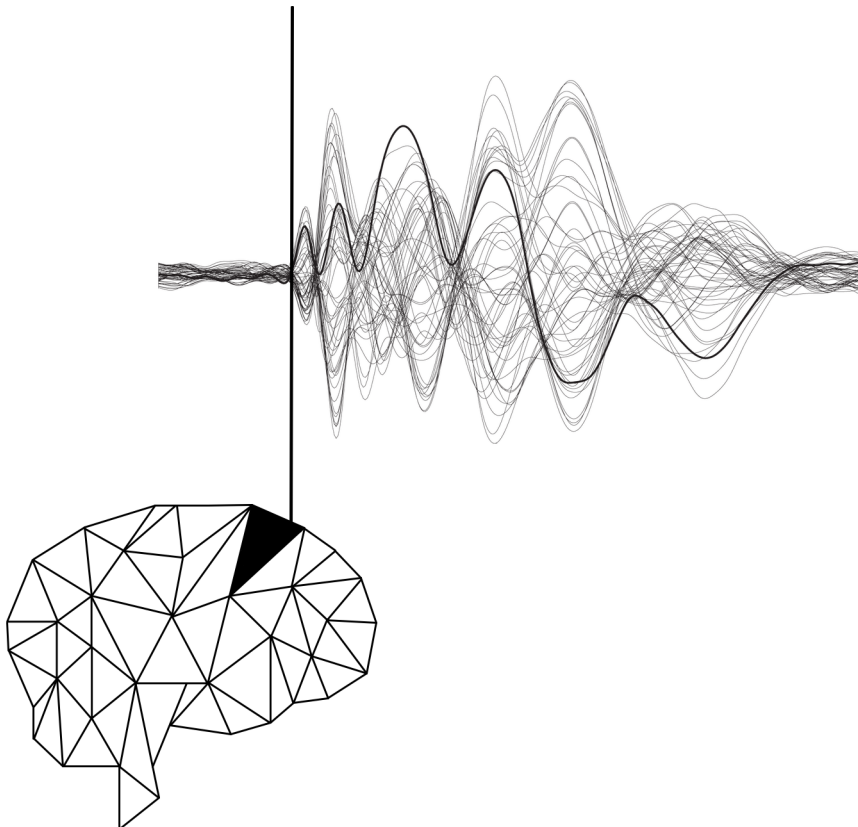Under the supervision of
Prof Steven LAUREYS,
Coma Science Group, GIGA-Consciousness

A TMS–EEG CONTRIBUTION TO THE MULTIMODAL ASSESSMENT OF BRAIN CONNECTIVITY AND CONSCIOUSNESS.

Olivier Bodart

THESE PRESENTEE EN VUE DE L'OBTENTION DU GRADE DE

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Content

List of publications

This thesis is based on the following publications:

Articles:

Bodart, O., Gosseries, O., Wannez, S., Thibaut, A., Annen, J., Boly, M., Rosanova, M., Casali, A.G., Casarotto, S., Tononi, G., Massimini, M., Laureys, S., 2017. Measures of metabolism and complexity in the brain of patients with disorders of consciousness. NeuroImage Clin. 14, 354–362. doi:10.1016/j.nicl.2017.02.002

Bodart, O., Amico E., Gomez F., Casali A.G., Wannez S., Heine L., Thibaut A., Annen J., Boly M., Casarotto S., Rosanova M., Massimini M., Laureys S., Gosseries O., Global structural and effective connectivity in patients with disorders of consciousness. Under review.

Napolitani, M.*, **Bodart, O.***, Canali, P., Seregni, F., Rosanova, M., Laureys, S., Massimini, M., Gosseries, O., 2014. Transcranial magnetic stimulation combined with high density EEG in altered state of consciousness. Brain Inj. 28, 1180–1189. doi:10.3109/02699052.2014.920524 * Contributed equally

Bodart, O., Laureys, S., Gosseries, O., 2013. Coma and disorders of consciousness: scientific advances and practical considerations for clinicians. Semin. Neurol. 33, 83–90. doi:10.1055/s-0033-1348965

Gantner, I.S., **Bodart, O.**, Laureys, S., Demertzi, A., 2012. Our rapidly changing understanding of acute and chronic disorders of consciousness: challenges for neurologists. Future Neurol. 8, 43–54. doi:10.2217/fnl.12.77

Book chapters:

Bodart, O., Charland-Verville, V., Laureys, S., Gosseries, O., 2013. Neuroimaging in Disorders of Consciousness, in: Filippi, M. (Ed.), Oxford Textbook of Clinical Neurology. Oxford University Press, London.

Bodart, O., Thibaut, A., Laureys, S., Gosseries, O., 2013. Disorders of Consciousness, in: Citerio, G., Smith, M., Kofke, A. (Eds.), Oxford Textbook of Neurocritical Care. Oxford University Press, London.

Gosseries, O., **Bodart, O.,** Massimini, M., 2015. Transcranial magnetic stimulation and electroencephalography, in: Rossetti, A., Laureys, S. (Eds.), Clinical Neurophysiology in Disorders of Consciousness: Brain Function Monitoring in the ICU and Beyond. Springer-Verlag Wien, doi:10.1007/978-3-7091-1634-0_10

Other publications as first or co-author, in alphabetical order:

Articles:

Amico, E., **Bodart, O.,** Rosanova, M., Gosseries, O., Heine, L., Van Mierlo, P., Martial, C., Massimini, M., Marinazzo, D., Laureys, S., 2017. Tracking dynamic interactions between structural and functional connectivity: a TMS/EEG-dMRI study. Brain Connect. 7, brain.2016.0462. doi:10.1089/brain.2016.0462

Bodart, O., Laureys, S., 2014. Predicting outcome from subacute unresponsive wakefulness syndrome or vegetative state. Crit. care 18, 132. doi:10.1186/cc13831

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Casarotto, S., Comanducci, A., Rosanova, M., Sarasso, S., Fecchio, M., Napolitani, M., Pigorini, A., Casali, A.G., Trimarchi, P.D., Boly, M., Gosseries, **O., Bodart,** O., Curto, F., Landi, C., Mariotti, M., Devalle, G., Laureys, S., Tononi, G., Massimini, M., 2016. Stratification of unresponsive patients by an independantly validated index of brain complexity. Ann. Neurol. 80. doi:10.1002/ana.24779

Gosseries, O., Charland-Verville, V., Thonnard, M., **Bodart, O.,** Laureys, S., Demertzi, A., 2014. Amantadine, Apomorphine and Zolpidem in the Treatment of Disorders of Consciousness. Curr. Pharm. Des. 20, 3893–3910.

Laureys, S., **Bodart, O.,** Gosseries, O., 2014. The Glasgow Coma Scale: time for critical reappraisal? Lancet Neurol. 13, 755–757. doi:http://dx.doi.org/10.1016/PII

Wannez, S., Gosseries, O., Azzolini, D., Martial, C., Cassol, H., Aubinet, C., Annen, J., Martens, G., **Bodart,** O., Heine, L., Charland-Verville, V., 2017a. Prevalence of Coma-Recovery Scale-Revised signs of

consciousness in patients in a minimally conscious state. Neuropsychol. Rehabil. in press. doi:10.1177/1352458506070750

Wannez, S., Hoyoux, T., Langohr, T., **Bodart, O.,** Martial, C., Wertz, J., Chatelle, C., Verly, J.G., Laureys, S., 2017b. Objective assessment of visual pursuit in patients with disorders of consciousness: an exploratory study. J. Neurol. doi:10.1007/s00415-017-8469-0

Book chapters:

Bodart, O., Laureys, S. 2016. Imaging the central nervous system in the critically ill, in: Webb, A., Angus, D., Finfer, S., Gattinoni, L., Singer, M. (Eds.), Oxford Textbook of Critical Care. Oxford University Press, London.

Di Perri, C., Cavaliere, C., **Bodart, O**., Thibaut, A., Bahri, MA., Soddu, A., Laureys, S. 2017. Sleep, Coma, Vegetative and Minimally Conscious States, in: Chokroverty, S. (Ed.) Sleep Disorders Medicine, Springer, Vienna.

Thibaut A., **Bodart, O.**, Laureys, S., Canavero S., 2015. Chronic Disorders of Consciousness, in: Canavero, S. (Ed.), Textbook of Cortical Brain Stimulation. De Gruyter, London.

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List of abbreviations

CRS-R – Coma recovery scale – revised

DMN – Default mode network

DOC – Disorder(s) of consciousness

DTI – Diffusion tractography imaging

DWI – Diffusion weighted imaging

EEG – Electroencephalography

EMCS – Emergence from the minimally conscious state

FA – Fractional anisotropy

FDG-PET – ^{18}fluoro-deoxyglucose positron emission tomography

fMRI – Functional magnetic resonance imaging

FOUR – Full outline of unresponsiveness

GCS – Glasgow coma scale

LIS – Locked-in syndrome

MCS – Minimally conscious state

MCS* – Non-behavioural minimally conscious state

MRI – Magnetic resonance imaging

PCI – Perturbational complexity index

TBI – Traumatic brain injury

TEP – TMS evoked potential

TMS – Transcranial magnetic stimulation

UWS – Unresponsive wakefulness syndrome

Abstract

Patients with chronic disorders of consciousness make a challenging population. On the clinical side, establishing an accurate diagnosis is arduous, as the signs of consciousness can be subtle, or even undetectable behaviourally. Both the families and the caregivers need truthful information to make tough decisions about the patient's management. Transcranial magnetic stimulation, coupled with high-density electroencephalography, is a promising technique to improve our diagnostic ability. The perturbational complexity index derived from this technique is able to distinguish between unconscious and conscious conditions. Its specificity remains to be determined. On the scientific side, the long-standing quest to discover the neural correlates of consciousness is still ongoing. Patients with disorders of consciousness have structural brain damage, and several areas may lose their ability to causally interact in complex patterns with long distance structure. The relation between this ability and structural integrity remains undetermined, despite a vast amount of neuroimaging studies on several networks and connectivities in this population.

Our objectives are i) to cross-validate the perturbational complexity index with other neuroimaging techniques, and to determine its specificity, and ii) to determine the relation between global structural integrity and the brain global ability to sustain complex long-range interactions.

To do so, we first combined transcranial magnetic stimulation with fluoro-deoxyglucose positron emission tomography, a validated technique studying the brain metabolism, in a population of patients behaviourally characterized by repeated assessments with the gold standard scale, the coma recovery scale – revised. To meet our second objective, we computed and compared the perturbational complexity index and the global fractional anisotropy, a magnetic

resonance imaging marker of structural integrity, in patients and in healthy subjects.

We found an excellent congruence between electrophysiological and metabolic results in our first study, even in behaviourally unconscious patients showing indirect signs of consciousness. In our second study, we demonstrated that structural integrity largely correlated with the perturbational complexity index, and did not depend on the time since onset or the aetiology.

This confirms the diagnostic value of transcranial magnetic stimulation and the perturbational complexity index. It is not only sensitive at the single subject level, but also highly specific. It can detect covert signs of consciousness, as confirmed by other neuroimaging techniques. As such, it could be integrated in diagnostic algorithms and improve their accuracy, leading to better management of these patients. Moreover, the brain's ability to sustain complex long-range interactions is highly dependant on the global structural integrity. By looking further in detail at the local correlation between these two parameters, our understanding of the emergence of consciousness from fixed structure with variable connectivity would improve. This would be one step forward in the quest for the neural correlates of consciousness.

Résumé

Les patients souffrant d'une altération chronique de la conscience constituent une population difficile mais motivante. Du point de vue clinique, établir un diagnostic précis est compliqué, car les signes de conscience peuvent être subtils, voire indétectables comportementalement. Tant les familles que les soignants ont besoin d'informations fiables pour prendre des décisions difficiles au sujet de la prise en charge du patient. La stimulation magnétique transcrânienne couplée à l'électroencéphalographie à haute densité est une technique qui pourrait améliorer notre précision diagnostique. L'index de complexité perturbationnelle dérivé de cette technique est capable de distinguer les sujets conscients et inconscients. Sa spécificité n'est pas encore démontrée. Du point de vue scientifique, la longue recherche des corrélats neurophysiologiques de la conscience est toujours en cours. Les patients avec une altération de la conscience ont des lésions cérébrales, et différentes aires peuvent perdre leur capacité d'interagir de façon complexe avec des structures distantes. La relation entre cette capacité et l'intégrité structurelle du cerveau n'est pas connue, malgré de très nombreuses études en neuroimagerie sur différents réseaux et leur connectivité.

Nos objectifs sont i) de valider l'index de complexité perturbationelle avec d'autres techniques de neuroimagerie, et d'en déterminer la spécificité, et ii) d'établir la relation entre l'intégrité structurelle globale et la capacité du cerveau à entretenir des interactions complexes et de longue distance.

Pour ce faire, nous avons d'abord associé la stimulation magnétique transcrânienne avec la tomographie à émission de positrons au glucose marqué, une technique validée d'étude du métabolisme cérébral, chez des patients bien caractérisés comportementalement par l'administration répétée d'une échelle

validée, l'échelle « coma recovery scale – revised ». Afin de rencontrer notre second objectif, nous avons calculé et comparé l'index de complexité perturbationelle et la fraction d'anisotropie globale, un marqueur d'intégrité structurelle dérivé de l'imagerie par résonnance magnétique, chez des patients et des sujets sains.

Nous avons trouvé une excellente concordance entre les données électrophysiologiques et métaboliques dans notre première étude, et ce même chez les patients comportementalement inconscients démontrant des signes indirects de conscience. Dans notre seconde étude, nous avons démontré que l'intégrité structurelle était largement corrélée avec l'index de complexité perturbationelle, et que cette interaction ne dépendait pas du temps écoulé depuis le coma, ou de son étiologie.

Ces résultats confirment la valeur de la stimulation magnétique transcrânienne comme outil diagnostic, ainsi que de l'index de complexité perturbationelle. Il est en effet non seulement sensible au niveau individuel, mais également hautement spécifique. Il est capable de détecter des signes indirects de conscience, comme confirmé par d'autres techniques de neuroimagerie. Dès lors, il pourrait être intégré dans les algorithmes diagnostiques et améliorer leur précision, menant à une meilleure prise en charge de ces patients. De plus, la capacité du cerveau de soutenir des interactions complexes et distantes est hautement dépendante de l'intégrité structurelle globale. En étudiant plus en détail la corrélation locale entre ces deux paramètres, notre compréhension de l'émergence de la conscience depuis une structure fixe mais avec une connectivité fonctionnelle variable en serait grandie. Ce serait un pas supplémentaire dans notre connaissance des corrélats neurophysiologiques de la conscience.

Chapter I

Introduction

Section based upon the following publications:

Bodart, O., Laureys, S., Gosseries, O., 2013. Coma and disorders of consciousness: scientific advances and practical considerations for clinicians. Semin. Neurol. 33, 83–90. doi:10.1055/s-0033-1348965

Gantner, I.S., **Bodart, O.**, Laureys, S., Demertzi, A., 2012. Our rapidly changing understanding of acute and chronic disorders of consciousness: challenges for neurologists. Future Neurol. 8, 43–54. doi:10.2217/fnl.12.77

Bodart, O., Charland-Verville, V., Laureys, S., Gosseries, O., 2013. Neuroimaging in Disorders of Consciousness, in: Filippi, M. (Ed.), Oxford Textbook of Clinical Neurology. Oxford University Press, London.

Bodart, O., Thibaut, A., Laureys, S., Gosseries, O., 2013. Disorders of Consciousness, in: Citerio, G., Smith, M., Kofke, A. (Eds.), Oxford Textbook of Neurocritical Care. Oxford University Press, London.

1. Disorders of consciousness

In the past fifteen years, thanks to increased interest and technological advances, scientific knowledge about disorders of consciousness (DOC) has risen tremendously (Figure 1). We will discuss that, as a result, the nosology has greatly expanded, the diagnostic criteria have been refined, and the neurophysiology underlying these disorders is better understood. Still, all the advances made in this field have not been translated to clinical practice yet, and several problems remain. Technology has limitations, the expanding nosology raises new interrogations, and the relationship between several findings often stays nebulous.
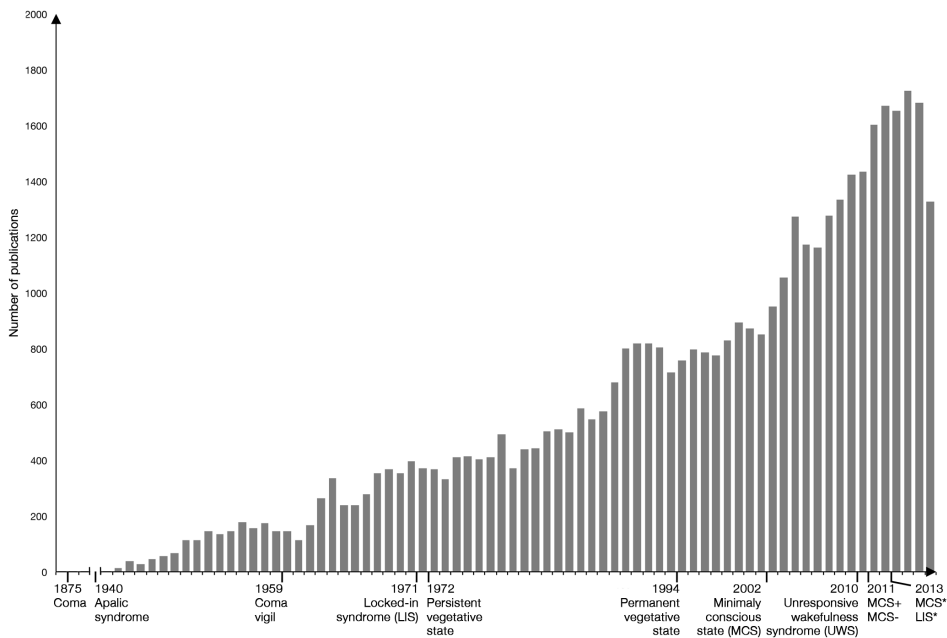
Figure 1 - Number of publications on DOC and evolution of nosology
This figure illustrates the huge increase in publications about disorders and consciousness, along with key definitions dates of the different states. Based on Pubmed research performed in September 2017 with the keywords "disorders of consciousness", "coma", "vegetative state", "minimally conscious state", "locked-in syndrome". *Updated from Gosseries, O., Zasler, N., Laureys, S., 2014. Recent advances in disorders of consciousness: focus on the diagnosis, Brain Inj., 28(9), 1141-50.*

1.1. Disorders of consciousness are better defined

More than 40 years after the first definition of the vegetative syndrome (1), the nosology of DOC has vastly expanded (2). DOC encompass a range of diseases characterized by altered state of arousal, awareness, or both. Arousal – the vigilance, the awakening – and awareness – the content of consciousness, of the self and the environment – are the two components of consciousness in its operational definition (3,4). DOC arise after a period of coma, a transient state of total lack of both arousal and awareness, even after stimulations. Coma is caused by a severe brain injury, but aetiologies are plentiful (3,5,6). They are usually grouped into anoxic brain injuries, traumatic brain injuries (TBI), and other non-anoxic non-traumatic aetiologies. Coma is a transient state: it lasts from one hour to some weeks at most. Patients then either die, or can gradually recover consciousness. The recovery of arousal without awareness characterizes the vegetative state (7,8). To better reflect the condition of these patients, awake but unresponsive, and avoid a negative connotation of being "vegetable-like", the new name "unresponsive wakefulness syndrome" (UWS) has been proposed in 2010 and used since then (9). These patients have fluctuating periods of arousal, although they do not actually sleep (10–13). When awake, they do not show any sign of consciousness, but may exhibit reflexive behaviours, spontaneously or in response to stimulations. However, subtle signs of consciousness can easily be missed (14–17). They are primordial to detect, as they tell apart the UWS from the minimally conscious state (MCS). MCS was defined as a new disorder of consciousness in 1997 (18), and the diagnostic criteria were outlined in 2002 (19). Patients in MCS show minor and fluctuant signs of consciousness, such as visual pursuit or fixation, object localization, localization of noxious stimulation, object manipulation, automatic motor reaction, and reproducible movement to command. MCS has been further categorized in MCS- and MCS+, as only the latter shows evidences of preserved language processing (20). Yet, by definition all these patients cannot communicate. The recovery of functional

communication, or of functional use of objects, are diagnostic criteria for the emergence of MCS (EMCS) (21). Sometimes, because they do not move nor speak, patients are mistakenly considered unconscious. They are in fact fully conscious, but unable to respond to stimuli consecutively to the complete interruption of the pyramidal tracts and most cranial nerves, leading to a complete paralysis of voluntary movements (22). When assessed carefully, these locked-in patients (LIS) are able to respond to command, and even communicate, through eyes movements and blinking. Some patients however have lost even the ability to move the eyes, and are behaviourally undistinguishable from UWS patients. The brain injury location should clue the caregivers in about the possibility of a total LIS, leading to further investigations (23).

Hence, instead of a few categories of altered states of consciousness – coma, vegetative state, and severe disability – , we now face a continuum of DOC – coma, UWS, MCS-, MCS+, EMCS (Figure 2). Moreover, these patients often fluctuate, and require dedicated and very sensitive tools to diagnose accurately.
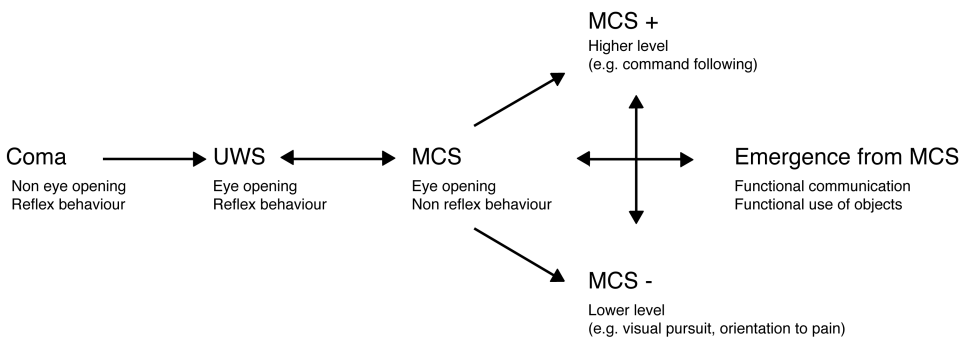
Figure 2 - Nosology of behavioural disorders of consciousness
This figure illustrates the natural evolution of consciousness in post-comatose patients, along with key diagnostic criteria for each step. Note that due to the common fluctuations in consciousness and vigilance, the patient may temporarily evolve to a less conscious state. *Adapted from **Bodart, O.**, Thibaut, A., Laureys, S., Gosseries, O., 2013. Disorders of Consciousness, in: Citerio, G., Smith, M., Kofke, A. (Eds.), Oxford Textbook of Neurocritical Care. Oxford University Press, London.*

1.2. *Behavioural tools have been refined*

The definition of these new DOC, and the development of clear diagnostic criteria, has been translated in sensitive and dedicated behavioural scales. Prior to these, the main scale to assess consciousness was the Glasgow Coma Scale (GCS) (24). Its purpose was to help managing acute patients with TBI, and having prognostic factors, by assessing eye opening, and motor and verbal response to stimulation. Thanks to its simplicity of use, the GCS became the scale to assess DOC, even after the acute stage, and even after non TBI (25). However, it has several limitations: the verbal scale cannot be scored in ventilated patients, a situation often encountered in the intensive care units, and except for some motor and verbal features, does not look for signs of consciousness, especially minor ones. That's why the Full Outline of UnResponsiveness scale (the FOUR) has been developed (26). Composed of four subscales, each scored from 0 to 4, this scale assesses motor responses as in the GCS, but also looks for visual pursuit, one of the first signs of consciousness recovery (21). Moreover, it can be applied to all patients in the intensive care units, by looking at the ventilation pattern instead of a verbal response. Finally, it can make the diagnostic of brain death as it evaluates brainstem reflexes. Outside of the intensive care units, the FOUR is not sensitive enough to minor signs of consciousness, and the gold standard behavioural scale is the Coma Recovery Scale – Revised (CRS-R) (Table 1) (27,28). Other scales have been developed, but none has the sensitivity and specificity of the CRS-R when it comes to detect MCS patients (29). The CRS-R is composed of six subscales, the auditory, the visual, the motor, the verbal, the oromotor, and the arousal scales, each assessing different items of increasing complexity. Some of these items are diagnostic criteria for MCS, and as the scale also assesses functional use of object and functional communication, it can also detect EMCS.

GCS	FOUR
Eye response	**Eye response**
4. Eyes open spontaneously	4. Eyelids open or opened, tracking or blinking to command
3. Eye opening to verbal command	3. Eyelids open but not tracking
2. Eye opening to pain	2. Eyelids closed but open to loud voice
1. No eye opening	1. Eyelids closed but open to pain
	0. Eyelids remain closed with pain
Motor response	**Motor response**
6. Obeys commands	4. Thumbs up, fist, or peace sign to command
5. Localizing pain	3. Localizing pain
4. Withdrawal from pain	2. Flexion response to pain
3. Stereotyped flexion to pain	1. Extension response to pain
2. Stereotyped extension to pain	0. No response to pain or generalized
1. No motor response	myoclonus status epilepticus
Verbal response	**Brainstem reflexes**
5. Oriented	4. Pupil and corneal reflexes present
4. Confused	3. One pupil wide and fixed
3. Inappropriate words	2. Pupil or corneal reflexes absent
2. Incomprehensible sounds	1. Pupil and corneal reflexes absent
1. No verbal response	0. Absent pupil, corneal, and cough reflex
	Respiration
	4. Not intubated, regular breathing pattern
	3. Not intubated, Cheyne-Stokes breathing pattern
	2. Not intubated, irregular breathing
	1. Breathes above ventilator rate
	0. Breathes at ventilator rate or apnea

CRS-R

Auditory function	Oromotor/verbal function
4. Consistent Movement to Command*	3. Intelligible Verbalization*
3. Reproducible Movement to Command*	2. Vocalization/Oral Movement
2. Localization to Sound	1. Oral Reflexive Movement
1. Auditory Startle	0. None
0. None	

Visual function	Communication
5. Object Recognition*	2. Functional: Accurate[+]
4. Object Localization: Reaching*	1. Non-Functional: Intentional*
3. Visual Pursuit*	0. None
2. Fixation*	
1. Visual Startle	
0. None	

Motor function	Arousal
6. Functional Object Use[+]	3. Attention
5. Automatic Motor Response*	2. Eye opening w/o Stimulation
4. Object Manipulation*	1. Eye opening with Stimulation
3. Localization to Noxious Stimulation*	0. Unarousable
2. Flexion Withdrawal	
1. Abnormal Posturing	
0. None/Flaccid	

Table 1 - Behavioural scales

This table illustrates 3 validated scales for the evaluation of acute comatose patients (GCS and FOUR) and the CRS-R, a validated and sensitive scale to diagnose UWS, MCS and EMCS.

The GCS is composed of 3 subscales (eye, verbal and motor responses) and can score from 3 to 15. The FOUR is composed of 4 subscales (eye, motor, brainstem and respiration) each composed of 4 items. The CRS-R is composed of 6 subscales (auditory, visual, motor, oromotor/verbal, communication and arousal). It can score from 0 to 23. In the CRS-R, * denotes MCS, and + denotes EMCS. *Adapted from* **_Bodart, O._**, *Thibaut, A., Laureys, S., Gosseries, O., 2013. Disorders of Consciousness, in: Citerio, G., Smith, M., Kofke, A. (Eds.), Oxford Textbook of Neurocritical Care. Oxford University Press, London.*

Despite the fact that the CRS-R was published with a detailed administration procedure, several improvements have been made since its publication. The systematic use of a mirror detects more often visual pursuit (31–33) and thus allows uncovering more patients in MCS. Visual pursuit however relies on the examiner appreciation, and objective measures are being developed (34). Similarly, using the patient's own name facilitates the detection of sounds localisation (35). Moreover, performing only one behavioural evaluation is not enough to establish a correct diagnosis. As the patients fluctuate, an optimal number of five CRS-R should be carried out in a relatively short period of time (36). In conclusion, good standardized behavioural scale – the CRS-R – exists, and the administration procedure has been refined to maximize its diagnostic power. However, consciousness may still be underestimated using behavioural tools only: the patients may be unable to understand the command due to aphasia (37,38), deafness, or dialect issues, be limited in their motor output due to paralysis, spasticity, hypotonic status, be unwilling to collaborate, be too drowsy to participate due to pathological fluctuation of vigilance or from drugs, …

To avoid these limitations and to try to provide objectives measures of consciousness, neuroimaging technologies have been developed.

1.3. Ancillary techniques are plenty …

Magnetic resonance imaging (MRI) is probably the most simple and efficient way to study the localization, severity, number, and nature of brain injuries. A relation between these parameters and the patient's state of consciousness has been looked for by several research groups. No distinction between UWS and MCS patients can be made using structural MRI only, as these patients sometimes share the same injuries despite their different levels of consciousness (39). However, a poor prognosis has been found in patients with lesions in the basal ganglia, the thalamus (especially if bilateral), and the brainstem (40–44). The number of lesions itself is correlated with the outcome (45). To have an

even sharper view of the brain's structural integrity, diffusion weighted imaging (DWI) and diffusion tractography imaging (DTI) can be used. Diffusion sequences measure the degree and direction of water molecules movements, movements that can be impeded by the presence of, for example, axons. DWI thus reflects the white matter structural integrity, and DTI reflects the structural connectivity within the brain (46). DWI features can differentiate between groups of UWS and groups of MCS, and correlate with the CRS-R total score (47). In UWS patients, white matter injuries not seen on conventional MRI can be detected with DTI, up to severed tracts in the brainstem of patients with TBI (48).

Functional MRI (fMRI) can look further than structure with the use of blood oxygenation level dependant sequences. By using the difference in signal between oxygenated and deoxygenated blood, blood oxygenation level dependant sequences can detect variations in local cerebral perfusion. Functional connectivity can be approached by looking at the statistical association between these variations in different areas. At rest, a network encompassing the anterior and posterior cingulate, the temporo-parieto-occipital junction and the thalamus is activated, and called the default mode network (DMN). The connectivity in this network correlates with the level of consciousness (49); it is null in brain dead patients (50) and nearly normal in patients with LIS (51). This network can be further divided between its internal and external parts, and these were respectively related to the awareness of the self and of the environment (52). The DMN is characterized by hyperconnectivity, which translates in a lack of anticorrelation between network components, in UWS and MCS but not in EMCS patients (53). Other resting state networks (executive control, salience, sensory-motor, auditory and visual) can be studied (54). The DMN and the auditory networks have very high accuracy in differentiating between healthy controls, UWS and MCS patients (55). Using active paradigms, in which subjects are asked to imagine performing tasks, fMRI can detect wilful

modulation of brain activity in healthy subjects (56) and patients with DOC (57–62), sometimes even in patients who are behaviourally unresponsive. By detecting a response to command, although in an indirect way, these paradigms are a great addition in the patients' diagnostic work-up.

Behind the brain structure and function, one can evaluate its metabolism. This is usually done with ^{18}fluoro-deoxyglucose positron emission tomography (FDG-PET), which demonstrates a global decrease of metabolism in UWS patients (63–65). Yet, recovery of consciousness is not related to a recovery of global metabolism (66,67). Indeed, the metabolism is impaired in a frontoparietal network, encompassing the prefrontal and posterior parietal associative cortices, the mesiofrontal area, the precuneus and the thalamus (68,69). Recovery of consciousness correlates with the metabolic activity in this network (70). Using the pattern of preserved or impaired metabolism in this network, it is possible to distinguish between UWS and MCS patients with a good accuracy (71,72).

Similarly to what has been done using MRI and FDG-PET, the injured brain has been studied by electroencephalography (EEG). At rest, entropy measures derived from EEG signal can distinguish between unconscious and minimally conscious patients (73). Alpha power in the occipital area is a positive prognostic factor in UWS patients (74). Event-related potentials (the EEG average of stimulus-induced time-locked activity) late components are thought to reflect the conscious processing of information (75). Passive paradigms can also reveal disruption of top-down connectivity (from higher order to primary cortices) in UWS patients but not in MCS patients (76). Aiming to reproduce fMRI results at the bedside, active EEG paradigms were developed. They can detect a response to command in a patient with total LIS (23), and in patients with DOC (77–80). Electromyography has also been used to detect subclinical motor response in DOC (81).

Numerous technologies (MRI, DWI, fMRI, FDG-PET, EEG, electromyography) have been developed and used to help establishing accurate diagnosis in DOC. On top of circumventing several limitations of behavioural evaluations, these techniques improved our understanding of the neural correlates of consciousness. Nonetheless, caution should be kept when interpreting the results for clinical purposes.

1.4.   … but still have limitations

Indeed, using active paradigms is cognitively very demanding, and some patients are unable to wilfully modulate their brain activity, sometimes despite being able to respond to command behaviourally. If a positive result from this technique is considered very similar to a behavioural sign of consciousness, the absence of response cannot be considered as an absence of consciousness (82). The opposite problem occurs with passive and resting states paradigms. Surely, the measures these techniques provide are quite far removed from the behavioural signs of consciousness. Caution should then be taken while interpreting positive results, and especially when translating group findings to the clinical single subject. Moreover, as these technologies rely on complex methodologies and statistics, errors can occur and modify the results, in both ways. For many of these techniques, the outcome still depends on the patients' performance, on their collaboration, on their understanding of the instructions. So if all these technologies have helped us progress in the understanding of these DOC, and in making accurate diagnosis, we have to keep in mind that they still have limitations.

1.5.   Why it matters

All these behavioural and technological tools have not been developed in vain. They are necessary to establish an accurate diagnosis of the residual level of consciousness in these non-communicating patients. An accurate diagnosis is

mandatory to provide the family and the caregivers with prognostic information, for example. Indeed, UWS and MCS patients do not have the same outcome, and there is a major role of the brain injury's aetiology. UWS patients have a worse outcome than MCS, and an anoxic aetiology as a poorer prognosis than TBI. Hence, UWS from anoxic brain injuries have very limited chance to recover signs of consciousness, and the situation is considered permanent after three months (7,8,83,84). On the other hand, MCS patients from TBI recover far more often, and can still do so 12 months after the original insult (83,84). Even though recovery after these traditional limits is observed, it usually coincides with very poor functional outcome (85,86). Ancillary techniques also provide valuable prognostic information. For example, the fractional anisotropy (FA), a feature of DWI reflecting the degree of anisotropy, hence, indirectly, of axonal integrity, is often used. This marker in specific tracts such as the corpus callosum and the internal capsule correlates with the functional outcome (87) and the diagnosis at the time of discharge (88). At the acute phase, a composite score based on the FA in different axonal tracts predict the outcome after traumatic (89,90) or anoxic (91) brain injuries. Using fMRI, the presence of connectivity within the DMN has positive prognostic value (92).

An accurate diagnosis is also crucial for appropriate therapeutic management, such as the treatment of pain. Dealing with pain in UWS and MCS patients is an issue, as by definition they are non-communicating. There is thus a risk of undertreatment, as the patient cannot report suffering, or overtreatment, with the risks of side effects it implies. The Nociception Coma Scale – Revised is an observational scale that has been developed to overcome these limitations, and can assess and monitor pain in these patients to guide the treatment (93–95). Moreover, we know that unresponsive patients do not "feel" pain, as noxious stimulations only activate the primary cortex, while MCS show a wide activation of the pain matrix for similar stimuli (96–98). However, some have underlined the risk that some behaviourally unresponsive patients could in fact feel pain

(99), while other demonstrated the major role of personal beliefs in managing pain in these patients (100). Treatments to improve level of consciousness also have different effects on UWS and on MCS patients. Amantadine, a dopaminergic agonist, can lower the mean disability rating scale score for several weeks in MCS but not in UWS patients in a placebo-controlled trial (101). Transcranial direct current stimulation, a non-invasive brain stimulation technique, can improve the CRS-R scores in MCS, but not in UWS patients (102).

Accurate diagnosis is also critical for ethical reasons. Indeed, given their different prognosis, end-of-life issues are treated differently for UWS and MCS by the caregivers, once again mainly depending on personal beliefs (103,104). The law is variable between states, sometimes allowing interruption of hydration and nutrition in UWS but not MCS patients, sometimes preventing any interruption of treatment (105–107).

Finally, accurate diagnosis is crucial to produce valid science. If different teams do not share the same diagnostic criteria, their results will not be comparable, and identifying the neural correlates of consciousness will not be possible. In conclusion, it is worth investing time and effort to achieve an accurate diagnosis, to be able to communicate correct prognosis, to tell the estimated effect of treatment, to adequately manage pain, to discuss end-of-life issues, and for science itself.

1.6. Several questions remain

Despite all these theoretical and technological advances, many issues remain unsolved. Currently, there is no technique able to differentiate UWS from MCS patients reliably, at the single patient level, and at the bedside. Indeed, fMRI active paradigms, while having specificity high enough to be accurate at the

single patient level, are not very sensitive. Many subjects who were able to behaviourally respond to command could not do so using this technique (108). FDG-PET has a good accuracy in discriminating between UWS and MCS patients (71), but is not a bedside technology, and exposes the patients to radioactive material. Moreover, it lacks sufficient accessibility. We will discuss later whether transcranial magnetic stimulation (TMS) coupled with high density EEG (TMS–EEG) is a good candidate to answer all these accuracy and accessibility issues, and is available at the bedside, or not.

All these neuroimaging techniques also revealed several cases where patients, behaviourally unambiguously unresponsive, had patterns of results more compatible with consciousness than with unconsciousness. This is the case with active fMRI, where some UWS patients are able to use the paradigm to respond to command (57,58,61,108). Active paradigms using EEG turned up some cases too (79,80). FDG-PET can also detect patterns of preserved metabolism in UWS patients, patterns usually only seen in patients at least minimally conscious. A majority of these patients recovered signs of consciousness at the 12 months follow-up (71). So far, little is known about this subpopulation of patients. Are they unable to behaviourally express their preserved consciousness, or are they subjected to the limits of neuroimaging in term of accuracy? Combining multiple techniques might help solve this question.

2. TMS–EEG

Among all the neuroimaging techniques, TMS–EEG is the one we will focus on in this thesis. The combination of a non-invasive brain stimulation technique with the high-density EEG temporal resolution offers novel ways to study the brain, even the injured one, and has many advantages over other technologies.

TMS, by generating brief magnetic pulses, induces electric field at the cortical surface, which in turn creates ionic currents depolarizing the neuronal membranes. The induced currents are the most likely to occur at the axonal level, as they have the lowest threshold for depolarization (109,110). This is especially true around bending axons, as it is where the induced electric field changes the most. As the magnetic field rapidly decreases with the distance, only the superficial cortical structures or the white matter just underneath can be depolarized directly (111,112). This depolarization of a small cortical area can however propagates through intra- and inter-hemispheric tracts to other cortical areas, and through projecting fibres to deeper cerebral structures. The ability of this perturbation to actively induce activity at a distance, hence the causal interaction between distant brain areas, reflects the effective connectivity. It differs from the functional connectivity, which is the statistical temporal correlation of the activation of several areas without a causal link, and from the structural connectivity, which reflects the anatomical connections that exist between structures, with no inference about the functionality of these connections (113). Recording the perturbation induced by TMS is possible with EEG, given its extremely high temporal resolution. However, for a long time EEG amplifiers were not able to deal with the large artefact induced by the TMS, and were saturated for several seconds, preventing the recording of any TMS evoked potential (TEP). Thanks to the development of sample-and-hold circuits, amplifiers are now able to record the EEG a few milliseconds only after the TMS pulse (114–116).

The TEPs obtained are reproducible and sensitive to changes in parameters (117). Each cortical area generates TEPs in its own frequency, respectively in the fast β/γ, β, and α frequency bands for the frontal, parietal, and occipital lobes (118). The generator for the α frequency of the occipital TEPs and for the spontaneous posterior α rhythm are the same, and share the same influence from visual attention (119). These TEPs are constrained by structure, as assessed by DTI, and the correlation between structural and functional connectivity decreases after the TMS pulse (120,121).

Several indices, reflecting the cortical excitability, effective connectivity, or the ability of TMS to modify the phase of on-going neuronal activity, have been developed (122). These have had several applications. While studying the neural correlates of cognitive tasks, TMS–EEG can detect an increase in effective connectivity between the frontal eye field and posterior brain areas during visual attention (123). Similarly, during motor attention, cortical excitability is greater (124). While performing spatial memory task, excitability and effective connectivity from the superior parietal lobule are rising as compared to resting conditions (125). This verifies in the visual memory network after long-term training (126). TMS–EEG also demonstrates specific neurophysiological changes in pathological states. In epileptic patients, cortical excitability as measured with TMS–EEG is increased (127), especially in the epileptogenic network (128), or before epileptiform discharges in generalized genetic epilepsies (129). The use of antiepileptic drugs, such as Lamotrigine and Levetiracetam, can modify the TEPs (130), and TMS–EEG is sometimes used to study the neurophysiological effect of drugs in development such as GABA-A antagonists (131). It can monitor the effect of non-invasive brain stimulation techniques, such as transcranial direct current stimulation that induces a rise in cortical excitability (132). This technique also has several applications in

psychiatric disorders. In schizophrenia, cortical excitability and effective connectivity from the premotor cortex is reduced, and correlates with cognitive deficits (133). These patients share with bipolar disorder and major depression a reduction of the TEPs natural frequency on the frontal lobe (134). In bipolar disorder, this reduction seems to be a biomarker of the disease, as it is present whether the patient is symptomatic or not (135). History of alcohol abuse is related with persistent changes in brain's connectivity, and with an increase in cortical excitability (136). Smartphone abuse with impaired attention is linked with decreased excitability of right dorsolateral prefrontal cortex (137). In patients with Alzheimer disease divergent results are found: there is a decreased excitability in the prefrontal cortex (138) but an increased excitability in the sensorimotor cortex (139).

Most importantly, TMS–EEG has incredible value in studying consciousness and its alterations, whether from physiological, pharmacological, or pathological origin. According to modern theories, consciousness arises from the brain's ability to integrate information (140), meaning that it has access to a large repertoire of possible states yet cannot be decomposed in smaller modules. One could look at how different brain areas causally interact (integrate information) and record the complexity of this interaction (to ensure it is not the sum of simple activities). TMS–EEG can perturb part of the thalamocortical system, and record how this perturbation causally interacts with distant areas over time. This spatio-temporal complexity could thus reflect the brain's ability to sustain consciousness (112). In wakeful healthy subjects, the TEPs recorded show that the initial depolarization affects multiple areas on both hemispheres, and the recorded response lasts for at least 300ms (141). While these subjects are in NREM sleep, and hence are physiologically unconscious, the TEPs are composed of a large slow wave that remains local, under the stimulated area (141,142). Using higher stimulation amplitude, the response can once again encompass both hemispheres; however, it remains a simple large slow wave,

hence without much complexity (141). Small variations in the TEPs are possible in this state: the impact of TMS on on-going activity is shorter, and the response larger, when people are not dreaming. This illustrates the effect of small changes in the thalamocortical system on consciousness (143). Other parameters can modify the recordings: cortical excitability increases with time spent awake, exemplifying the effect of an increased sleep pressure (144), yet it remains under the influence of circadian rhythm (145). In REM sleep, when subjects can report the content of their dreams, the TEPs are similar to those observed in wakefulness, involving both hemispheres in a complex pattern (142).

Unconsciousness can also be obtained using general anaesthesia. The responses acquired using Midazolam (146), Propofol, and Xenon (147) all share the same reduction in the spatio-temporal complexity, and all remain slow, local and of short duration. However, the cortical excitability (in this case, the amplitude of the first component of the response) differs between these molecules (large with Xenon, small with Propofol) (146,147). Using Ketamine, subjects seem as unconscious as with the other tested drugs, yet upon awaking they can report vivid conscious dream-like experiences. The TEPs obtained under Ketamine anaesthesia are much more complex and closely alike those observed in wakefulness and REM sleep (147).

Unconsciousness, or altered consciousness, can also arise from severe brain injuries, such as those observed in patients with DOC. When patients are completely unconscious, in UWS, the TEPs are slow, local, and short lasting, as in unconscious healthy subjects, during NREM sleep or general anaesthesia (148). They can also be inexistent despite high stimulation intensity, especially in patients with anoxic brain injuries, or if stimulation lands on cortical lesions in TBI (149). This reminds the major importance of neuronavigation while performing TMS–EEG in this population. In MCS patients, the responses are more complex than in UWS, spreading away from the stimulation site (148,150).

In LIS, the TEPs are similar to those observed in awake healthy subjects (148). When unconscious patients recover – minimal – signs of consciousness, the complexity of the response increases, sometimes before the behavioural changes (148). TMS–EEG thus generate either TEPs with fast oscillations, that spread bilaterally, away from the stimulation area, in wakeful healthy subjects, during REM sleep, Ketamine anaesthesia, or in LIS patients, or TEPs with slow oscillations, that remain local under the stimulation area, in NREM sleep, Midazolam, Xenon, and Propofol anaesthesia, or in UWS patients. MCS patients have TEPs between normal subjects and UWS. Figure 3 illustrates the morphologies of TMS responses in different states of consciousness.

Distinguishing between UWS and MCS by looking at the TEPs shapes only is not very reliable, though. An objective measure of the spatio-temporal complexity of TEPs was thus designed: the perturbational complexity index (PCI). This measure is normalized by the source entropy, and is above 0.31 when consciousness is present (normal wakefulness, REM sleep, Ketamine anaesthesia, LIS), and below 0.31 when consciousness is absent (NREM sleep, Propofol, Midazolam, and Xenon anaesthesia, UWS). Interestingly, PCI is also above 0.31 in MCS patients, and thus seems to be able to distinguish between UWS and MCS at the single subject level (151). Table 2 summarizes the advantages and inconveniences of TMS–EEG over other neuroimaging techniques (152).
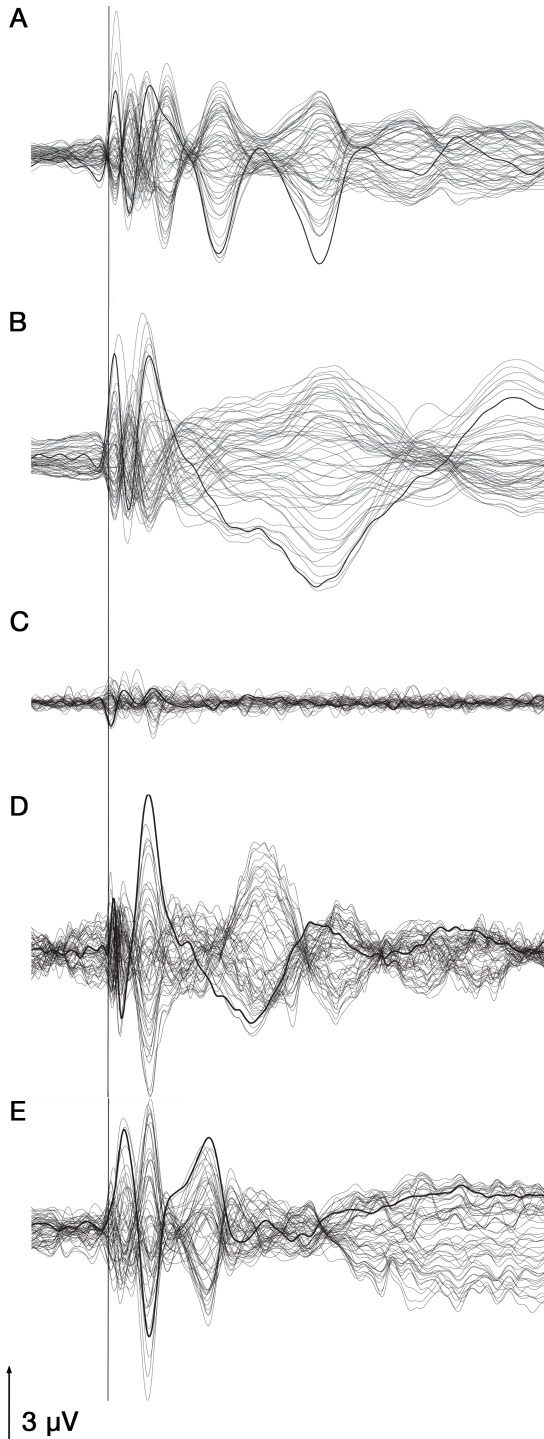
Figure 3 - TEPs in different states of consciousness

Typical response to TMS in different physiological, pharmacological, and pathological conditions. (A) Healthy subject, awake. (B) Same healthy subject under Dexmetedomidine deep sedation. (C) Anoxic UWS patient – no visible response. (D). MCS patient. (E) LIS patient. For each condition, the vertical line indicates the TMS pulse. Amplitude scale is 3μV/cm, and time span from -100ms before the TMS pulse to +400ms after it.

Advantages	Disadvantages
Bypass afferent sensory pathways.	Dependent of the subject cortical excitability, lowered in case of brain atrophy and with several drugs (including antiepileptic).
Does not require functioning efferent pathways.	Requires stable state of wakefulness.
Does not require subject active participation.	Limited spatial resolution.
Does not require language processing.	Acute patients assessment limited by the presence of metallic implant, external CSF drain, or uncontrolled epilepsy.
Highly reproducible within subject.	Requires heavy logistic and subject preparation.
Can be use at the patient bedside.	Source modeling possibly inaccurate in cases of extensive brain lesions or scalp deformations.
Sensitive to changes in stimulation parameters.	
Good temporal resolution.	
Probes effective connectivity.	
Discrimination between conscious and unconscious conditions.	
Supported by recent theories of consciousness.	

Table 2 - Advantages and disadvantages of TMS–EEG

This table summarizes general advantages and disadvantages of TMS–EEG over other diagnostic tools (behavioural or neuroimaging). CSF: Cerebrospinal fluid. *Adapted from Napolitani, M.*,* **_Bodart, O.*_**_, Canali, P., Seregni, F., Rosanova, M., Laureys, S., Massimini, M., Gosseries, O., 2014. Transcranial magnetic stimulation combined with high density EEG in altered state of consciousness. Brain Inj. 28, 1180–1189. doi:10.3109/02699052.2014.920524 (* contributed equally)_

TMS–EEG consequently is an attractive technique, especially in patients with DOC. It bypasses several limitations that other neuroimaging techniques have. It can discriminate between conscious and unconscious conditions at the single subject level, which is mandatory for a potential clinical diagnostic tool. It is also the sole technology to assess the perturbational effective connectivity, which could be combined to other connectivities (structural and functional) and with underlying brain metabolism to increase our understanding of the neural correlates of consciousness.

3. Objectives

Global understanding of DOC has greatly improved, as we discussed above. The nosology expanded from a few grained to a continuum of alteration of consciousness. This leads to more difficulties in making accurate diagnosis, despite considerable technological advances. TMS–EEG, through PCI, seems to be a fantastic tool to discriminate between UWS and MCS. Our first objective is to validate this technique with other validated neuroimaging technologies.

We have seen that some UWS patients have neuroimaging patterns compatible with consciousness, not congruent with their behaviour. Our second objective is to settle if these results are artefacts, or if they actually reflect covert cognition.

Finally, TMS–EEG allows measuring the perturbational effective connectivity: our last objective is to establish how effective connectivity behaves compared to structural connectivity.

Chapter II

Shared TMS–EEG methodology

Section based upon the following publications:

Napolitani, M.*, **Bodart, O.*,** Canali, P., Seregni, F., Rosanova, M., Laureys, S., Massimini, M., Gosseries, O., 2014. Transcranial magnetic stimulation combined with high density EEG in altered state of consciousness. Brain Inj. 28, 1180–1189. doi:10.3109/02699052.2014.920524 * Contributed equally

Gosseries, O., **Bodart, O.,** Massimini, M., 2015. Transcranial magnetic stimulation and electroencephalography, in: Rossetti, A., Laureys, S. (Eds.), Clinical Neurophysiology in Disorders of Consciousness: Brain Function Monitoring in the ICU and Beyond. Springer-Verlag Wien, doi:10.1007/978-3-7091-1634-0_10

The studies conducted for this thesis were performed on the same equipment, using the same set up, and similar processing steps.

1. Acquisitions

EEG is recorded using a 60 channel EEG cap, whose size (small, medium, or large) is adapted to the participant's head. Electrodes are organized according to the 10-20 international positioning system. Reference and ground are located on the forehead, one centimetre apart. Two more channels are used to record the electrooculogram, by positioning one electrode on the upper external side of the left eye and one on the lower external side of the right eye. The electrodes are made of carbon, shaped as open ring, with a low profile, to further decrease TMS induced artefacts. The subject's skin is prepared by first removing the hair inside the electrode with a cotton tip, then scrubbing the scalp with a bit of abrasive gel (Nuprep®, Weaver and Company, Aurora, Colorado, USA), and finally filling the electrode with conductive gel (ECI Electro-Gel™, Electro-Cap International, Eaton, Ohio, USA). This allows the impedances to stay below 5 kΩ. The amplifier provides a colour coded impedance check, but whose relation with actual impedance value is not available (electrodes are shown in green when below 5 kΩ, and black when disconnected, but also in yellow and red when impedances values are greater than 5 kΩ). EEG is recorded using an Eximia sample-and-hold amplifier gating the TMS artefact 100µs prior and 2ms after the stimulus (Nexstim Plc., Helsinki, Finland). The signal is then sampled at 1450Hz and band pass filtered between 0.1 and 350Hz.

Stimulations are performed using an air-cooled figure-of-eight coil (inner diameter 50mm, outer diameter 70 mm, focal area of stimulation 0.68 cm^2) driven by a mobile TMS unit (Nexstim Plc, Helsinki, Finland). The biphasic pulses last 280µs and reach an intensity of 1 to 2 T (maximum electric field 20 mm below the coil 199V/m), and are performed automatically with an

interstimulus interval jittering randomly between 2000ms and 2300ms. The coil charging is delayed outside of the recording interval (after 1000ms). The intensity of stimulation is set as a percentage of the maximum output, and adapted to reach an estimated evoked electric field of 120V/m at the cortical level. This estimation is provided by the neuronavigation system based on a spherical head model. This intensity can be adapted according to artefacts or signal-to-noise ratio, reaching up to 150V/m, hence an intensity well above threshold for an EEG response (50V/m).

To ensure reproducibility, the recordings are performed with neuronavigation (Eximia NBS, Nexstim Plc, Helsinki, Finland). This system uses an infrared camera tracking reflective sensors on goggles and the coil, and the subject's 3D T1 MRI on which landmarks were selected (nasion and both tragi) and co-registered in real life. Using a software-aiming device, this allows the stimulator to prevent any pulse going from the coil if the latter is not exactly in the same position as the repeated stimulus (location, distance to scalp, rotation and angle) with an error less than 2mm. Similarly, this allows to precisely locate the stimulation targets, on the participant's own brain. We aim to stimulate the medial part of the left or right superior parietal lobule (BA7) and the medial part of the right or left superior frontal gyrus (BA6), while avoiding obvious structural lesions. The exact stimulation site can vary slightly if the original target cannot be stimulated without major artefacts. Each target is stimulated between 200 and 400 times. All the stimulation sites, the MRI landmarks and the electrodes locations are registered at the end of the experiment.

To avoid the recording of auditory evoked potentials, as the TMS pulses are quite loud, subjects are listening to a constant white noise through inserted earphones. Healthy subjects are asked to report if they can still hear the TMS pulse and the white noise volume is adapted accordingly. In non-communicating patients, the volume is set at a fixed value such that the experimenters can hear

the noise. To prevent bone conduction of the TMS "click", a light layer of foam is placed between the coil and the EEG cap. All the subjects are installed as comfortable as possible, either in their bed or on a chair, and keep their eyes open during the recordings. If necessary, an arousal protocol is performed on patients with DOC (27). Figure 4 illustrates our TMS–EEG setup, with a neuronavigation and targeting system.

Figure 4 – Our TMS–EEG setup
Example of our setup, combining a neuronavigation system (a) , the stimulation coil with tracking elements (b), a compatible high-density EEG net (c) and a compatible EEG amplifier (d). The neuronavigation system is composed of 3D brain reconstruction (a1), an infrared tracking camera (a2) and tracking googles (a3). *Adapted from Napolitani, M.*, **Bodart, O.***, Canali, P., Seregni, F., Rosanova, M., Laureys, S., Massimini, M., Gosseries, O., 2014. Transcranial magnetic stimulation combined with high density EEG in altered state of consciousness. Brain Inj. 28, 1180–1189. doi:10.3109/02699052.2014.920524 (* contributed equally).*

2. Initial data processing

Data were initially processed in Matlab R2007b (Matworks, Natick, MA), using SSP 2.0e (scripts provided by *Casali et al.*). First, all the artefacted trials (electrode movement, eye movement, overwhelming muscle activity) were visually identified and discarded. At least 150 good trials per session were kept for further analysis. Isoelectric channels, or channels with constant or major artefacted activity were also visually selected and discarded. No more than 10 channels were removed. EEG was then band-pass filtered at 0.1-45Hz, down sampled at 362.5Hz, and split using a -800 +800ms window around the TMS pulse. The EEG activity was then averaged to get the TEP, and the signal baseline was corrected according to the EEG activity from -400 -100ms. Additional artefacted channels could be identified and discarded at this stage. ICA was sometimes used to remove further artefacts, such as 50Hz line noise, muscle activity, blinks, or TMS pulse. The EEG signal up to 400ms after the TMS pulse was then analysed using D30 (version October 2014), another script provided by *Casali et al.* The EEG sources were computed using weighted minimal norm constraint to solve the inverse solution, and a 3-spheres BERG model of head volume conductance. Significant source activation was assessed using non-parametric bootstrap statistic (486 bootstraps, distribution size 70000, significant level $\alpha = 0.01$). Then a matrix of the spatial distribution of these significant sources against time was computed. This matrix was then compressed using Lempel-Ziv algorithm. The resulting value was normalized by the source entropy of the initial matrix to obtain the PCI. When the TMS could not elicit a sufficient EEG response (entropy <0.08 or signal to noise ratio <1.4), the PCI was set to 0, reflecting the fact that the stimulation could not significantly engage a neuronal activity.

Chapter III

TMS–EEG and neuroimaging

Section based upon the following publications:

Bodart, O., Gosseries, O., Wannez, S., Thibaut, A., Annen, J., Boly, M., Rosanova, M., Casali, A.G., Casarotto, S., Tononi, G., Massimini, M., Laureys, S., 2017. Measures of metabolism and complexity in the brain of patients with disorders of consciousness. NeuroImage Clin. 14, 354–362. doi:10.1016/j.nicl.2017.02.002

Bodart, O., Amico E., Gomez F., Casali A.G., Wannez S., Heine L., Thibaut A., Annen J., Boly M., Casarotto S., Rosanova M., Massimini M., Laureys S., Gosseries O., Global structural and effective connectivity in patients with disorders of consciousness. Under revision.

1. TMS–EEG and FDG-PET

1.1. Combining TMS–EEG and FDG-PET

As we have seen, several questions remain open while dealing with DOC, to which TMS–EEG could contribute to answer. Given the need for an accurate diagnostic tool usable at the patient bedside, and given the apparent ability of the PCI to discriminate between UWS and MCS patients, further validation and cross-validation of TMS–EEG is required. FDG-PET is a validated neuroimaging method, contributing to establish correct diagnosis (71,72). TMS–EEG and FDG-PET are not redundant, as the first evaluates dynamically the brain's ability to sustain complex interactions, while the second assesses the brain's residual metabolism at rest, especially in the fronto-parietal network. In the present study, the first objective is thus to cross-validate TMS–EEG and FDG-PET, against the behavioural gold standard, the CRS-R.

Several neuroimaging techniques have unveiled UWS patients with abnormally good results, results usually observed in subjects with – at least minimal – consciousness. However, none of these patients were assessed using multiple imaging methods, to validate that the results were the sign not of a lack of specificity, but rather of covert consciousness in this subpopulation. Hence, identifying signs of covert consciousness using both TMS–EEG and FDG-PET is the second objective of this study.

1.2. Methodology

In order to meet our cross-validation objective, we performed FDG-PET then TMS–EEG five days apart in a population of 24 adult patients (13 male, 12 TBI, time since injury 52.5 weeks (5-1371), age 35 ±12 years). To limit the impact of spontaneous recovery, patients were included at least five weeks after the brain injury. However, we wanted to reflect the diversity of time since-onset observed in the clinical setting, and thus did not set an upper limit for the time since injury. FDG–PET was acquired and analysed as in (71,153,154). Briefly, after being kept awake in a quiet dark room for at least 10 minutes, patients received 150 to 300MBq of ^{18}FDG. They were then kept awake in the same quiet dark room for fixation for 30 minutes, before being scanned for 12 minutes on a Philips Gemini TF PET-CT scanner. At this stage, some patients required light sedation to avoid movement that could have prevented the acquisitions. This could not have influenced the results, as it took place after the radiotracer had been fixed in the brain by glucose uptake at wakeful rest. Patterns of preserved or globally decreased metabolism were visually identified by experts of our team on statistical maps computed by SPM8 (www.fil.ion.ucl.ac.uk/spm), using a contrast consisting of 39 age-matched healthy controls (significance threshold of $p<$ .05 uncorrected in all contrast for single subject analyses). More precisely, when the statistical tool did not detect a single voxel of preserved metabolism in the whole associative fronto-parietal network bilaterally, the pattern was set as compatible with unconsciousness; when at least some significantly preserved metabolic activity could be detected in the fronto-parietal network, the pattern was considered as compatible with consciousness. TMS–EEG acquisition protocol is described in detail in chapter II, and is similar to the one used in (148,151,155). One year after the study, the subjects' outcome was assessed using the Glasgow outcome scale extended (156). The behavioural diagnosis was reported as obtained by the best CRS-R, while FDG-PET and TMS–EEG

results were classified as compatible with either consciousness or
unconsciousness.

1.3. FDG-PET and TMS–EEG are congruent

To cross-validate FDG-PET and TMS–EEG, we performed these two techniques
in a behaviourally well-defined DOC population. A final table containing the
behavioural, metabolic, and electrophysiological diagnosis, as well as the one-
year outcome, summarizes the results (Table 3).

Independently of the behavioural diagnosis, FDG-PET and TMS–EEG classified
the patients as conscious or unconscious with almost no mismatch. In the 15
patients who were behaviourally – minimally – conscious (two LIS, two EMCS,
seven MCS+, four MCS-), FDG-PET was always able to detect preserved
metabolism in at least some part of the fronto-parietal network, and the best PCI
was above 0.31 for all but one patient (discussed below). Four behaviourally
UWS patients had preservation of only the brainstem or cerebellum metabolism
in FDG-PET, and none in the fronto-parietal network, and their best PCI ranged
between 0 and 0.27, hence below 0.31. These results are illustrated in Figure 5.

Patient	Demography		TSI	Best CRS-R		TMS		PET	Outc.
	Age	Aetiol.	(weeks)	Score	Diag.	PCI max	Diag.	Results	GOSE
LIS1	25	NTBI	200	23	LIS	0.62	Cons.	LIS	3 =
LIS2	35	NTBI	163	23	LIS	0.48	Cons.	LIS	3 =
EMCS1	23	TBI	60	23	EMCS	0.5	Cons.	Cons.	3 =
EMCS2	52	NTBI	21	22	EMCS	0.45	Cons.	Cons.	NA
MCS1	39	NTBI	37	17	MCS	0.43	Cons.	Cons.	3 =
MCS2	53	TBI	460	15	MCS	0.46	Cons.	Cons.	3 =
MCS3	46	Mixed	1371	12	MCS	0.44	Cons.	Cons.	3 =
MCS4	32	TBI	200	11	MCS	0.43	Cons.	Cons.	1 -
MCS5	26	TBI	145	11	MCS	0.38	Cons.	Cons.	NA
MCS6	19	TBI	27	11	MCS	0.33	Cons.	Cons.	3 =
MCS7	21	TBI	209	11	MCS	0.38	Cons.	Cons.	3 =
MCS8	54	NTBI	23	13	MCS	0.39	Cons.	Cons.	3 =
MCS9	26	TBI	630	8	MCS	0.4	Cons.	Cons.	3 =
MCS10	50	Mixed	33	7	MCS	0.4	Cons.	Cons.	3 =
MCS11	19	TBI	188	10	MCS	0.22	Uncons.	Cons.	3 =
UWS1	40	TBI	45	5	UWS	0.49	Cons.	Cons.	1 -
UWS2	31	TBI	207	7	UWS	0.4	Cons.	Cons.	2 =
UWS3	25	TBI	33	5	UWS	0.37	Cons.	Cons.	1 -
UWS4	27	NTBI	13	5	UWS	0.38	Cons.	Cons.	3 +
UWS5	21	TBI	25	7	UWS	0.25	Uncons.	Cons.	2 =
UWS6	34	NTBI	1116	7	UWS	0.2	Uncons.	Uncons.	NA
UWS7	44	NTBI	14	6	UWS	0	Uncons.	Uncons.	1 -
UWS8	47	NTBI	5	6	UWS	0	Uncons.	Uncons.	1 -
UWS9	43	NTBI	9	5	UWS	0	Uncons.	Uncons.	2 =

Table 3 - Behavioural diagnosis, imaging results, and outcome

Previous page:

This table reports the results of the 24 patients. Patients are arranged from best evidence for consciousness to no evidence for consciousness. Behavioural (CRS-R), FDG-PET, PCI, and outcome are reported. Results not compatible with consciousness are greyed. The two patients with mismatches between FDG-PET and PCI are highlighted. Note that 4 patients who are behaviourally unresponsive have FDG-PET and PCI compatible with consciousness. Aetiol.: aetiology; TSI: time since injury; diag.: diagnosis; outc.: outcome; GOSE: Glasgow outcome scale extended; cons.: conscious; uncons.: unconscious. Outcomes at 12 months: NA not available; + improved; - declined; = did not change. *Adapted from **Bodart, O.,** Gosseries, O., Wannez, S., Thibaut, A., Annen, J., Boly, M., Rosanova, M., Casali, A.G., Casarotto, S., Tononi, G., Massimini, M., Laureys, S., 2017. Measures of metabolism and complexity in the brain of patients with disorders of consciousness. NeuroImage Clin. 14, 354–362. doi:10.1016/j.nicl.2017.02.002*

Two patients had discordant results: one was MCS- according to a visual pursuit observed on one occasion before the FDG-PET, which indeed revealed preserved metabolism of the left internal fronto-parietal network. However, best PCI was 0.22, not compatible with consciousness. It is worth mentioning that the day of TMS–EEG, the patient was behaviourally unresponsive. Moreover, PCI could only be computed from one out of four spots, due to the presence of a cerebrospinal fluid shunt and severe brain injuries. The second patient was behaviourally unresponsive, and still was five years after the brain injury occurred. Despite stimulating over the right hemisphere, which had shown preserved metabolism, PCI were all below 0.31.
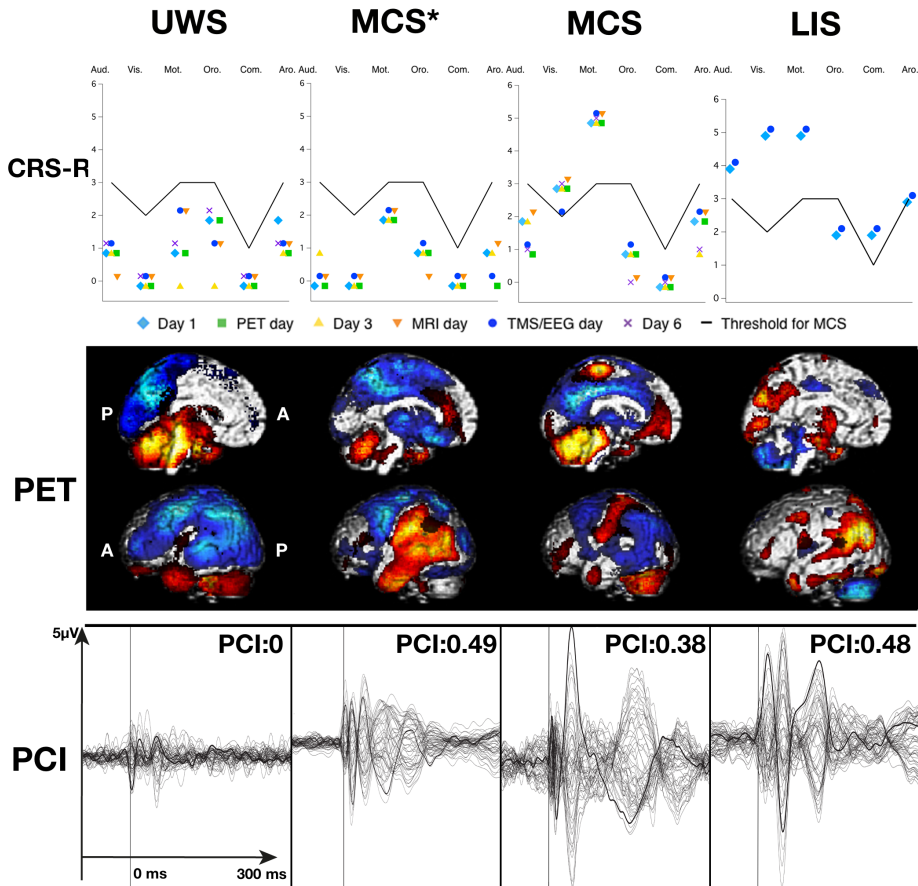
Figure 5 - Typical behavioural, metabolic, and PCI findings in UWS, MCS, MCS*, and LIS patients

In this figure, the top row illustrates the behavioural scores of each subscale for all the assessments with the black line representing the threshold for MCS. The second row illustrates the areas on the left hemisphere in which FDG-PET finds significantly impaired (blue) or preserved (red) metabolism compared to 39 controls ($p < .05$). The third row illustrates the TMS evoked potential traces at the cortical level, which are later used to compute PCI (reported in the top right corner). Note that while behaviourally UWS and MCS* are alike, MCS* TEPs and FDG-PET patterns are more similar to those observed in MCS and LIS patients. Aud. Vis. Mot. Oro. Com. Aro. are the six CRS-R subscales. A: Anterior. P: Posterior. *Adapted from **Bodart, O.,** Gosseries, O., Wannez, S., Thibaut, A., Annen, J., Boly, M., Rosanova, M., Casali, A.G., Casarotto, S., Tononi, G., Massimini, M., Laureys, S., 2017. Measures of metabolism and complexity in the brain of patients with disorders of consciousness. NeuroImage Clin. 14, 354–362. doi:10.1016/j.nicl.2017.02.002*

Overall, FDG-PET and TMS–EEG were congruent in 22 out of 24 patients, a
rate of agreement better than with behavioural data (Table 4).

Test(s) in favour of …	CRS-R	PET	PCI	All
Consciousness	15 (62.5%)	20 (83,3%)	18 (75%)	14 (58.3%)
Unconsciousness	9 (37.5%)	4 (16.7%)	6 (25%)	4 (16.7%)
Total	24 (100%)	24 (100%)	24 (100%)	18 (75%)
	CRS-R PET	CRS-R PCI	PET PCI	
Consciousness	15 (62.5%)	14 (58.3%)	18 (75%)	
Unconsciousness	4 (16.7%)	5 (20.8%)	4 (16.7%)	
Total	19 (79.2%)	19 (79.2%)	22 (91.7%)	

Table 4 - Concordance of CRS-R, PET, and PCI results
This table summarizes the number of cases in which each technique, or the combination of all of
them, provided results compatible with consciousness or not (top part of the table). On the bottom
part, the number of cases where any couple of two techniques shared results concurring with
consciousness or unconsciousness is reported. The association of FDG-PET and PCI is the one
with the most concordance with 22 out of 24 samples concurring. *Adapted from **__Bodart, O.,__**
*Gosseries, O., Wannez, S., Thibaut, A., Annen, J., Boly, M., Rosanova, M., Casali, A.G., Casarotto,
S., Tononi, G., Massimini, M., Laureys, S., 2017. Measures of metabolism and complexity in the
brain of patients with disorders of consciousness. NeuroImage Clin. 14, 354–362.
doi:10.1016/j.nicl.2017.02.002.*

1.4. MCS* patients share metabolic and electrophysiological features

So far, 18 patients (LIS, EMCS, MCS, and UWS) had congruent TMS–EEG and FDG-PET results, and two have shown discordance. The remaining four patients were behaviourally unambiguously UWS. However, their best PCI were above 0.31, hence in the distribution of consciousness. In these patients, we detected metabolic patterns compatible with consciousness, with the preservation of either the left, right or bilateral fronto-parietal network. We can moreover safely infer the presence of covert cognition in these non-behavioural MCS patients (MCS*) as one of them was able to wilfully modulate his brain activity in the active fMRI task (the other three were sedated and did not undertake the test). At one year, the only patient who improved in our study had these metabolic and electrophysiological features. All these findings suggest that in these patients, the brain had kept the ability to sustain consciousness, but it did not translate behaviourally. To reflect their specific neurophysiological features and prognosis, the behavioural diagnosis of UWS was thus changed to MCS* for this group.

2. TMS–EEG and DTI

2.1. Combining TMS–EEG and DTI

Patients who suffered from brain injuries severe enough to cause a prolonged DOC have altered brain structure. We have seen that global FA was a good marker of structural integrity in these patients (47,157,158). It could thus be used as a surrogate marker of residual structural connectivity. Furthermore, TMS–EEG assesses causal interactions between distant areas induced by the TMS perturbation (112). Accordingly, it is a good marker of the perturbational effective connectivity. Our objective in this study is to explore the relation between effective connectivity and increasing level of damage in the underlying structure.

2.2. Methodology

To study the potential relationship between structural integrity and effective connectivity, we included 39 patients more than four weeks after a brain injury that lead to a coma, and 14 healthy subjects. Behavioural diagnosis was established after repeated assessments with the CRS-R, including the day of MRI and TMS–EEG. Sixteen patients were excluded from further analysis as PCI, FA, or both could not be computed, leaving us with 23 patients (13 males, 11 TBI, median time since injury 33 weeks (5-1371), mean age 37 ± 15 years) and 14 healthy subjects (five males, mean age 25 ± 4 years old) for a total of 37 participants. Healthy subjects and patients were scanned in a 3T MRI scanner (Allegra, Siemens, DWI: 64 non-collinear directions using a b-value = 1000 s/mm^2, two b=0, TR= 5700ms, TE= 87ms, matrix size = 128x128, 45 slices, slice thickness = 3 mm, gap= 0.3 mm; and T1 3D MPRAGE). To avoid excessive movement artefacts, 15 patients required light sedation during the MRI. DWI was analysed as in (120,159), using FSL diffusion toolbox 2.0 (FSL 5.0, FMRIBs Software Library, http://www.fmrib.ox.ac.uk/fsl, Oxford, UK) to

correct eddy current distortion (160), the brain extraction tool (161) and masking to isolate the white matter, and weighted linear least squares fitted to the log-transformed data to estimate the FA. Volumes with vibration artefacts were visually identified (diffusion gradient in the x direction greater than 0.8) and discarded (162). After the tensor eigenvalue maps were computed, we used FSL maths (163) to average the FA values of the white matter mask's voxels to get the global FA (158). We performed TMS–EEG as described in details in chapter II, and kept the best PCI obtained (PCI max) for further analyses. Expecting a linear positive correlation between FA and PCI in this population, we tested it using one-tailed Pearson's correlation. We then verified our hypothesis that structural integrity, the global FA, could predict effective connectivity, the PCI, using a linear regression model. Gender and age were added as co-predictors in a hierarchical entry design. We verified our results on the patients' subpopulation, excluding that the model was only driven by healthy subjects, adding this time the CRS-R total score and the time since injury as additional co-predictors. All the statistical analyses were performed in SPSS 20 (IBM Corp., Armonk, NY, USA).

2.3. Structural integrity support TMS–EEG complexity

To verify our hypothesis that structural integrity support TEP complexity, we computed the global FA and the PCI in a behaviourally well-defined DOC population and in healthy subjects. We then tested the relationship of these two parameters in a linear correlation and in a linear regression model (Figure 6). We found a significant correlation between PCI and FA ($r = .86$, $p<.0001$). Global FA could predict 74% of PCI max variance in the whole group (F(1,35)=100.45 $p<.001$). Taking only the patients into account, we still found a significant correlation between structural integrity and TEPs complexity ($r = .75$, $p<.0001$), and according to our regression model, global FA could still predict 56% of PCI

max variance (F(1,21)=27.17 $p<.001$). Neither age, gender, total CRS-R, nor time since injury did have any significant effect on the models (Table 5).

Whole group	B	SE B	ß
Step 1			
Constant	-0.66	0.11	
FA	3.43	0.34	.86*
Step 2			
Constant	-0.6	0.13	
FA	3.35	0.36	.84*
Age	0	0	-.11 [N.S.]
Gender	-.01	0.02	-.05 [N.S.]

Note: R^2 for step 1 =.74. *$p<.001$. ΔR^2 for step 2 = .01 (p=.48)

Patients	B	SE B	ß
Step 1			
Constant	-0.49	0.17	
FA	2.85	0.55	.75*
Step 2			
Constant	-0.63	0.5	
FA	3.45	0.91	.91*
Age	0	0	-.08 [N.S.]
CRS-R total score	0	0	-.18 [N.S.]
Time since injury	0	0	.14 [N.S.]
Gender	0	0.03	-.02 [N.S.]

Note: R^2 for step 1=.56. ΔR^2 for step 2 = .03 (p=.84) *$p<0.001$. N.S. : not significant

Table 5 - Regression models

This table reports the unstandardized coefficients B and their standard error for the constant and the predictor, as well as the standardized coefficient ß for the predictor. R^2 and significance is also reported for each model. The first model applies to the whole sample, including patients and controls. The second model applies to patients only. *Adapted from **Bodart, O.,** Amico E., Gomez F., Casali A.G., Wannez S., Heine L., Thibaut A., Annen J., Boly M., Casarotto S., Rosanova M., Massimini M., Laureys S., Gosseries O., Global structural and effective connectivity in patients with disorders of consciousness. Under review.*
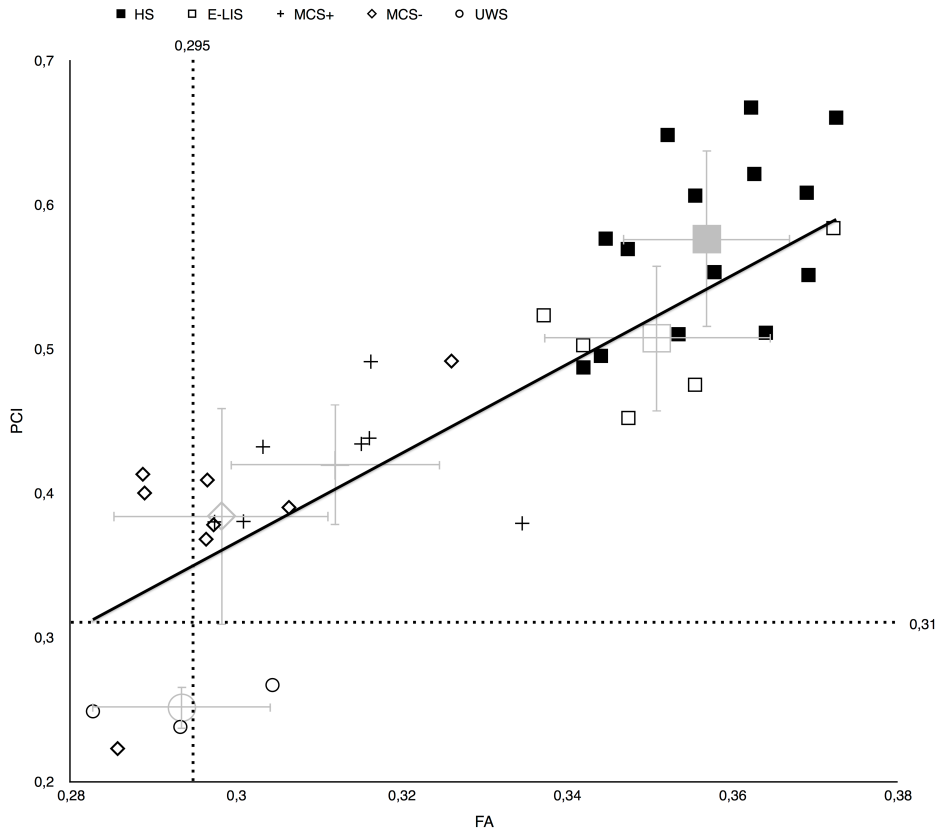
Figure 6 - PCI over global FA in subjects and subgroup

This scatter plot illustrates the positive linear relationship between FA and PCI in patients and controls ($r = .86$ $p < .0001$, $R^2 = .74$). Subjects are plotted with different symbols according to their diagnosis: circle for UWS, diamond-shape for MCS-, cross for MCS+, empty square for EMCS and LIS (E-LIS), and black square for healthy subjects (HS). The mean and standard deviation for each subgroup are plotted in light grey. Dot lines mark the threshold for PCI (horizontal, 0.31) and the global FA (vertical, 0.295). *Adapted from **Bodart, O.,** Amico E., Gomez F., Casali A.G., Wannez S., Heine L., Thibaut A., Annen J., Boly M., Casarotto S., Rosanova M., Massimini M., Laureys S., Gosseries O., Global structural and effective connectivity in patients with disorders of consciousness. Under review.*

Overall, we found that structural integrity, represented by the global FA, is positively and linearly correlated with brain's ability to sustain complex responses to a stimulation, as reflected by the PCI, in a population of brain injured patients and healthy subjects.

3. Discussion

The first objective of this thesis was to demonstrate the validity of TMS–EEG, and its derived index the PCI, as a diagnostic tool for patients with DOC. In our first study, we validate TMS–EEG results with another validated neuroimaging technique, FDG-PET (164). Indeed, PCI and FDG-PET find matching results compatible with the presence or absence of consciousness in 22 out of our 24 patients cohort. This includes four patients, LIS and EMCS, 10 MCS, and eight UWS, four of which have PCI max above 0.31 and preserved metabolism in the fronto-parietal network. The rate of agreement between PCI and FDG-PET is even better than the association of any of those techniques with the current diagnostic gold standard, the CRS-R (Table 4). The PCI threshold to distinguish between unconscious and conscious conditions (0.31) is also validated. It was initially found in a limited number of patients and healthy subjects with varying level of consciousness (151). It is confirmed as the optimal cut-off (with 100% accuracy) between conscious and unconscious conditions (respectively wakefulness, REM sleep, Ketamine anaesthesia, LIS, EMCS, and patients with stroke on the one hand, and NREM sleep, Midazolam, Xenon, and Propofol anaesthesia on the other hand) in a benchmark population of 150 subjects including some with brain injuries. Applied to a population of 38 MCS and 43 UWS, this PCI threshold has a 94.7% sensitivity to detect MCS patients, and also identifies 9 UWS patients with high PCI (155). TMS–EEG, via the PCI, is thus validated internally and against FDG-PET as a well founded diagnostic tool to discriminate conscious from unconscious conditions, thanks to our results.

Such a tool is indeed absolutely necessary in clinical practice. The frontier between UWS and MCS patients is blurred. Behaviourally, despite using standardized scales such as the CRS-R (27,29), the rate of misdiagnosis is prohibitive (around 30-40%) (14,16,17,165), partly due to the high rate of

fluctuation over short periods of time (36,166). Behavioural evaluations are moreover inherently dependent upon the patient's capacity to express his/her consciousness. This ability may be hindered by sensory and motor limitations, aphasia, fatigue, pain, … Currently, the tools to overcome these limitations are based on active paradigms. In fMRI, wilful activation of the supplementary motor area and of the parahippocampal gyrus upon command (respectively when asked to "imagine playing tennis" and "imaging navigating around the house") can be detected in healthy subjects (56). Using the same paradigm or variations in commands, the ability to follow simple commands can be found in patients with DOC (57,58,62,108,167–169). This technology is not easily transferable to clinical practice: fMRI is not available outside hospitals, is expensive, requires heavy logistics to transport the –sometimes critically ill – patients, is not accessible to patients with metallic implants, or to those who move too much and would require sedation. Active paradigms are thus developed using EEG, a portable, widely accessible and relatively cheap technology without contraindications. By using power spectrum and spectral analysis, command following can be detected in this challenging population (79,80,170–173). The issue remains that these technologies rely on data that need to be analysed and interpreted. The statistical methods to validate a result as compatible with a response to command are variable, and this can lead to different interpretations of the same dataset (e.g. (80) vs (172)). Assessing the accuracy of these neuroimaging techniques, hence their sensitivity and specificity, is also challenging (174,175). Indeed, patients with DOC cannot, by definition, communicate their consciousness consistently, therefore establishing true positive and true negative is complex. It can be approached by first validating the methods on healthy subjects, or brain injured subjects that can report their consciousness; this is exactly what has been done for the PCI (155). Poor sensitivity in this population would likely raise the risk of false negative in DOC patients. In active fMRI, the sensitivity is great in healthy subjects, but most behaviourally MCS patients were unable to respond to command using this

paradigm (58). It might be due to an absence of capacity to respond to command, also encountered behaviourally (MCS- patients), or because these patients are able to respond to simple oral commands, but the cognitive charge of active paradigms is too high and hinder their capacity to react appropriately. Sensitivity is poor in active EEG paradigms, even in healthy subjects, with sufficiently accurate classification in at most 50 to 75% of the participants (80,173,176). Specificity is another potential issue, especially when both fMRI and EEG can detect activity where there is none, given propitious statistics (177,178). Given its block design and appropriate correction of the statistical threshold, active fMRI has a good specificity. EEG on the other hand depends greatly on the statistical method used. Nonetheless, both techniques can see their specificity lowered, and the rate of false positive increased, by the numerous confounds they are subject to (motion artefacts, statistical noise, environmental artefacts, …). These issues may be solved by confronting the data to other evidences, especially by comparing the results of different techniques. Combining rest and sleep EEG, active fMRI, and FDG-PET in 44 patients, a relation between spared brain metabolism, preserved sleep architecture and EEG background, and the ability to use mental imagery to respond to command was noted (179). Active fMRI was combined to FDG-PET and compared to behavioural diagnosis in 41 UWS and 81 MCS patients. In this study, FDG-PET had a better sensitivity to MCS than fMRI (93% vs 45%, respectively), and was better at predicting the 12 months outcome of these patients, especially when it was poor (71).

Moreover, among the 41 UWS patients, 13 had neuroimaging results compatible with consciousness (12 identified by FDG-PET and three with active fMRI). The majority of this subpopulation improved at the 12 months follow-up. By coupling active fMRI and active EEG paradigms in six patients, some have detected one UWS able to respond to command at both occasions. Two more patients could only express their consciousness through fMRI, and one using

EEG (180). This suggests that these patients have preserved cognition not accessible through behavioural testing. Such patients are detected since the beginning of active paradigms (57,58,80,168,169), more frequently in patients with TBI (171). Given their ability to use technology to respond to command and their better outcome than expected for this population (84), considering these patients unconscious is incorrect, and the term MCS* was proposed to describe this specific population (181). The mechanism of this cognitive motor dissociation is only being approached (182). The ability to respond to command through mental imagery, but not behaviourally, relies upon selective disruption of tracts between the thalamus and the primary motor cortex, leading to a lack of excitatory coupling between these two regions (183).

Our second objective is to investigate if the unresponsive patients with high PCI that might correspond to MCS*, detected by TMS–EEG, are the sign of a lack of specificity of the technique (155). In our study, by combining TMS–EEG with FDG-PET, we detect four UWS patients that have preserved brain metabolism and high PCI. One of these even has the ability to use mental imagery in fMRI (164). Our multimodal study thus demonstrates that high PCI in UWS patients is not a lack of specificity of the technique, but rather the proof that it is sensitive enough to detect MCS* patients. This excellent accuracy of TMS–EEG and PCI in discriminating between conscious and unconscious conditions is not only validated on a large population of healthy and brain injured subjects where consciousness could be truly assessed (155). Indeed, we here cross-validated these results using FDG-PET imaging, and demonstrated the better outcome of MCS* patients detected by TMS–EEG. To conclude, by combining TMS–EEG, FDG-PET, CRS-R, and outcome assessment in patients with DOC, we have demonstrated that PCI is a valid clinical diagnostic tool, able to detect cognitive motor dissociation. As we discussed previously, having accurate diagnostic tool is vital for the clinical management of DOC patients. Indeed, misdiagnosis may interfere with pain management (96,98,100,184), may lead to false hopes or at

the contrary loss of belief in families and caregivers, influencing their decisions (82,185–187), especially regarding end-of-life issues (103), or even access into rehabilitation centres (188).

There are some limitations to these results, and to the conclusion that can be drawn from them. The first is that TMS–EEG and FDG-PET do not agree on the diagnosis in 100% of the patients. We did not expect a perfect agreement between these techniques however, as they both reflects very different aspects of brain physiology, at different temporal and spatial resolution. Indeed, TMS–EEG evaluates the very dynamic complexity of neuronal interactions over milliseconds, after a perturbation. This perturbation is applied locally, and a single site is stimulated at a time, although its effects propagate to most cortical areas. Each of these areas responds with their own natural frequency, as discussed above. FDG-PET on the other hand evaluates the patterns of impaired or preserved brain metabolism over several minutes, at rest. It samples the whole brain at once. As we cannot probe all the brain with TMS–EEG, especially in patients with brain injuries, we cannot infer the exact relation between TMS–EEG on one site and its metabolism. This would be an interesting subject for a future study. In two subjects, the FDG-PET is compatible with consciousness while the PCI max is in the distribution of unconsciousness. The first patient is behaviourally UWS, even after a follow-up of five years. Despite the preservation of the whole right hemisphere metabolism, the PCI from multiple sessions on this side are in the range of unconsciousness. One could infer that while preserved metabolism is necessary, it might not be sufficient for the emergence of complex neurophysiological responses to stimulations. Due to a lack of connectivity, to an altered balance between excitatory and inhibitory drive, or to sleep-like neuronal bistability caused by increased potassium conductance, the active neurons may be unable to engage in complex network interactions. The second patient has a global behavioural diagnosis of MCS-, thanks to the presence of visual pursuit on one occasion, the day of the PET-CT.

On all the other assessments of the week, including the day of TMS–EEG, the patient was UWS. Due to the presence of a cerebrospinal fluid shunt and a large frontal lesion, PCI has only been computed from one session, and was in the range of unconsciousness. The discrepancy between PCI and FDG-PET can thus be explained either by the technical inability of TMS–EEG to elicit a correct response on the selected area, or by a fluctuation of consciousness the day of this test. The second limitation to our results is our limited sample size, especially in UWS patients. Indeed, in our 24 subjects, nine are behaviourally UWS, but five were reconsidered as MCS*. Having this few number of true UWS patients limits the generalization of our results, especially given the wide possible variability in aetiology, time since onset, pattern of lesions, and the presence of low versus no PCI. We cannot assure that all the different conditions are identified. This poor representation of unconscious patients is also due to a high proportion of MCS* in our population, greater than in the literature so far, involving all the UWS patients with a TBI aetiology. However, the 24 subjects were included as they were prospectively assessed for clinical reasons, meaning that they represent the population of patients one could encounter in a clinical setting. Moreover, despite our high proportion of MCS* among the behaviourally UWS patients, we find the same preponderance of TBI aetiology (five TBI versus one non TBI). A further imperfection to the generalization of our study is the limited access to both TMS–EEG and FDG-PET. Both require specific expertise, and are not readily accessible in the clinical settings. FDG-PET is expensive, ionizing, and requires to transport the patient to the appropriate unit. TMS–EEG can be tough to perform in uncooperative patients, the analyses take time, and a lot of artefacts can hinder obtaining the PCI. This weak point can be improved by prioritizing the access to patients most likely to benefit from the results, such as TBI patients in whom an end-of-life decision has soon to be taken.

The third objective of this thesis is to explore the relationship between effective and structural connectivity in brain injured patients. Using PCI and the global FA as surrogate markers, we find a strong positive linear correlation between the brain structural integrity and its ability to sustain complex long range interactions. Global FA explains 74% of PCI variability in our population, and 56% if accounting only for brain-injured patients. At the best of our knowledge, there is no available scientific report about the relationship between these types of connectivity in patients with DOC. Nonetheless, the importance of structural connectivity for the emergence of consciousness has been explored. The structural integrity of key structures, such as the DMN and the anterior forebrain mesocircuit (a network encompassing thalamus, globus pallidus, putamen, and caudate nucleus) is impaired in patients with DOC (189–192). More precisely, the degree of connectivity disruption between the thalamus and cortical areas (192), especially the posterior cingulate cortex/precuneus (189), or between the precuneus and the anterior forebrain mesocircuit (190), correlates with the alteration of consciousness, and can be used to classify groups of patients as unresponsive or minimally conscious (191,192). Global marker of structural damage, such as the mean diffusivity of the subcortical white matter, can differentiate UWS from MCS group of patients (47). The strength of structural connectivity within the DMN correlates with its functional connectivity in healthy subjects (193). There is indeed a positive relationship between structural integrity and metabolic rates in the DMN of DOC, which is significantly larger in the thalamo-parietal tracts if the patients emerge from the MCS (194). In patients with DOC, consciousness is correlated with the degree of functional connectivity, metabolic rates and grey matter volumes in the DMN. Negative connectivity within this network is only found in EMCS and healthy subjects (53). In one UWS patient recovering signs of consciousness, the functional connectivity in the DMN increased between the first and the second scan, while the global structural integrity remained near normal (195). On the other hand, recovery of signs of consciousness was paralleled with markers of axonal

regrowth in DTI in one patient (196). These multimodal studies emphasize the role of combining modalities: the same functional, metabolic, and electrophysiological findings can be found in patients with very different level of consciousness, differing only by the amount of structural damage (153).

Effective connectivity is also impaired in patients with DOC, as seen using EEG (76,197), fMRI (198), and TMS–EEG (148,150,151,155,164). Combining structural and effective connectivity can lead to better understanding in normal or pathological neurophysiology: new tracts in the language processing networks are discovered thanks to a strong effective connectivity between distant areas (199). Impaired effective connectivity between the thalamus and cortical areas such as the insula and the superior frontal gyrus is found in patients with schizophrenia, despite preserved functional and structural connectivity in these structures (200). However, we are the first to combine these techniques in patients with DOC, revealing a strong linear correlation between structural and effective connectivity. In epileptic patients, the correlation is only modest (201), and, by combining fMRI and DTI in anaesthetized monkeys, correlation between functional and structural connectivity in negatively associated with the level of consciousness (202). We could have expected a lack of correlation in our population. Indeed, while the structure remains the same, PCI varies according to physiological or pharmacological loss of consciousness in healthy subjects (141,142,146,147). Moreover, in DOC patients, preserved structure can be non-functional (203,204), due to abnormal hyperpolarization (sleep-like bistability (205)), or to neurotransmitters depletion (206). This explains why there is not a perfect correlation between structure and effective connections. Still, the relationship between the PCI and the global FA is strong in our population. In our opinion, this has several causes. First, contrary to the studies that looked at the structural-functional or structural-effective correlations, we did so at the global level, and not at the local level. Thus, while there is a global correlation between structure and function, the relationship may be different

within specific networks, and may vary according to the level of consciousness. Secondly, we excluded acute cases, where the impact of dysfunctional but structurally preserved tracts is the greatest. Indeed, wallerian degeneration takes time, and the structural damage can be delayed compared to the loss of function. Third, we included a range of DOC, from UWS to EMCS and LIS, and healthy subjects. The local variability in the correlation between structure and effective connectivity at one level of consciousness might be superseded by the global relation that we demonstrated. Finally, contrary to the study in anaesthetised monkeys that demonstrated a stronger correlation between structure and function as the level of consciousness decreases (202), we here looked at the relation between structural and effective, not functional, connectivity. As this had never been done before, we did not know how causal interactions would behave when the level of structural damage increased, and the level of consciousness decreased. Studying in detail this relationship further our understanding of the neural correlates of consciousness. The importance of networks in DOC has been underlined decades ago, and connectivity studies have followed. However, there is no integrative study of structural, functional, and effective connectivity to date. Our study is a step forward in the process of identifying the sufficient structure for the emergence of consciousness. Furthermore, by combining validated techniques such as the PCI with global FA, a threshold of structural integrity could be identified, disentangling between UWS and MCS patients. At the group level, this is already possible using the mean diffusivity (47), or global DTI histograms (157). In our study, a global FA threshold of 0.295 has a good sensitivity (91%) but only moderate specificity (67%) at the single patient level to distinguish between UWS and – at least minimally – conscious conditions (Figure 6). This might be due to the heterogeneity in the extent of brain damage and its aetiology.

These results suffer some limitations. Once again, our sample is not large, as several patients had to be dropped out due to artefacted TMS–EEG and/or DTI.

More problematic is the low number of UWS patients, causing an imbalance between the unconscious and conscious conditions. The fact that several aetiologies are included might also be an issue, as the mechanisms of structural damage and loss of function differ vastly between TBI and anoxic brain injuries, for example. We did not meet all the possible combinations of conditions. Larger sample would allow subgroup analysis based on the aetiology, and identifying specific key structure or structural damage to explain the patient's current level of consciousness. The last limitation is that we approached this problem with global metrics, and not local ones. This prevents identifying specific structures. A local approach, combining structural connectome with an effective one, is currently being tested in healthy subjects during increasing level of anaesthesia. Once validated, we could test again this DOC population.

To conclude, we demonstrated that there is a strong linear correlation between structural integrity and the brain's ability to sustain long-range complex interaction, from UWS patients to healthy subjects, independently of the aetiology or the time since onset.

4. Perspectives

In this thesis, we demonstrated the diagnostic ability of PCI. We also underlined the major importance of a correct identification of consciousness in the management of DOC patients. For these reasons, we feel that TMS–EEG should take a greater place in diagnostic algorithms (Figure 7). To be clinically useful though, TMS–EEG should be more accessible, both in term of time and financial costs and in expertise needed to get to the PCI. Indeed, so far the equipment is costly, and is of limited access. Getting enough data in these tough non-collaborative patients takes hours, even for seasoned researchers. The analyses are performed off line, and require a lot of data cleaning before applying the statistical and mathematical tools needed to compute the PCI. The equipment

costs are already coming down, as more companies are embracing the technology. Exposing its potential will lead to a more widespread use, which could also lower the entry fee to TMS–EEG equipment.

Unidentified disorder of consciousness

Behavioural assessment: CRS-R

UWS   MCS, EMCS, LIS, ...

FDG-PET

Severely impaired metabolism
Confirms UWS, poor prognosis

Partially preserved metabolism
Potential for consciousness

TMS-EEG

Potential for consciousness unexpressed at the time of exam (fluctuation of vigilance?)   ← PCI < 0.31   PCI > 0.31 → MCS*
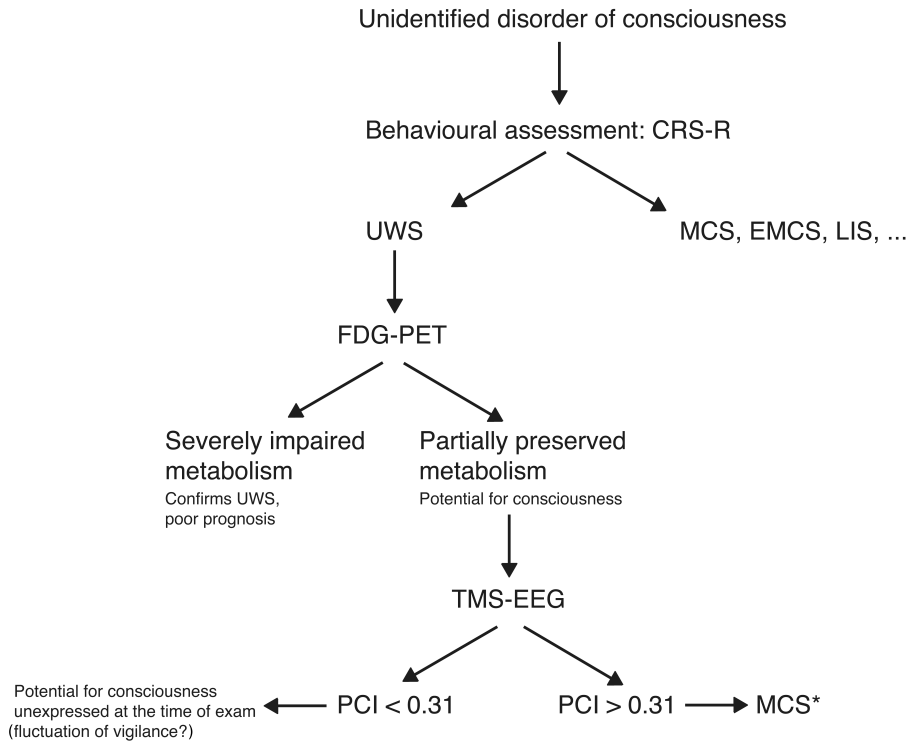
Figure 7 – Potential diagnostic algorithm
Patients with unidentified DOC should be first repeatedly assessed using validated standardized behavioural scale, such as the CRS-R. If no signs of consciousness can be detected, potential for consciousness can be identified using FDG-PET. In case this exam shows at least partial preservation of the fronto-parietal network metabolism, TMS-EEG could be used to detect the presence of covert consciousness. This would be the case if the PCI were above the distribution found in unconsciousness (>0.31). MCS* patients could then be assessed using active paradigm, aiming at establishing communication. *Adapted from **Bodart, O.,** Gosseries, O., Wannez, S., Thibaut, A., Annen, J., Boly, M., Rosanova, M., Casali, A.G., Casarotto, S., Tononi, G., Massimini, M., Laureys, S., 2017. Measures of metabolism and complexity in the brain of patients with disorders of consciousness. NeuroImage Clin. 14, 354–362. doi:10.1016/j.nicl.2017.02.002.*

In the meantime, careful selection of patients can circumvent the issue of cost and accessibility. Indeed, some patients are more susceptible to benefit from TMS–EEG assessment than others. This includes UWS patients after a TBI, who

are more likely to be MCS* than after anoxic brain injuries. Patients in whom an accurate diagnosis would not change much in their management should not be assessed with TMS–EEG, as opposed to those, for example, where an end-of-life decision has to be taken. As more and more scientists around the world are using this technology, the global level of expertise is rapidly growing. But to ensure that the results are reproducible between teams, the acquisition and analyses stages should be standardized, optimally using different brands of equipment. With the exponential progresses in the field of robotics, one could imagine the future development of a robotic arm to support the – heavy – TMS coil. By coupling it with the neuronavigation system, it could stay on target, following the micro- (or macro) movements of the patient's head. Analyses can also be simplified. A new version of the PCI computing software is already being developed in this purpose. By computing this index at the scalp level, instead of going through source modelling to get the signal at the cortical level, a lot of analyses steps could be avoided. Moreover, source modelling bears the potential to introduce errors in the algorithm, as it has to deal with sometimes severely damaged brains. Ultimately, TMS–EEG could be used as other clinical evoked potentials, simple and robust, and give immediate results at the end of the recording.

MCS* detected this way could then benefit from more extensive assessments, including active paradigms, and rehabilitation programs would focus on establishing means of communication. TMS–EEG could also be used to monitor the spontaneous recovery of these patients, but also the effect of diagnosis modulating therapeutic interventions.

In this thesis, using a global approach, we also demonstrated the positive correlation between structural integrity and the brain's ability to sustain long-range complex interactions. This relationship between structural and effective connectivity should be further studied at the local level. We could first do so in

healthy subjects, where the structure is intact, looking at the structure-effective correlation in key networks, such as the DMN and the mesocircuit, or in the whole brain, in varying levels of consciousness (in sleep and general anaesthesia). We would obtain a map of the millisecond dynamics of brain activity, of loss of consciousness and recovery of consciousness, on a detailed brain structure. This would allow identifying key structures and networks and shed light to their behaviours during variations of the level of consciousness.

Our understanding of consciousness would be even more extensive by combining other neuroimaging techniques, such as fMRI for functional connectivity, and FDG-PET for metabolism. Analysing local effective and functional connectivity, regional brain metabolism, on a fixed structure, during conscious and unconscious conditions, could provide a multimodal connectome of the brain, and overall a much better understanding of the brain physiology. Indeed, by combining the TMS–EEG millisecond time scale resolution, the fMRI and FDG-PET spatial precision, on the DTI anatomical structure, an accurate brain model could be generated.

Such a tool, along with the key structures and networks identified in healthy subjects, could then be used in patients with DOC. Their brain physiology could be compared on multiple levels to other patients and to healthy subjects, both in conscious and unconscious condition, providing valuable information for diagnosis, prognosis, or even therapeutic purposes.

Chapter IV

Conclusion

In the challenging field of DOC, better diagnostic tools are always needed, especially given the expanding nosology and the impact misdiagnosis can have on the management of these patients. TMS–EEG, through PCI, was suggested as a potential diagnostic tool. Its ability to disentangle unconscious from conscious conditions has been demonstrated. Here, we provide further validation of this technique by comparing its results against FDG-PET, a neuroimaging technique supported by a vast literature. By combining these technologies, we support our hypothesis that high PCI in behaviourally UWS patients is in fact a sign of covert consciousness. A hypothesis further corroborated by the ability of one of these patients to follow simple command in fMRI's active paradigm. This study thus demonstrates that PCI is a specific diagnostic tool, accurate at the single subject level, and that can be used for monitoring the recovery of consciousness. This is the first study to validate TMS–EEG, and PCI, with another neuroimaging technique, and it confirms the added value of multimodal assessments of patients with DOC.

These patients also exacerbate the scientific interrogations about the neural correlates of consciousness. While important networks, such as the DMN, the anterior forebrain mesocircuit, and the fronto-parietal network, have been studied, the relationship between structural and effective connectivity, both within these systems and at the global level, remained unclear. Here we provide the first demonstration of a positive correlation between the global structural integrity and effective connectivity, in DOC and healthy subjects. This relation at the global level might unveil in future studies a threshold under which structure is too damaged to sustain complex long-range interactions. These

findings can lead to more work on the local level, studying the correlation between a structural and an effective connectome. Doing so could shed light upon key structural nodes supporting the effective networks allowing the emergence of consciousness.

Globally, this thesis focused on multimodal approaches of patients with DOC, using TMS–EEG along with metabolic and structural neuroimaging techniques. This multimodal method proved to be a great tool, both for clinical studies (such as the validation of PCI's ability to detect MCS* patients) and in research (by combining perturbational effective connectivity with other connectivities in the study of the neural correlates of consciousness).

While still evolving, TMS–EEG is a fantastic technology that, I am sure, will continue to provide amazing scientific and clinical results.

Appendix E

Paper V

Bodart, O., Amico E., Gomez F., Casali A.G., Wannez S., Heine L., Thibaut A., Annen J., Boly M., Casarotto S., Rosanova M., Massimini M., Laureys S., Gosseries O.

Global structural and effective connectivity in patients with disorders of consciousness.

Under review.

Global structural and effective connectivity in patients with disorders of consciousness.

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Conclusion: We here demonstrated that structure supports effective connectivity even in brain-injured patients. Increased structural damage level decreases effective connectivity, which prevents the emergence of consciousness. This may be the first step in unveiling the role of specific structural-effective networks in the emergence and loss of consciousness.

Keywords

Abbreviations

Coma recovery scale-revised – CRS-R

Diffusion weighted imaging – DWI

Default mode network – DMN

Emergence from the minimally conscious state – EMCS

Fractional anisotropy – FA

Functional MRI – fMRI

Locked-in syndrome – LIS

Minimally conscious state – MCS

Perturbational complexity index – PCI

Transcranial magnetic stimulation – TMS

Unresponsive wakefulness syndrome – UWS

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Abstract

Background: Previous studies have separately reported impaired functional, structural, and effective connectivity in patients with disorders of consciousness (DOC). Little is known however about how these different kinds of connectivity relate and support each other.

Objective: We aimed at testing that structural connectivity supports effective connectivity, and confirm that they are both impaired in patients with DOC.

Methods: We assessed 23 patients with severe brain injury more than 4 weeks post-onset, leading to DOC or locked-in syndrome, and 14 healthy subjects. We calculated the perturbational complexity index (PCI) using repeated single pulse transcranial magnetic stimulation coupled with high density electroencephalography, and used it as a surrogate of effective connectivity. For structural connectivity, we computed the global fractional anisotropy (FA) using diffusion weighted imaging. We used linear regression modelling to test our hypothesis.

Results: Global FA and PCI are lower in DOC patients than in healthy subjects ($0.31 < 0.36$, $p < .001$, and $0.4 < 0.58$, $p < .001$, respectively). Global FA can predict 74% of PCI variance in the whole sample and 56% in the patients' group.

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Introduction

After a severe brain injury leading to a coma, patients can present transient or permanent disorders of consciousness (DOC), such as the unresponsive wakefulness syndrome (UWS), which is characterized by the recovery of wakefulness without awareness [1]. The minimally conscious state shows fluctuating signs of awareness such as visual tracking, localization to noxious stimulation, or contextual emotional responses [2]. MCS can be divided into MCS- and MCS+ depending respectively on the absence or presence of clear evidence of language function, such as the ability to follow simple commands [3,4]). Patients who recover functional communication or functional use of objects have emerged from the MCS (EMCS [5]). On the other hand, some patients are thought to have a DOC, as they are unable to move or speak, but they in fact remain fully conscious and suffer from locked-in syndrome (LIS) [6,7].

The differential diagnosis of these conditions, which remains challenging [8], is currently mainly behavioural, based on standardized scales such as the Coma Recovery Scale-Revised (CRS-R [5]). However, the underlying physiopathology remains poorly understood [9]. In the attempts to identify the neural correlates of consciousness, functional, structural, and more recently effective connectivity patterns and impairments have been studied in patients with DOC. Briefly, structural connectivity reflects the anatomical connections between neurons, which can for example be assessed using diffusion weighted imaging (DWI). Functional connectivity is a statistical measure of correlation between neuronal activities that has been tremendously used with functional MRI (fMRI) but also with EEG. Effective connectivity is defined as the causal link between neurophysiological events [10], which can be measured with transcranial magnetic stimulation coupled with high density EEG (TMS-EEG).

Individually, each kind of connectivity has demonstrated altered patterns in DOC.

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Using fMRI, impaired functional connectivity was found at the whole brain level [11–13], between hemispheres [12,14], and in the default mode network (DMN) [15–19] of patients with DOC. The DMN functional connectivity negatively correlated with the level of consciousness, especially for its inter-hemispheric features [20,21], and remained stronger in the precuneus of MCS than in UWS patients [20]. The connectivity in this network has been shown to have prognostic value, as patients who remained UWS or MCS showed hyper connectivity in the DMN as compared to those who emerged from the MCS [22]. Light sedation with Propofol (used to avoid movement artefacts during fMRI acquisitions) seemed, however, to have a limited impact on the DMN connectivity in DOC patients [23].

Structural connectivity can be studied with fractional anisotropy (FA), a measure of the water diffusion anisotropy in the brain, which is restricted by axonal tracts, and thus may also reflect white matter integrity [24]. For example, the FA estimate of white matter damage predicted the functional outcome after a cardiac arrest [25]. Using DWI, structural damage has also been reported in DOC, affecting the tracts connecting the precuneus with the anterior forebrain [26], and the tracts connecting the thalamus to the posterior cingulate, whose integrity was correlated with the residual level of consciousness in DOC [27]. More generally, the thalamo-cortical structural connectivity has been shown to be the most affected in UWS patients, while MCS+ exhibited preserved connections with the temporal lobe and the premotor areas, as compared to MCS- patients [28]. Damaged white matter tracts, assessed by lower mean diffusivity peak, has been used to disentangle UWS from MCS patients [29].

Finally, effective connectivity has been shown, with TMS–EEG, to be globally decreased in DOC [30–33]. Using this approach, the ability of a stimulus to perturb distant area has been studied in UWS and MCS patients, showing it was only partially preserved in the MCS, while it was lost in the UWS population [31,32]. Later, the perturbational complexity index (PCI) was designed to summarize the capacity of the brain to sustain complex interaction after a

perturbation, hence its global effective connectivity [33]. This index was validated on a large population and a threshold value of 0.31 has proven its faculty to disentangle unconscious from –minimally– conscious conditions at the single patient level [34]. We subsequently cross-validated this index against cerebral 18-fluorodeoxyglucose positron emission tomography [35]. Using EEG data and dynamic causal modelling [36] or partial direct coherence [37], effective connectivity was also shown to be altered in UWS patients. Dynamic causal modelling assessment of effective connectivity was also performed in fMRI, demonstrating an altered connectivity of the posterior cingulate within the DMN in UWS more than in UWS patients as compared to healthy controls [38].

Despite all these recent connectivity studies, little is known about how structural connectivity supports functional or effective connectivity in severely brain-injured patients. A better understanding of the relationship between these connectivities may contribute to unravel the neural correlates of consciousness, and of brain physiology in general. Is the TMS–EEG complexity supported by global structural connectivity? Multimodal approaches, studying different kinds of connectivity, are unfortunately scarce in this population. Recently, using TMS–EEG and DWI, we demonstrated a temporary decrease of the structure-function correlation in awake healthy volunteers [39]. A multimodal approach was used to study two DOC patients with functional hemispherectomy [40], showing that the same functional, metabolic, and electrophysiological dysfunction can be underlined by different structural damage (major loss of tracts versus relatively preserved architecture) and lead to different disorders of consciousness (UWS versus MCS). Annen *et al* demonstrated, using PET and DWI, that the relationship between functional and structural connectivity in the DMN of DOC patients remained, and was even stronger in the thalamus of those patients who emerged from the MCS [41]. Multimodal without integrative approaches, using structural and functional

connectivity [42], or using functional connectivity, metabolic and structural data were also reported [43], but lacked any insight into the structure-function relationship.

Our aims in this study are to non-invasively investigate the link between global structural connectivity (assessed with FA) and effective connectivity (assessed with TMS-EEG) in patients with DOC, and confirm that both connectivities are impaired in this population.

Materials and methods

Population

Thirty-nine non-acute patients were assessed using TMS–EEG and DWI in our University Hospital. Twenty-four were included in previous TMS–EEG studies [33,35]. All patients suffered from an acquired brain injury leading to a period of coma, then to various levels of impaired consciousness or LIS. All patients were included more than 4 weeks after the injury, when deemed medically stable. They were excluded if they had prior neurological, neurosurgical or psychiatric disorders, or if they had any contraindication to TMS–EEG and MRI (i.e., active epilepsy, electronic implanted devices, external ventricular drain). We also recruited 14 healthy subjects as control population on the University campus, with similar exclusion criteria. All participants or their legal surrogates gave their informed consent to take part to the study. The Ethics Committee of the Medical School of the University of Liege approved the study.

Behavioural assessments

Behavioural diagnosis was established using the CRS-R repeatedly [5,44]. The CRS-R is a standardized and validated scale to study the residual level of consciousness of brain-injured patients. It consists of six subscales (auditory, motor, visual, oromotor/verbal,

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communication, and arousal), each comprising items of increasing complexity, allowing to detect subtle signs of consciousness (MCS) or of functional communication or object use (EMCS) [2]. MCS was further divided between MCS+ when the patients were able to respond to command, and MCS- when they showed other signs of minimal consciousness [3]. LIS diagnosis was performed prior to the inclusion in this study, and was confirmed by the ability of these patients to communicate using eye-movements [6]. All patients were assessed multiple times by accredited experts, including the days when MRI and TMS–EEG were performed.

TMS–EEG

Single pulse TMS–EEG was performed and recorded similarly to our previous studies [32–34]. We used a figure-of-eight coil driven by a mobile stimulator (Nexstim Ltd., Finland) to stimulate the left or right superior parietal lobule and superior frontal lobule, avoiding obvious structural lesion as detected on the subjects T1 [45]. These two targets were identified with a neuronavigation system (Nexstim Ltd., Finland) using infrared camera and a software aiming device preventing any stimulation more than 2 mm away from the target. We recorded 200 trials on each site for healthy subjects, and 400 trials for the patients (to preserve sufficient data quality despite the expected artefacts in this population), with an intensity adjusted for optimal signal-to-noise ratio (evoked electric field of 100 to 150 V/m). By using a 60 channel sample and hold amplifier (Eximia, Nexstim Ltd., Finland), we recorded EEG while avoiding the large artefact evoked by the TMS pulse. Auditory evoked potentials were also prevented using a white noise masking throughout the stimulation sessions. Further artefact removal (channel movement, ocular movement, overwhelming muscle,…) was performed during data pre-processing on MATLAB 2007 (Matworks, Natick, MA). The EEG signal was transposed from the scalp to the cortical level using source reconstruction (based on a 3-spheres BERG

method and weighted minimum norm constraint), then the perturbational complexity index (PCI) was computed as in Casali *et al* [33] (**FIGURE 1**). The best PCI of each subject was kept for analysis (PCI max).

MRI

MRI was acquired with a 3T MRI scanner (Allegra, Siemens, DWI: 64 non-collinear directions using a *b*-value = 1000 s/mm^2, two *b*=0, *TR*= 5700 ms, *TE*=87 ms, matrix size = 128x128, 45 slices, slice thickness = 3 mm, gap= 0.3 mm; and T1 3D MPRAGE). Light sedation was sometimes required to obtain movement artefact free data in patients with DOC. We used typical pre-processing steps [39,46] to analyse DWI data, employing eddy current distortion correction [47] utilizing FSL diffusion toolbox 2.0 (FSL 5.0, FMRIBs Software Library, http://www.fmrib.ox.ac.uk/fsl, Oxford, UK). We applied the same rotations to diffusion-weighted volumes and their corresponding gradient directions. We then stripped the skull and used a mask to isolate the white matter with the brain extraction tool [48]. We used weighted linear least squares fitted to the log-transformed data to estimate the FA image for each subject. If needed, after visual inspection, we removed any vibration artefact by excluding the volumes with the highest FA values (diffusion gradient in the x direction greater than 0.8) [49]. Finally, we computed the tensor eigenvalue maps for each subject. The global FA value for each subject was obtained by averaging the FA values of the voxels in the white matter mask, using FSL maths [50], as in [51]. Figure 1 illustrates the main analyses steps necessary to obtain both the PCI and the global FA (**FIGURE 1**).

Statistics

Differences in PCI and FA between healthy subjects and patients were tested using independent sample t-test. The Levene's test was used to assess the homogeneity of variance.

Linear correlation between FA and PCI, in the whole group and in the patients, was tested using one-tailed Pearson's correlation, as we expected a positive relationship. We used a linear regression model with a single predictor to test if structural connectivity, approached with global FA, could predict effective connectivity, represented by PCI. To verify the effect of gender and age as potential predictors, we created a second model with a hierarchical entry design. To check that the model was not only driven by healthy subjects, we performed the same analysis again using only the patients' group. In this model, we assessed the effect of the CRS-R total score and the time since injury as potential co-predictors, in a hierarchical entry, and checked again for a potential effect of age and gender in this subpopulation. We assessed the assumptions of errors independence using the Durbin-Watson statistics, and checked the assumption of no multicollinearity. All analysis were performed using SPSS 20 (IBM Corp., Armonk, N.Y., USA). Results were considered significant at $p < .05$, and corrected for multiple comparisons (Bonferroni) when necessary.

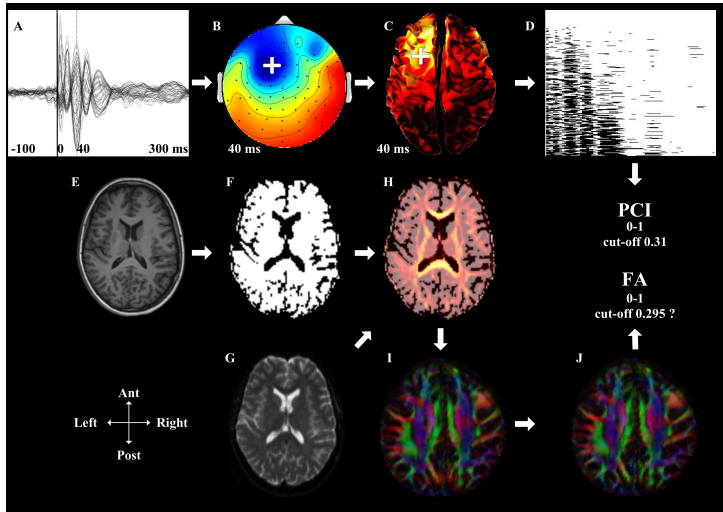Figure 1: Main analyses steps to obtain PCI and global FA.

Figure 1 illustrates the various steps necessary to obtain both the PCI and the global FA.

The first row illustrates the TMS–EEG processing. Once the TMS–EEG evoked potential is generated and artefact free (A, the vertical line showing the TMS pulse time, and a dotted line at 40ms), a scalp amplitude map can be generated (B, at 40ms, the white cross showing the location of TMS pulses). From there, source reconstruction algorithm can be used to get a map of the significant cortical sources evoked by TMS (C, at 40ms, the white cross showing the location of TMS pulses). These sources are plotted against time in a binary matrix (D), which is compressed to get the PCI (that ranges between 0 and 1 with a cut-off of 0.31). The second and third rows illustrate the MRI processing. The T1 (E) of the subject is segmented to generate a mask (F), and the diffusion images (G) are fused with the mask (H) to compute the FA (I). Vibration artefacts are then removed (J), and the global FA estimated (with a proposed optimal value of 0.295).

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Results

Out of the 39 patients enrolled, we had to exclude 16 of them because patients moved too much and we aborted the TMS–EEG session (n=7), or because we could not compute the global FA (n=5) (when the patients moved too much, and one had an extremely deformed brain), or the PCI (n=7) (when the signal-to-noise ratio was too low). Some patients had multiple issues and neither the global FA nor the PCI could be obtained. For the following analyses, we used the remaining sample of 23 adult patients (13 males, 11 traumatic brain injuries, median time since injury 33 weeks (5-1371), mean age 37 ± 15 years) and 14 healthy subjects (5 males, mean age 25 ± 4 years old) for a total of 37 participants.

Based on behavioural assessments, 5 patients could communicate (2 LIS and 3 EMCS, grouped for analysis as E-LIS). Seven patients were MCS+, 8 were MCS-, and 3 were UWS. Healthy subjects and patients did not significantly differ by gender ($\chi^2(1)$=1.508, p=.187), but the controls were significantly younger (mean (M)=24.8, standard error (SE)=0.94) than patients (M=37.8, SE=3.16) ($t(26)$=-3.83, p=.001).

PCI max was significantly lower in patients than in healthy subjects (M=0.4, SE=0.02 and M=0.58 SE= 0.02 respectively) ($t(35)$=6.15, p<.001). Four patients (3 UWS and 1 MCS-) had a PCI max under 0.31. FA was also significantly lower in patients than in healthy subjects (M=0.31, SE=0.01 and M=0.36, SE=0.003 respectively) ($t(32)$=7.6, p<.001). In other words, PCI was decreased by 31% in patients as compared to healthy subjects, while FA was 14% lower in the patient group. Demographical data and neuroimaging results (PCI and FA) are reported in table 1 (**TABLE 1)**.

Table 1: Demographical data and neuroimaging results

Subject	Gender	Age	Best diagnosis	Best CRS-R	Aetiology	Weeks (Onset)	PCI max	FA
HS1	F	24	HS	-	-	-	0.495	0.344
HS2	F	26	HS	-	-	-	0.606	0.356
HS3	M	20	HS	-	-	-	0.648	0.352
HS4	M	23	HS	-	-	-	0.608	0.369
HS5	M	27	HS	-	-	-	0.576	0.345
HS6	F	30	HS	-	-	-	0.569	0.347
HS7	F	24	HS	-	-	-	0.487	0.342
HS8	M	32	HS	-	-	-	0.510	0.353
HS9	F	25	HS	-	-	-	0.660	0.373
HS10	F	22	HS	-	-	-	0.553	0.358
HS11	M	28	HS	-	-	-	0.621	0.363
HS12	F	24	HS	-	-	-	0.511	0.364
HS13	F	22	HS	-	-	-	0.551	0.369
HS14	F	20	HS	-	-	-	0.667	0.362
Pat1	F	35	LIS	22	Ischemic	163	0.475	0.355
Pat2	M	45	LIS	15	Ischemic	6	0.584	0.372
Pat3	M	23	EMCS	23	TBI	60	0.502	0.342
Pat4	F	60	EMCS	16	Haemorrhage	7	0.523	0.337
Pat5	M	51	EMCS	22	Ischemic	21	0.452	0.347
Pat6	F	32	MCS+	11	TBI	200	0.434	0.315
Pat7	F	26	MCS+	11	TBI	145	0.380	0.297
Pat8	M	39	MCS+	17	Haemorrhage	37	0.432	0.303
Pat9	M	40	MCS+	5	TBI	45	0.491	0.316
Pat10	M	20	MCS+	11	TBI	190	0.380	0.301

Pat11	M	27	MCS+	19	Anoxic	5	0.379	0.335
Pat12	M	46	MCS+	12	Mixed	1371	0.438	0.316
Pat13	M	31	MCS-	7	TBI	207	0.409	0.297
Pat14	F	50	MCS-	7	Mixed	33	0.400	0.289
Pat15	F	25	MCS-	5	TBI	33	0.368	0.296
Pat16	F	38	MCS-	10	TBI	15	0.491	0.326
Pat17	F	27	MCS-	5	Haemorrhage	13	0.378	0.297
Pat18	M	54	MCS-	13	Anoxic	23	0.390	0.306
Pat19	F	19	MCS-	10	TBI	188	0.223	0.286
Pat20	M	26	MCS-	10	TBI	630	0.413	0.289
Pat21	F	44	UWS	6	Anoxic	14	0.267	0.304
Pat22	M	81	UWS	6	Ischemic	5	0.238	0.293
Pat23	M	21	UWS	7	TBI	24	0.249	0.283

Demography (gender, age, diagnosis, aetiology of the brain injury, and time since onset in weeks) is provided in table 1, along the results of max PCI and global FA. *F: Female; M: male; HS: Healthy subject; -: Not applicable; TBI: Traumatic brain injury.*

Our linear regression model showed that FA could significantly predict 74% of PCI max value in the whole sample ($F_{(1,35)}=100.45$ $p<.001$; $R^2=0.74$). PCI was significantly correlated with FA ($r = .86$, $p < .0001$) (**FIGURE 2**). In the patients' subpopulation, the model was still significant ($F_{(1,21)}=27.17$ $p<.001$) and FA predicted 56% of PCI max ($R^2=0.56$). There was a significant relationship between PCI and FA in this subgroup ($r = .75$, $p < .0001$). Neither age nor gender did improve the model in the whole sample ($\Delta R^2=0.01$, $p=.48$). To account for potential effect of behaviour (CRS-R total score) and time (time since onset) in the patients group, we introduced these predictors in the model, at the same level as age and gender. This did not improve the model significantly either ($\Delta R^2=0.03$, $p=.84$) (**TABLE 2**).

Table 2: Regression models

Whole group	B	SE B	ß
Step 1			
Constant	-0.66	0.11	
FA	3.43	0.34	.86*
Step 2			
Constant	-0.6	0.13	
FA	3.35	0.36	.84*
Age	0	0	-.11 [N.S.]
Gender	-.01	0.02	-.05 [N.S.]

Note: R^2 for step 1 =.74. *p<.001. ΔR^2 for step 2 = .01 (p=.48)

| Patients	B	SE B	ß
Step 1			
Constant	-0.49	0.17	
FA	2.85	0.55	.75*
Step 2			
Constant	-0.63	0.5	
FA	3.45	0.91	.91*
Age	0	0	-.08 [N.S.]
CRS-R total score	0	0	-.18 [N.S.]
Time since injury	0	0	.14 [N.S.]
Gender	0	0.03	-.02 [N.S.]

Note: R^2 for step 1=.56. ΔR^2 for step 2 = .03 (p=.84) *p<0.001. N.S. : not significant

Table 2 reports the unstandardized coefficients B and their standard error for the constant and the predictor, as well as the standardized coefficient beta for the predictor. R square and significance level is also reported for each model. First model applied to the whole sample, including patients and controls. FA significantly predicted PCI variance (R^2=.74). Age and gender did not significantly improve the model (change in R^2=0.01, p=.48). In the second model using patients' data only, FA still significantly predicted PCI variance (R^2=.56). We here used a hierarchical approach on the patients to check for behavioural and time since onset effects, and checked again for potential effect of age and gender in this subgroup. These co-predictors did not have a significant influence on the model (change in R^2=.03, p=.84).

In our sample, the PCI cut-off value of 0.31, proposed in Casali *et al* [33] and recently validated on a large cohort [34], had 97% accuracy in detecting patients with at least minimal consciousness (97% sensitivity and 100% specificity). Only one MCS- patient was not detected, but was clinically UWS on the TMS-EEG testing day despite applying the arousal protocol [5]. Moreover, PCI could be computed on only one of the two targets, the left prefrontal area, due to the presence of a cerebrospinal fluid shunt and severe brain lesions on other areas of interest, encompassing the whole right hemisphere.

No a priori cut-off value for global FA was available. In our dataset, an optimal value of 0.295 had 89% accuracy (91% sensitivity, and 67% specificity) to distinguish between unconscious and conscious conditions (**FIGURE 2**). Figure 3 (**FIGURE 3**) illustrates the white matter tractography derived from DWI and TMS evoked potential of a representative subject in each diagnostic category. It clearly shows that structural integrity and TMS evoked potential complexity decrease in a parallel fashion from normal in healthy controls and LIS to very impaired in UWS, with intermediate aspect in the MCS.

Figure 2: PCI over global FA in subjects and subgroups.
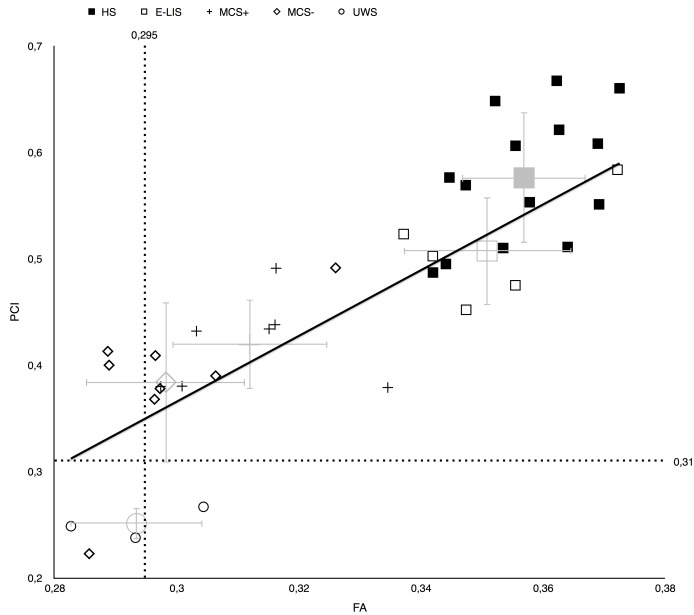
This scatter plot illustrates the positive linear relationship between FA and PCI in patients and controls ($r = .86$ $p < .0001$, $R^2 = .74$). Subjects are plotted with a different symbol according to their diagnosis (circle for UWS, diamond-shape for MCS-, plus for MCS+, empty square for EMCS and LIS, and black square for healthy subjects). The mean and standard deviation for each subgroup are plotted using lighter grey. Dot lines represent the threshold for PCI (horizontal, 0.31) and global FA (vertical, 0.295). *HS: Healthy subjects; E-LIS: EMCS and LIS group.*

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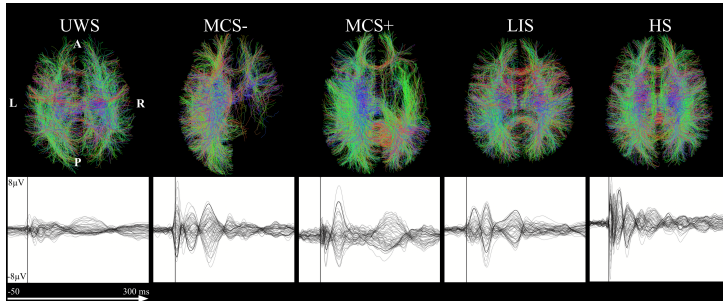Figure 3: Tractography image and TMS evoked potential in UWS, MCS-, MCS+, LIS, and healthy subject.

Figure 3 illustrates the tractography image (top view, minimal tract length 50mm) and TMS evoked potential of subjects in each category. Although there is a large variability in the aetiology and severity of brain injuries, there is a clear increase in track density from UWS to controls. The TMS evoked potentials are almost flat in this particular UWS subject, and become more complex both in shape and times as we progress to MCS, LIS, and healthy subjects. *A: Anterior; P: Posterior; L: Left; R: Right; UWS: Unresponsive Wakefulness Syndrome; MCS: Minimally Conscious State; LIS: Locked-in Syndrome; HS: Healthy Subject*

Discussion

In this study, our aims were to investigate the link between structural and effective connectivity at the global level in patients with DOC, and to confirm that effective and structural connectivity are impaired in this population. With 23 patients and 14 healthy subjects, we demonstrated that structural connectivity, approached with global FA, could explain 74% of the effective connectivity variability, represented by PCI. In other words, during wake condition, brain's causal interactions are strongly dependant on structure, at the global level. This might not be true using a more regional approach. Indeed, Barttfeld and

colleagues demonstrated that the correlation between structural and functional connectivity in monkeys was maximal under deep sedation, while it was actually quite limited during wakefulness [52]. When considering only the patients subgroup, PCI max variance was still mainly explained by FA. Interestingly, we found that adding the time since onset and behavioural assessment (CRS-R best score) did not improve the model. Although patients with better diagnosis have better structural and effective connectivity, we found no effect of behavioural scores alone. This suggests that both better effective connectivity and better CRS-R scores are supported by preserved brain structures. Although wallerian degeneration after structural damage can lead to drastic changes in FA over time [53], we could not find an effect of the time period between the brain injury and the examination. This might be due to the wide range of type and severity of structural damage in our population, including traumatic, anoxic, ischemic, haemorrhagic and mixed brain injuries, ranging from limited pons stroke to diffuse cortical and subcortical contusions or major diffuse anoxic lesions, for example.

The fact that there is a structure-function relationship might seem trivial. Indeed, clinical neurology has for a long time viewed the brain as a sum of functional areas anatomically delimitated (e.g., [54]). Networks are now the centre of much more attention, (e.g., [10]), which partly explains the amount of studies of brain connectivity in various conditions, including (un)consciousness. However, the structural-effective connectivity relationship has not yet been demonstrated in this brain-injured population. Indeed, the level of structural damage, and the potential inherent deformations, may hinder appropriate measure of structural connectivity. That is also the reason why we approached it with a global index (FA), as opposed to tractography. Nevertheless, we still had to exclude one patient as her brain was too deformed and segmentation failed. Similarly, PCI requires dedicated TMS-EEG

equipment that is not widely available, and it can be tricky to assess non-collaborating patients, who can present lots of artefacts (e.g., involuntary eye movements, head movements, perspiration) and limited number of target areas to stimulate (areas median enough to avoid muscle artefacts can be severely damaged, or inaccessible due to the presence of shunts, for example). Nonetheless, we here demonstrate that PCI, thus the perturbational effective connectivity, the most straightforward causal link between brain areas, is strongly supported by structure in the whole population. We show that the level of structural damage parallels the level of effective connectivity impairment. When considering only the patient's group, there was still a moderate relationship. Thus, structural connectivity accounts for more than half of the effective connectivity variance, but not all of it. There are several potential explanations for that. For example, some neural tracts might be damaged but remain functional. The opposite might also occur, with fully preserved but not functional or disconnected structures, which prevents them to contribute to effective connectivity [55,56]. Other factors might negatively influence effective connectivity in presence of a preserved brain structure. Some neurons, or brain areas, might be incapable to react to stimulations due to prolonged hyperpolarization [57]. Neurotransmitters depletion may also impair function despite preserved structure [58], and might be approached using magnetic resonance spectroscopy [59]. This underlines the necessity to use multimodal imaging in this challenging population, and to preferably combine techniques able to study structure, function, and effective connectivity, when trying to unveil the complex neurophysiology of DOCs.

The interest of effective-structural connectivity relationship has been studied in other populations. In a multimodal TMS, fMRI, and DWI study on schizophrenia, impaired effective connectivity between thalamus and insula and between thalamus and superior frontal gyrus was found. This deficit was not associated with impaired structural connectivity, and

functional connectivity was also preserved [60]. The authors suggest that the underlying pathology might be located within the thalamus itself, thus not accessible using DWI and fMRI. This illustrates the added value of multimodal imaging studies in such complex disease, as some information can be accessible only by one of the techniques. Using implanted electrodes in a pre-surgical set-up, a structural and an effective connectomes were obtained in patients suffering from refractory epilepsy, showing a modest (ρ = .21) correlation between structure and effective connectivity at the local level [61]. While studying language processing in healthy subjects, using Granger causality on fMRI data, effective connections between the primary auditory cortex and the lateral planum temporale and anterior superior temporal gyrus, and between the lateral planum temporale and the posterior superior temporal gyrus were detected. This lead to the discovery of fibre tracts structurally connecting these regions, once again underlying the potential of multimodal neuroimaging [62].

We confirmed that structural and effective connectivity were both decreased in patients with brain injuries, as previously reported. The structural damage in DOC patients is in line with previous studies [29,51,63], as is the effective connectivity impairment [32–34,36,38]. While no threshold for FA has been described to distinguish between unconscious and –minimally – conscious states, we found that in our sample, setting the threshold at a global FA value of 0.295 would have a very good sensitivity, but a moderate specificity. Previous studies have underlined the importance of preserved structure in specific networks such as the DMN [27], or between the anterior forebrain mesocircuit and the DMN [26]. It is thus possible that a good structural global index might have to take into account the relative preservation of these specific regions. It is also not known whether sufficient complexity can be reached only through the activation of fronto-parietal networks, or any other network supported by a relatively preserved structure.

Future studies should further investigate this combined structural-effective connectivity approach with local, rather than global, values. Indeed, building a structural-effective connectome would allow exploring the networks that matters for consciousness, and the underlying structure that would be necessary to do so. Doing so in healthy subjects under anaesthesia would allow studying the dynamics of effective connectivity modifications on a stable structural connectome. This would shed light on important mechanisms behind the loss and recovery of consciousness in a healthy brain. Larger dedicated studies might also be able to find a global FA threshold distinguishing between unconsciousness and –minimal– consciousness.

Our study has some limitations. The first one is the sample size, and especially the low number of UWS. Despite the exclusion criteria, the limited number of patients with chronic DOC, and the difficulty to perform TMS–EEG in this challenging population, we managed to include 39 patients and to compute PCI and global FA in 23 of them. Increasing that number might have increased the number of the UWS subgroup, but without guarantee. Indeed, UWS has a very poor prognosis, even when compared to other DOC [64], and are thus less represented in the chronic DOC population. Another limitation of our study is the significant age difference between our control and the patients' groups. However, effective connectivity as measured with TMS–EEG does not change significantly with physiological aging as demonstrated by Casarotto *et al* [65]. There is an age-related modification of global FA, as reported in [66,67], but the change is small, and unlikely to drive the association we found between global FA and PCI.

Conclusion

Despite a vast literature on the importance of structural and effective connectivity in patients with DOC, no study explored how brain anatomy is related to effective connectivity. Here we demonstrated that the majority of effective connectivity variance is explained by structure, as approached by PCI and FA, respectively. This result underlines that both structural and effective connexions need to be relatively preserved for consciousness to emerge. We also confirmed that effective and structural connectivity are both severely impaired in patients with DOC. There might be a minimal amount of structural connectivity below which no consciousness can be observed, and specific networks in a structural-effective connectome may need to be preserved, but this is the object of future studies.

Conflict of interest

Acknowledgement

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