the primary motor cortex, and middle temporal region (BA22). Our data also revealed increased connectivity between BA40 and the visual areas BA18 and hMT+ (**Figure 3**).

Broca's area showed an increased functional connectivity with ventral visual stream areas hOC3v and hOC4v, as well as with area BA10 in the left anterior prefrontal cortex (**Figure 4**). Finally, no significant group differences in functional connectivity were found for the primary or secondary auditory cortex.

Decreased Functional Connectivity in Blind

Although the largest amount of the observed changes concerned increases in functional connectivity, blind subjects also showed decreases in connectivity in a number of brain areas. More specifically, blind participants showed reduced functional connectivity between ventral visual areas hOC3v, hOC4v and fusiform gyrus and dorsal stream area hMT+ on the one hand, and between fusiform gyrus and MI on the other hand. For the somatosensory seeds, decreases in functional connectivity were observed between BA40 and the inferior temporal area BA21, and between SI and the cerebellum. Blind participants also had a reduced functional connectivity between Broca's area and its contralateral homolog in the right hemisphere.

Discussion

We investigated alterations in resting state functional connectivity in congenital blindness using a seed-based

TABLE 3 | Group differences in functional connectivity (congenitally blind vs. sighted controls).

Seed		Brodmann area	MNI coordinates (mm)			Cluster	p-FWE
			X	*Y*	*Z*	Size	Cluster
REGION OF INCREASED CONNECTIVITY IN CB							
hOC3d	Middle cerebellum		0	−60	−38	224	0.0486
	Middle and inferior frontal (R)	BA44,45	52	32	28	275	0.0238
	Inferior frontal and middle frontal (L)	BA45	−44	46	−8	233	0.0427
	Cerebellum (L)		−14	−72	−46	373	0.0067
hOC3v	Inferior frontal (L)	BA44,45	−48	34	26	272	0.0296
	Inferior temporal (L)	BA20	−62	−50	−12	323	0.0155
hOC4v	Thalamus (bilateral)		10	−4	−8	287	0.0193
	Inferior frontal (L)	BA44,45	−50	32	24	454	0.0024
	Cerebellum		2	−80	−16	339	0.0097
FG	Inferior frontal and middle frontal (L)	BA44,45	−40	16	26	989	0
	Inferior temporal (L)	BA20	−56	−46	−10	449	0.003
	Middle and inferior frontal (R)	BA44,45	44	32	20	435	0.0035
	Inferior temporal (R)	BA20	62	−42	−10	381	0.0067
	Inferior parietal (L)	HIP1,2,3, IPC	−32	−58	42	316	0.0149
MT	Inferior frontal (L)	BA44,45	−52	22	22	823	0
BA40	Middle and inferior temporal (R)	MT	48	−60	0	426	0.0026
	V2	BA18	12	−80	42	393	0.0039
BA7pc	Middle cingulate (L)	SPL	−8	−60	58	847	0
	Supramarginal and inferior parietal (L)	BA40	−58	−36	32	221	0.0492
S1	Middle cingulate (R)	BA4,6,SPL	6	−22	46	1213	0
	Supramarginal (L)	BA40, IPC	−50	−32	24	454	0.0016
	Pre and post−central (L)	BA1,2,3,4,6	−46	−20	44	290	0.0143
	Middle and superior temporal (R)	BA22	58	10	−6	233	0.0331
Broca	Middle and inferior occipital (L)	hOC3v, hOC4v	−36	−82	12	1574	0
	Prefrontal (L)	BA10	2	60	10	248	0.0255
REGION OF DECREASED CONNECTIVITY IN CB							
hOC3v	Middle temporal (R)	MT,OP,IPC	60	−60	14	371	0.0061
hOC4v	Superior, middle and inferior temporal (R)	MT,OP,IPC	46	−58	12	866	0
FG	Middle temporal (R)	MT	52	−62	4	568	0.0008
	pre and post−central, precuneus	BA4,6	6	−34	58	361	0.0085
BA40	Middle and inferior temporal (R)	BA21	68	−42	−2	344	0.0074
	Middle temporal (L)	BA21	−64	−44	6	328	0.0091
S1	Cerebellum and VI		10	−78	0	209	0.048
Broca	Inferior frontal (R)	BA44,45	52	8	44	299	0.0121

approach with *a priori* defined ROIs. Although our data revealed a mixture of increases and decreases in functional connectivity in the blind brain, the increases strongly prevailed. The most striking findings of this study were the increases in functional connectivity in the congenital blind brain within the ventral and dorsal visual streams, and between visual cortical regions and Broca's area. In sharp contrast, functional connectivity between dorsal and ventral visual areas was reduced. **Figure 4** summarizes the observed increases and decreases in functional connectivity of the congenitally blind brain.

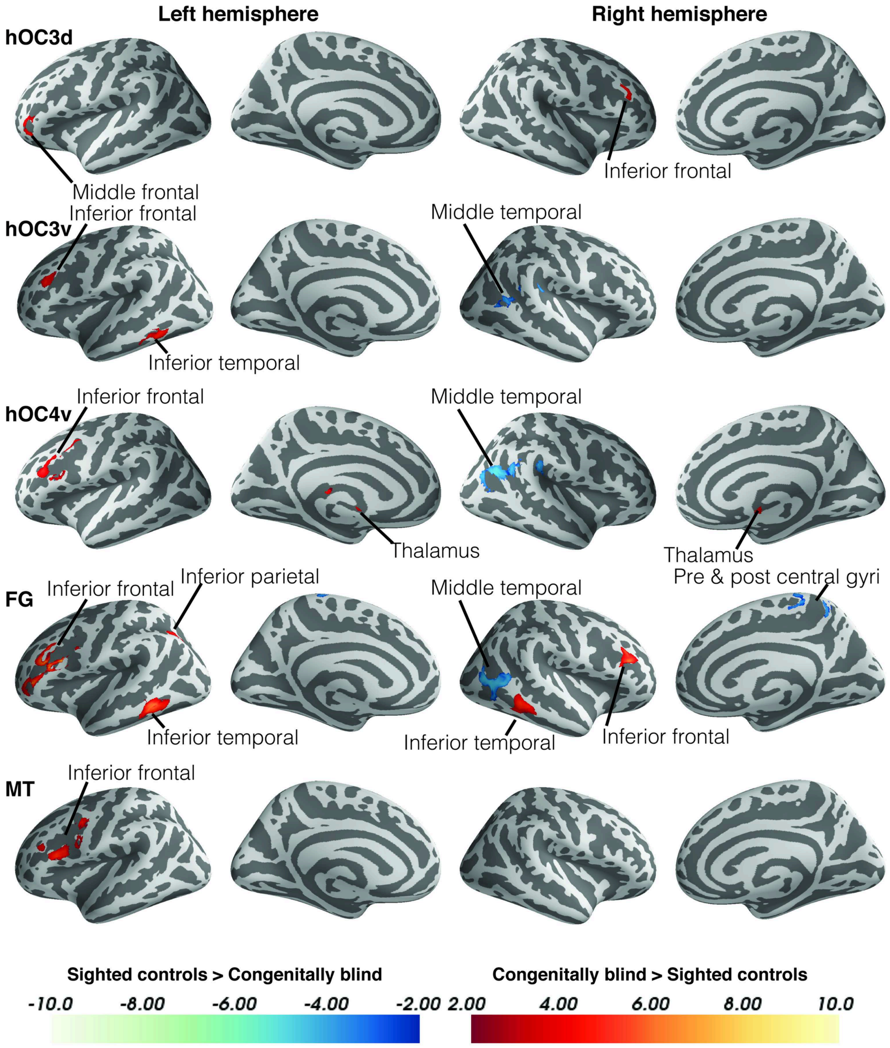

FIGURE 2 | Differences in resting state functional connectivity between blind and sighted controls (visual ROIs). Increases in functional connectivity in the blind group are indicated in red, whereas decreases in functional connectivity compared to controls are shown in blue. Cluster-level FWE-corrected $p < 0.05$. Scale bars indicate Z-values. Abbreviations: CB, congenitally blind; SC, sighted controls.

Increased Functional Connectivity within the Visual Streams

Our data show evidence of increased functional connectivity in the ventral visual stream in congenitally blind subjects, more specifically between ventral stream areas hOC3v and fusiform gyrus and the inferior temporal gyrus (BA20). The ventral stream consists of a complex recurrent network between visual areas V1–V4 and the inferior temporal cortex (Kravitz et al., 2013). In sighted subjects, this pathway is implicated in the processing of object quality, object representation or object category (Kravitz et al., 2013). These processes are necessary for object and scene comprehension that form the contents of visual awareness. The fact that this pathway is preserved in blind subjects adds new evidence to the notion that the ventral visual stream holds representations of object shape which are supramodal in nature, and not necessarily visual (Kupers et al., 2011a). For example, non-visual recruitment of the ventral temporal cortex was seen after haptic (Pietrini et al., 2004), non-haptic (Ptito et al., 2012) and auditory (Amedi et al., 2007) exploration of objects in congenital blind subjects.

Congenitally blind subjects also showed increased functional connectivity in the dorsal visual stream, more specifically between BA40 and the secondary visual cortex (V2), as well as between somatosensory areas (BA7pc) and BA40. In normal

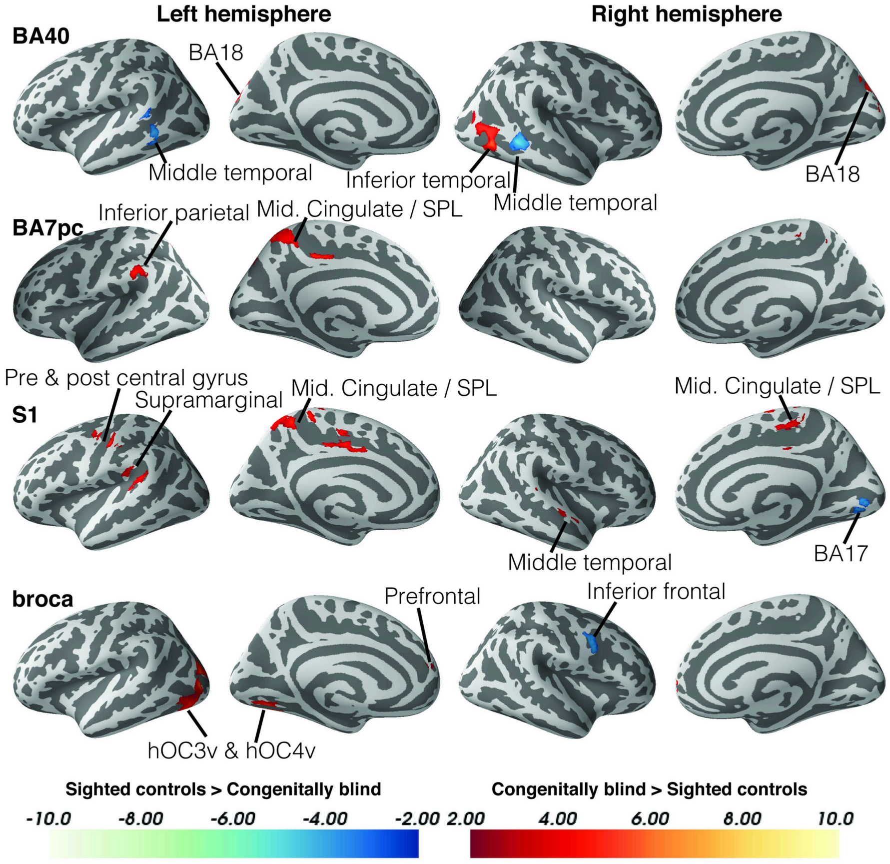

FIGURE 3 | Differences in resting state functional connectivity between blind and sighted controls (somatosensory and language ROIs). Increases in functional connectivity in the blind group are indicated in red, whereas decreases in functional connectivity compared to controls are shown in blue. Cluster-level FWE-corrected $p < 0.05$. Scale bars indicate Z-values. Abbreviations: CB, congenitally blind; SC, sighted controls.

sighted individuals, the dorsal visual stream is heavily implicated in the visual guidance of action, and consists of a set of projections from the visual cortex to the superior parietal lobule. From there, the dorsal stream splits into the parieto-prefrontal, parieto-medial temporal and parieto-premotor pathway (Kravitz et al., 2011). The parieto-prefrontal pathway connects the parietal cortex to prefrontal regions (e.g., BA46) and is important in top-down control of eye movements and spatial working memory. The parieto-medial temporal pathway, connecting to the posterior cingulate cortex via parahippocampal substructures, is implicated in spatial navigation. Finally, the parieto-premotor pathway connects to premotor regions and is involved in visually-guided actions such as reaching and grasping (Kravitz et al., 2011). Our finding of increased functional connectivity between BA40 and V2, as well as between BA7pc and BA40, are indicative of a fast pathway for information processing from higher order somatosensory to lower level visual areas. This conjecture is in line with results of a recent MEG study indicating that somatosensory information reaches the occipital cortex in the blind via somatosensory and posterior parietal areas (Ioannides et al., 2013), and with results of functional activation studies showing occipital cortex activation following somatosensory stimulation in blind individuals (Ptito et al., 2005). Finally, applying TMS over the occipital cortex can induce tactile sensations in blind subjects trained in the use of a tactile sensory substitution device or in Braille reading (Kupers et al., 2006; Ptito et al., 2008a).

Our results of increased functional connectivity within both the dorsal and ventral visual streams in congenitally blind subjects are in line with results of a recent functional connectivity density mapping study (Qin et al., 2014). Functional activation studies also support the finding of increased connectivity within the dorsal and ventral streams in the blind brain (Kupers et al., 2011a for a review). For instance, congenitally blind subjects trained in the use of the tongue display unit (TDU) showed stronger connectivity between the cuneus and areas within the dorsal and ventral streams (Ptito et al., 2005). In addition, a dynamic causal modeling study showed that the activation of the occipital cortex in blind individuals during an auditory discrimination task is mediated via enhanced corticocortical

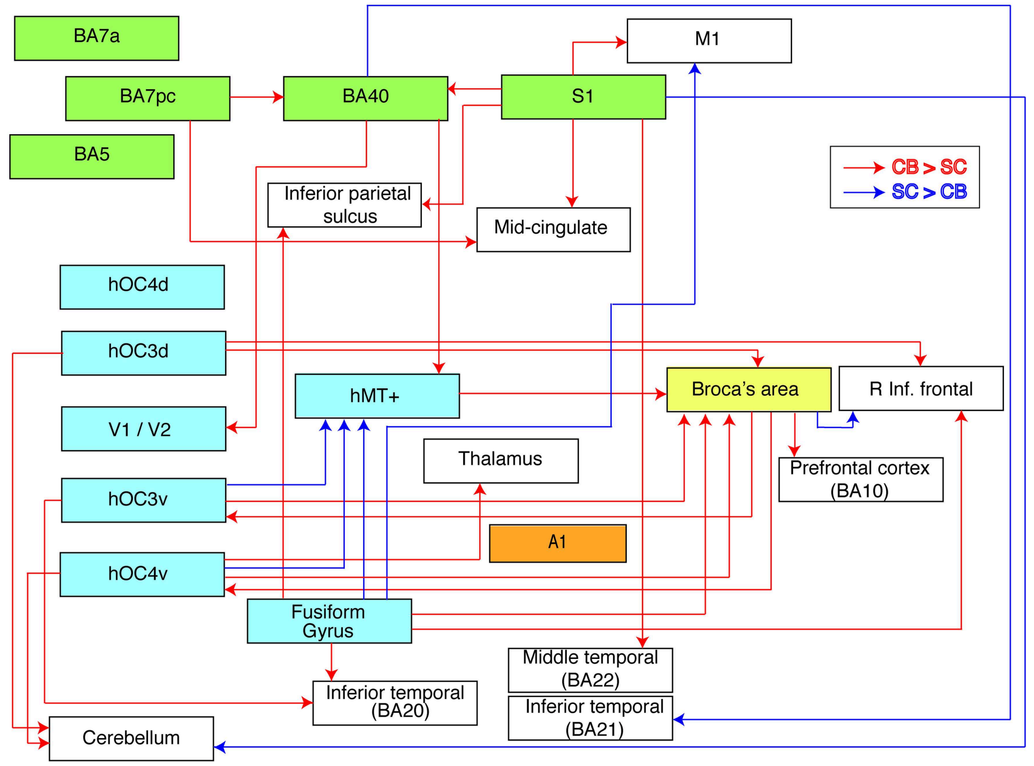

FIGURE 4 | Flow chart summarizing group differences in functional connectivity. The used ROIs are represented by colored boxes (blue, visual seeds; green, somatosensory seeds; yellow, language seed; orange, auditory seed). Red arrows indicate an increased connectivity in blind compared to sighted between our seed and the boxes' corresponding brain area. Blue arrows indicate the opposite, i.e., a decreased connectivity in blind compared to sighted individuals.

connections from the auditory to the occipital cortex (Klinge et al., 2010). These functional changes are probably due to anatomical reorganization of the pathways that funnel non-visual information to the visual cortex of the blind (Kupers et al., 2011a). Thus, our rsfMRI data of increased connectivity in the visual streams are supported by results of various functional activation studies showing that the visual streams of the congenitally blind undergo compensatory plasticity and are able to process non-visual information in conjunction with the visual cortex (Dormal et al., 2012; Kupers and Ptito, 2014).

Decreased Connectivity between the Ventral and Dorsal Visual Stream

In sharp contrast with the increase in functional connectivity *within* the visual streams, our data revealed decreases in connectivity *between* the two streams in blind participants. Connectivity of ventral areas hOC3v, hOC4v and fusiform gyrus with dorsal stream area hMT+ was decreased, as well as that between BA40 and the inferior temporal cortex (BA21). There is growing evidence that the dorsal and ventral streams are less independent than originally thought (Schenk and McIntosh, 2010). Although these streams have clear independent functional roles, there is functional and structural evidence that they do not function in an independent manner (Mahon et al., 2007; Borra et al., 2008; Rosa et al., 2009; Schenk and McIntosh, 2010; Zanon et al., 2010). Our data suggest that in the congenitally blind brain the two streams are less interconnected than in the sighted brain. We hypothesize that this may be due to increases in functional connectivity within the two streams. An alternative explanation is that cross-modal non-visual sensory information processing in extrastriate cortex reduces the need for functional connectivity between the streams. Future structural imaging and voxel based morphometric assessment might shed light on the changes in white matter structure within the dorsal and ventral stream to assess whether there is a structural or only functional differentiation between the two streams.

Connectivity of the Primary Visual Cortex

We did not find evidence for changes in connectivity in primary visual cortex (V1 and V2). This is in agreement with several other functional connectivity studies (Bedny et al., 2011; Watkins et al., 2012; Butt et al., 2013; Burton et al., 2014). A recent study reported decreased functional connectivity density only in primary visual areas of late blind subjects, while congenitally blind showed increased connectivity between lower tier visual areas and somatosensory areas (Qin et al., 2014), overlapping with the small cluster of increased functional connectivity between BA40 and the primary visual areas observed in this study. However, the literature on changes in functional connectivity of primary visual areas in blind individuals is incongruent. Thus, several fMRI studies reported a correlation between damage to the optic radiation and an event-related fMRI response in visual areas (Seghier et al., 2004), or decreased functional connectivity of primary visual areas with the rest of the brain Liu et al., 2007; Yu et al., 2008; Shu et al., 2009; Wang et al., 2013. These results were explained by the general loss hypothesis. However, this proposed mechanism cannot explain the ubiquitous role of the primary visual cortex in non-visual perceptual and cognitive tasks (Sadato et al., 1996; Amedi et al., 2003, 2004; Burton et al., 2003; Ptito et al., 2005, 2007; Karlen et al., 2006; Voss et al., 2008; Kupers et al., 2010, 2011a; Matteau et al., 2010; Sani et al., 2010; Bedny et al., 2011; Collignon et al., 2011; Watkins et al., 2012). Nor can it explain enhanced effective connectivity with other regions (Wittenberg et al., 2004; Ptito et al., 2005; Klinge et al., 2010). Furthermore, a recent review on structural changes as measured with diffusion concluded that although the literature is inconsistent, it suggest that neither strength nor macro-scale topographic organization is changed in blind individuals (Bock and Fine, 2014). This is congruent with new research showing that functional connectivity based topographic organization of the visual cortices is indistinguishable from sighted controls, and increased functional connectivity to frontal and posterior temporal areas (Striem-Amit et al., 2015).

Visual Cortex and Language Processing

Broca's area (BAs 44 and 45) was the cortical area with the largest amount of alterations in functional connectivity in congenitally blind participants. A total of five visual seeds, hOC3d, hOC3v, hOC4v, hMT+ and fusiform gyrus, showed increased functional connectivity with this area. In addition, Broca's area also showed stronger connectivity with ventral visual areas hOC3v, hOC4v, and with medial prefrontal cortical area BA 10. The current consensus is that the occipital cortex of blind individuals is involved in language processing, showing similar properties as "classical language related areas" (Bedny et al., 2011). Braille reading in blind subjects activates an extensive network of brain areas, including posterior and medial occipital areas, fusiform gyrus, area hMT+, inferior temporal gyrus, inferior frontal, prefrontal, intraparietal sulcus, and somatosensory motor areas (Burton et al., 2002a). More specifically, the increased functional connectivity between visual areas and Broca's area in congenitally blind individuals might relate to the role of the occipital cortex in semantic processing. Whereas semantic processing activates the inferior frontal cortex in both sighted and blind subjects, it activates additionally visual cortical areas in the latter group (Burton et al., 2003; Noppeney et al., 2003; Amedi et al., 2004; Bedny et al., 2011; Watkins et al., 2012). These results expand earlier findings of increased connectivity of the occipital cortex with Broca's area in congenital blindness (Liu et al., 2007; Bedny et al., 2011; Watkins et al., 2012; Butt et al., 2013; Wang et al., 2013; Burton et al., 2014; Deen et al., 2015). The co-activation with Broca's area extends to most of the occipital cortex (Burton et al., 2003; Deen et al., 2015), and might next to language also functionally correlate to working memory (Deen et al., 2015). These results also relate to findings of increased white matter volume within the tracts between prefrontal and occipital areas. More specifically in the fronto-occipital fasciculi (Ptito et al., 2008b; Bock and Fine, 2014).

The increased functional connectivity between Broca's area and hMT+ might be explained by the role of tactile flow processing in Braille reading (Ricciardi et al., 2007). All our congenitally blind were reading braille from when they were children (see **Table 1** for speed of braille reading), and Burton et al. (2002a) showed that this area is linked to braille reading only in early bind subjects. Furthermore, the role of the occipital cortex in language processing is further supported by studies showing that rTMS over the mid-occipital cortex not only reduces accuracy of verb-generation (Amedi et al., 2004), but also impairs Braille reading performance (Kupers et al., 2007). Finally, it is worth mentioning that a bilateral occipital stroke in an early blind patient resulted in the loss of Braille reading skills (Hamilton et al., 2000). It is thus interesting for further studies to examine braille performances and related functional connectivity within hMT+ as well as other areas in both congenitally and late blind subjects.

In line with previous results (Bedny et al., 20111), congenitally blind subjects also showed increased functional connectivity between occipital area hOC4v and the thalamus. This finding suggests a thalamo-cortical implication in language processing in the congenitally blind, a conjecture that is supported by the observation that stimulation of left thalamic regions produces language deficits in blind subjects (Johnson and Ojemann, 2000). Our data also revealed a decrease in functional connectivity between Broca's area and its homolog in the right hemisphere. In sighted but not in congenitally blind individuals, the right inferior frontal area is also activated during language tasks (Burton et al., 2002b). Blind subjects might use the visually deprived occipital cortex instead because it is more cost-effective.

Somatosensory Areas

Our results indicate increased functional connectivity between the supramarginal gyrus (BA40) and secondary visual cortex and area hMT+, and between SI and BA40. As stated above, the supramarginal gyrus, occipital, middle temporal and somatosensory cortices are activated by Braille reading (Burton et al., 2002a; Gizewski et al., 2004; Sadato, 2005). We explain the co-activation of somatosensory regions by the tactile input of Braille reading. Indeed, tactile stimuli activate inferior and ventral temporal, as well as somatosensory regions in blind individuals (Pietrini et al., 2004; Ptito et al., 2005, 2012; Matteau et al., 2010; Ricciardi et al., 2013). This co-activation of parietal and visual areas may be at the basis of the superior tactile acuity in blind individuals (Kupers and Ptito, 2014), this might also be related to the increases in white matter volume found in somatosensory and motor areas (Noppeney et al., 2005).

Other rsfMRI studies have reported a decrease of functional connectivity between visual and somatosensory regions (Liu et al., 2007; Yu et al., 2008; Bedny et al., 2011; Qin et al., 2013). However, this finding is at odds with results of several other activation studies indicating strong connectivity between these areas. For instance, functional connectivity was shown to be increased between hMT+ and somatosensory areas (Sani et al., 2010). Furthermore, a recent MEG study from our group revealed activation of the occipital cortex following median nerve stimulation in congenitally blind individuals (Ioannides et al., 2013). A connectivity analysis further suggested that median nerve stimulation first activated primary somatosensory cortex, then the posterior parietal cortex and finally visual areas V3 and V5 (Ioannides et al., 2013). Using somatosensory-evoked potentials, we reported that tactile stimulation of the tongue in blind individuals trained in the use of the tongue display unit first activated the somatosensory cortex and then the occipital cortex (Kupers et al., 2006). Finally, a combined PET-TMS study showed that TMS of the primary somatosensory cortex leads to increased blood flow in the occipital cortex in congenitally blind subjects only (Wittenberg et al., 2004). Together, these findings argue in favor of an enhanced parieto-occipital connectivity in congenital blindness which is supported by the present rsfMRI data.

Auditory and Motor Areas

Although many studies have indicated superior auditory abilities in congenitally blind individuals (Kupers and Ptito, 2014), we did not find significant group differences in functional connectivity of auditory areas. Active tasks have indicated stronger cooperation between the auditory and occipital cortices in congenital blindness (Klinge et al., 2010; Collignon et al., 2011). It is possible that in the present study, scanner noise masked a purported increase in resting state functional connectivity between auditory and occipital cortices in blind individuals (De Martino et al., 2014).

We found decreased functional connectivity between the fusiform gyrus and pre- and post-central areas. This is in agreement with several other studies that found decreases between visual areas and motor-related regions, a finding that was explained by the loss of eye-hand coordination in blind subjects (Liu et al., 2007; Yu et al., 2008; Wang et al., 2013). Eye-hand coordination in sighted individuals leads to co-activation of visual and motor areas (Winstein et al., 1997), which is reduced in conditions of congenital blindness.

Methodological Considerations

Several rsfMRI studies have explored changes in functional connectivity in the blind brain. The reported results are not very consistent and sometimes even conflicting. These differences in results might be due to spurious samples or protocol bias. For instance, some studies included blind subjects with residual light perception (Li et al., 2013; Wang et al., 2013; Burton et al., 2014), or had a mixture of congenitally blind and late-onset blind participants (Butt et al., 2013). Our study cohort was a homogeneous group of congenitally blind participants without any light perception. Furthermore, contrary to some (Liu et al., 2007; Yu et al., 2008), our study used subjects that are not previously used for any analysis, nor was there any active paradigm during the scanning session (Bedny et al., 2011). Another explanation for the inconsistency between studies relates to differences in used methodologies for assessing functional connectivity in rsfMRI data. Early studies used a more exploratory method with atlas-based regions of interest (Liu et al., 2007; Wang et al., 2008; Watkins et al., 2012; Li et al., 2013; Qin et al., 2014), or one or a few hypothesis-driven ROIs, mostly the primary visual area (Yu et al., 2008; Li et al., 2013; Qin et al., 2013). In contrast, our investigation focussed on small areas that are not present in current atlases. Information about the time course (and therefore its functional correlation) of these small areas could also be missed when the time courses of all voxel in an atlas based area are averaged. Our research focused on brain areas with known functional or structural changes in blind subjects in the visual, somatosensory, auditory and language domain, and seed placement was done according to architectonical studies.

We combined the time-series of homologous areas from both hemispheres. For this reason we are unable to draw any conclusions on purported hemispheric differences in functional connectivity. Further, we excluded "increased" or "decreased" correlations in our second level analysis that were caused by anti-correlating time-series in our first level analysis. As with all resting state functional connectivity studies, we are only able to show correlations between different areas, and not any causality. Thereto, DCM or granger causality studies are needed.

Conclusion

In summary, our data reveal increased functional connectivity *within* both the ventral and the dorsal visual streams in congenitally blind participants. However, connectivity *between* the two visual streams was reduced in blind subjects. In addition, our data revealed stronger functional connectivity in blind participants between the occipital cortex and areas implicated in language and tactile (Braille) processing such as the inferior frontal gyrus (Broca's area), the thalamus, the supramarginal gyrus and the cerebellum. Our results underscore the extent of cross-modal reorganization and the supra-modal function of the occipital cortex in congenitally blind individuals.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnana.2015.00086

Figure S1 | Resting state functional connectivity within blind and sighted controls (visual ROIs). Within group functional connectivity for sighted controls (left) and congenitally blind (right). Cluster-level FWE-corrected $p < 0.05$. Scale bars indicate Z-values.

Figure S2 | Resting state functional connectivity within blind and sighted controls (somatosensory and language ROIs). Within group functional connectivity for sighted controls (left) and congenitally blind (right). Cluster-level FWE-corrected $p < 0.05$. Scale bars indicate Z-values.

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Corrigendum: Prevalence of increases in functional connectivity in visual, somatosensory and language areas in congenital blindness

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Author contributions: RK and MP designed the study. NR recruited participants, collected psychometric and brain imaging data. LH, MB, CC, and AS analyzed the rsfMRI data. LH, RK, and MP wrote the manuscript. SL and AS edited the manuscript and provided scientific expertise.

All the other files remain unchanged. This error does not change the scientific conclusions of the article in any way.

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This thesis explores brain connectivity and sensory stimulation in patients with disorders of consciousness (DOC). These are serious conditions where massive brain damage can lead to a dissociation between arousal and awareness (e.g., UWS and MCS).

Part I explores brain connectivity. We highlight that brain function and structure are intimately related to each other, and to consciousness. The decrease in brain function can be used to distinguish between the clinically indicated states of consciousness.

Part II evaluates passive sensory stimulations.
Preferred stimuli may have the power to
momentarily enhance brain function,
and behavioral responses.