UNIVERSITE DE LIEGE

Faculté de Médecine

Therapeutic challenges in disorders of consciousness

THIBAUT Aurore

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Sous la direction du Professeur LAUREYS Steven

Thèse présentée en vue de l'obtention du grade
de Docteur en Sciences Médicales
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To Gauthier, my friends and my family

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SCIENTIFIC PUBLICATIONS

This thesis is based on the following publications:

Articles

1. Thibaut A, Bruno MA, Chatelle C, Gosseries O, Vanhaudenhuyse A, Demertzi A, Schnakers C, Thonnard M, Charland-Verville V, Bernard C, Bahri M, Phillips C, Boly M, Hustinx R, Laureys S. *Metabolic activity in external and internal awareness networks in severely brain-damaged patients.* Journal of Rehabilitation Medicine. 2012; 44(6):487-94
2. Thibaut A, Chatelle C, Gosseries O, Laureys S, Bruno MA. *Transcranial direct current stimulation: a new tool for neurostimulation.* Revue Neurologique. 2013;169(2):108-20.
3. Thibaut A, Chatelle C, Ziegler E, Bruno MA, Laureys S, Gosseries O. *Spasticity after stroke: physiology, assessment and treatment.* Brain Injury. 2013; 27(10):1093-105.
4. Thibaut A*, Chatelle C*, Whyte J, De Val MD, Laureys S, Schnakers C. *Equally contributed *Pain issues in disorders of consciousness.* Brain Injury. 2014; 28(9):1202-8.
5. Thibaut A*, Chatelle C*, Gosseries O, Bruno MA, Demertzi A, Bernard C, Hustinx R, Tshibanda L, Bahri MA, Laureys S. *equally contributed *Changes in cerebral metabolism in patients with a minimally conscious state responding to zolpidem.* Frontiers in Human Neuroscience. 2014; 8:917.
6. Thibaut A, Bruno MA, Ledoux D, Demertzi A, Laureys S. *tDCS in patients with disorders of consciousness: sham-controlled randomized double-blind study.* Neurology. 2014; 82(13):1112-8.
7. Thibaut A, Chatelle C, Wannez S, Deltombe T, Stender J, Schnakers C, Laureys S, Gosseries O. Spasticity in disorders of consciousness: A behavioural study. European Journal of Physical Rehabilitation Medicine. 2014 [Epub ahead of print]
8. Thibaut A, Deltombe T, Wannez S, Gosseries O, Ziegler E, Dieni C, Deroy M, Laureys S. *Impact of soft splints on upper limb spasticity in chronic patients with disorders of consciousness: A randomized, single-blind, controlled trial.* Brain Injury. 2015, in press.
9. Thibaut A,* Di Perri C*, C. Chatelle, M. A. Bruno, S. Wannez, C. Bernard, R. Hustinx and S. Laureys. *equally contributed *Clinical response to tDCS depends on residual brain metabolism and grey matter integrity in patients with minimally conscious state.*

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10. Chatelle C, Thibaut A, Bruno MA, Boly M, Bernard C, Hustinx R, Schnakers C, Laureys S. *Nociception coma scale-revised scores correlate with metabolism in the anterior cingulate cortex.* Neurorehabilitation and Neural Repair. 2014 Feb;28(2):149-52.
11. Di Perri C, Thibaut A, Heine L, Soddu A, Demertzi A, Laureys S. *Measuring consciousness in coma and related states.* World Journal of Radiology. 2014; 6(8):589-97
12. Bruno MA, Vanhaudenhuyse A, Thibaut A, Moonen G, Laureys S. *From unresponsive wakefulness to minimally conscious PLUS and functional locked-in syndromes: recent advances in our understanding of disorders of consciousness.* Journal of Neurology. 2011;258(7):1373-84.

Book chapters

1. Thibaut A, Chatelle C, Stender J, Demertzi A, Bernard C, Hustinx R, Laureys S, Bruno M. *Positron emission tomography imaging in altered states of consciousness: Coma, sleep and hypnosis.* In PET and SPECT in Neurology.Ed. Dierckx, Otte, Vries, Waarde, Leenders, Paris, Springer-Verlag France, 2014.
2. Thibaut A, Di Perri C, Bodart O, Laureys S. *Behavioural Diagnosis of Disorders of Consciousness.* In Clinical Neurophysiology in Disorders of Consciousness. Ed. Laureys S, Rossetti A.O., Paris, Springer-Verlag France. In press.
3. Bodart O, Thibaut A, Laureys S, Gosseries O. *Disorders of consciousness.* In Oxford Textbook of Neurocritical Care. Eds. M. Smith and F. Richardson. In press.
4. Thibaut A, Bodart O, Laureys S. *Brain stimulation for patients with disorders of consciousness.* In Surgical Principles of Therapeutic Cortical Stimulation. Ed. S. Canavero, De Gruyter. 2015

Other publications:

Articles

1. Cruse D, Thibaut A, Demertzi A, Nantes J, Bruno MA, Gosseries O, Vanhaudenhyse A, Bekinstein T, Owen A, Laureys S. *Actigraphy Assessments of Circadian Sleep-Wake Cycles in the Vegetative and Minimally Conscious States.* BMC Neuroscience, 2013; 11:18

2. Gosseries O., Demertzi A., Ledoux D, Bruno MA., Vanhaudenhuyse A., Thibaut A., Laureys S., Schnakers C.
   Burnout in healthcare workers managing patients recovering from coma.
   Brain Injury, 2012, 26(12):1493-9
3. Thonnard M., Gosseries O., Demertzi A., Lugo Z., Vanhaudenhuyse A., Bruno MA., Chatelle C., Thibaut A., Charland-Verville V., Habbal D., Schnakers C., Laureys S.
   Effect of zolpidem in chronic disorders of consciousness: a prospective open-label study.
   Functional Neurology 2013;28(4):259-64.
4. Gosseries O, Thibaut A, Boly M, Rosanova M, Massimini M, Laureys S.
   Assessing consciousness in coma and related states using transcranial magnetic stimulation combined with electroencephalography.
   Annales Francaises d'Anesthésie et Réanimation, vol 33, 2014
5. Stender J., Gosseries O., Bruno MA., Charland-Verville V., Vanhaudenhuyse A., Demertzi A., Chatelle C., Thonnard M., Thibaut A., Heine L., Soddu A., Boly M., Schnakers C., Gjedde A., Laureys S.
   Diagnostic precision of PET imaging and functional MRI in disorders of consciousness: a clinical validation study.
   Lancet, 2014. 384(9942):514-22.
6. Di H, Nie Y, Hu X, Tong Y, Heine L, Wannez S, Huang W, Yu D, He M, Thibaut A, Schnakers C, Laureys S.
   Assessment of visual fixation in vegetative and minimally conscious states.
   BMC Neurology. 2014;14:147.
7. Stender J, Kupers R, Rodell A, Thibaut A, Chatelle C, Bruno MA, Gejl M, Bernard C, Hustinx R, Laureys S, Gjedde A.
   Quantitative rates of brain glucose metabolism distinguish minimally conscious from vegetative state patients.
   Journal of Cerebral Blood Flow & Metabolism. 2015;35(1):58-65

Book chapters

1. Demertzi A., Gosseries O., Bruno MA., Schnakers C., Vanhaudenhuyse A., Chatelle C., Charland V., Thibaut A., Thonnard M., Laureys S.
   Désordres de la conscience: aspects éthiques.
   In Coma et états de conscience altérée, Springer, Paris, Springer-Verlag France, 2011
2. Bruno MA., Ledoux D, Vanhaudenhuyse A., Gosseries O., Schnakers C., Thibaut A., Chatelle C., Thonnard M., Charland V., Laureys S.
   Pronostic des patients récupérant du coma.
   In Coma et états de conscience altérée, Springer, Paris, Springer-Verlag France, 2011
3. Vanhaudenhuyse A., Boly M., Bruno M-A., Gosseries O., Demertzi A., Kirsch M., Tshibanda J-F., Thonnard M., Charland-Verville V., Chatelle C., Thibaut A., Laureys S, Soddu A.
   Functional imaging and impaired consciousness.
   In Coma and Consciousness, Springer, London, Springer-Verlag France, 2012

4. Demertzi A., Gosseries O., Bruno MA., Schnakers C., Vanhaudenhuyse A., Chatelle C., Charland V., Thibaut A., Thonnard M., Laureys S.
The ethics of managing disorders of consciousness.
In Coma and Consciousness, Springer, London, Springer-Verlag France, 2012

5. Bruno MA., Ledoux D, Vanhaudenhuyse A., Gosseries O., Thibaut A., Laureys S. *Prognosis of patients with altered states of consciousness.*
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SUMMARY

Managing pain and promoting recovery in patients with disorders of consciousness (DOC) is a real clinical challenge. The first aim of my thesis was to improve pain management by improving our knowledge of (i) potential pain assessment tools and (ii) spasticity, a potential source of pain that may also prevent further recovery in this population. The second aim of my work was to investigate potential pharmacological and non-pharmacological treatments for promoting recovery in patients with DOC.

1. Symptomatic treatments: pain and spasticity

Pain management in non-communicative patients is a real challenge. Clinically, it is difficult to adapt treatment, since it is not possible to obtain feedback from the patients. Previous studies have shown that the Nociception Coma Scale-Revised (NCS-R), a scale to assess pain and nociception in patients with DOC, is a sensitive tool to assess responses to noxious stimulation. To further investigate the neural correlates of the scale, we assessed whether NCS-R scores could reflect nociceptive brain processing in this population. We investigated the correlation between NCS-R total scores and cerebral metabolism in areas involved in pain processing. Results showed a positive correlation between NCS-R total scores and brain metabolism in the posterior part of the anterior cingulate cortex - an area known to be involved in the cognitive and affective aspects of pain processing. This result supports the hypothesis that the NCS-R is related to cortical processing of pain and may constitute an appropriate behavioural tool to assess the efficacy of treatment and monitor nociception and pain in non-communicative patients.

Apart from detecting pain in this population, there is also the challenge of identifying and treating the possible sources of pain. One potential source of discomfort is spasticity, which may even reduce the patients' ability to show signs of consciousness at the bedside. Though spasticity is known to be very common in patients following a stroke or acquired brain damage, we know very little about its driving mechanisms and prevalence in DOC. As a result, there is also a lack of guidelines regarding pharmacological treatment and rehabilitation. In a cross-sectional study involving 65 patients in unresponsive wakefulness syndrome/vegetative state (UWS/VS) and minimally conscious state (MCS), we reported that 89% of the patients showed spasticity in at least one limb and 62% of the patients had severe invalidating spasticity based on the Modified Ashworth Scale. Interestingly, we also observed a positive correlation between the

severity of spasticity and pain scores observed during care (as measured by the NCS-R), highlighting the importance of standardized management of pain and spasticity in this population. Finally, we identified a linear positive correlation between the severity of spasticity and time since injury, emphasizing the importance of prolonged and revised treatments in chronic stages. Recently, we conducted a single-blind randomized sham-controlled trial aiming to assess the efficacy of soft splints on upper limb spasticity in chronic patients with DOC. A positive effect of the splints on intrinsic hand muscle spasticity (as assessed by the Modified Ashworth Scale) and hand opening was observed after wearing the splints for 30 minutes. These findings suggest that soft splints, through their positive effects on muscle hypertonia, their comfort and simple application, could be easily and efficiently added to the patient's daily management to decrease spasticity.

2. Curative treatments: zolpidem and transcranial direct current stimulation

Zolpidem, a short-acting non-benzodiazepine hypnotic drug, has been shown to induce paradoxical effect in some rare cases of patients in DOC, promoting recovery of behavioural signs of consciousness. Using Positron Emission Tomography (PET), we assessed zolpidem-induced changes in regional brain metabolism in three chronic MCS patients with known zolpidem response (i.e. temporary emergence from MCS). The aim of this study was to better understand the neural mechanisms underlying such a recovery. Our results highlighted increased metabolism within the prefrontal areas following zolpidem intake as compared with placebo. This finding corroborates the key role of the prefrontal cortices in the recovery of consciousness.

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that has been previously reported to transiently improve working memory and attention by stimulating the left dorsolateral prefrontal cortex (DLPF) in patients with stroke as well as Parkinson's and Alzheimer's disease. However, no studies have investigated its ability to promote recovery in patients with DOC. We performed the first double-blind randomized placebo-controlled clinical trial in patients in UWS/VS and MCS and assessed the effect on the level of consciousness (as measured by the Coma Recovery Scale-Revised) of a single session of anodal tDCS over the left DLPF cortex. This crossover trial showed that, at the group level, tDCS could promote recovery of behavioural signs of consciousness in patients in MCS but not in UWS/VS. Forty three percent of patients in MCS (13/30) responded to the stimulation – i.e. showed a new sign of

consciousness (e.g. visual pursuit, object localization or recognition, localisation to pain or command following) after the real tDCS that was not present before, nor after the sham tDCS. Out of these 13 patients, 5 were in this state for more than one year, suggesting that tDCS could still be effective after long time periods in a MCS. In order to improve our understanding of the underlying mechanisms of tDCS, we then retrospectively investigated why some patients showed tDCS-induced improvement (i.e. responders), while others did not. Neuroimaging data (PET and structural magnetic resonance imaging – MRI) allowed us to compare residual brain metabolism and grey matter volume in responders versus non-responders. We found that the transient recovery of signs of consciousness following tDCS in patients with chronic MCS (> 3 months) seems to require residual metabolic activity and residual grey matter in (i) the presumed stimulated area (i.e. left DLPFC), (ii) distant cortical areas (i.e. precuneus), and (iii) subcortical brain regions (i.e. thalamus) known to be involved in awareness and arousal. These findings suggest that tDCS is a feasible treatment that may promote recovery of new signs of consciousness in patients with DOC, although it also suggests that some patients may be more suited to benefit from tDCS than others. We therefore need to deepen our understanding of the neuronal correlates underlying its effect, especially in patients with brain lesions, in order to provide guidelines for clinicians.

In this work, we highlighted several potential pharmacological and non-pharmacological treatments that may be helpful for improving patients' quality of life and promoting recovery in DOC. Future studies should provide guidelines for standardized management and treatment in DOC in order to improve both motor and cognitive rehabilitation in this population.

RÉSUMÉ

Traiter la douleur et stimuler la récupération des patients en état de conscience altérée (ECA) est un vrai défi pour les chercheurs et les cliniciens. Le premier but de ma thèse visait à améliorer la gestion de la douleur en enrichissant nos connaissances (i) sur les outils cliniques d'évaluation de la douleur et (ii) sur la spasticité, une source potentiel de douleur pouvant entraver la récupération des patient en ECA. Le second objectif de mon travail était d'investiguer les effets de traitements pharmacologiques et non-pharmacologiques sur l'amélioration de la récupération de la conscience au sein de cette population de patients.

1. Traitements symptomatiques : douleur et spasticité

Cliniquement, il est difficile d'adapter de manière adéquate un traitement chez les patients non-communicants, puisqu'il est impossible d'obtenir un feed-back de leur part sur leur ressenti. Des études ont montré que la *Nociception Coma Scale Revised (NCS-R*), évaluant la douleur et la nociception des patients en ECA, est un outil adéquat permettant d'apprécier les réponses des patients à des stimuli douloureux. Afin d'approfondir l'étude des corrélats neuronaux de cette échelle, nous avons évalué si les scores de la NCS-R pouvaient refléter les processus neuronaux du traitement de la douleur au sein de cette population de patients. Pour ce faire, nous avons étudié la corrélation entre les scores totaux de la NCS-R et le métabolisme cérébral régional des aires impliquées dans le traitement de la douleur chez des patients en ECA. Les résultats ont montré une corrélation positive entre les scores totaux de la NCS-R et le métabolisme cérébral de la partie postérieure du cortex cingulaire antérieur, une région connue pour son implication dans les processus cognitifs et affectifs de la gestion de la douleur. Ces résultats confirment l'hypothèse que la NCS-R est liée au traitement cortical de la douleur. Cette échelle pourrait ainsi constituer un outil comportemental approprié pour le contrôle de la douleur chez les patients non-communicants.

En parallèle à la détection de la douleur au sein de cette population, notons également le défi d'identifier et de traiter les possibles sources de douleur. L'une d'elles est la spasticité, qui en plus d'être inconfortable pour le patient, peut également réduire les capacités de celui-ci à exprimer des signes de conscience. Bien que la spasticité soit courante chez les individus ayant été victime d'un accident vasculaire

cérébral ou d'autres lésions cérébrales, nous en connaissons peu sur les mécanismes qui induisent la spasticité, ni sur sa prévalence chez les patients en ECA. En conséquence, on observe un manque de recommandations thérapeutiques par rapport à la prise en charge de ces patients, qu'elles soient pharmacologique et/ou rééducative. C'est pourquoi nous avons réalisé une première étude évaluant la sévérité de la spasticité chez des patients en ECA. Dans cette étude portant sur 65 patients en éveil non-répondant/état végétatif (ENR/EV) et en état de conscience minimale (ECM), nous avons observé que 89% des patients démontrent un signe de spasticité au niveau d'au moins un membre et que 62% d'entre eux souffrent d'une spasticité sévère invalidante (mesurée avec l'échelle d'Ashworth modifiée). De manière intéressante, nous avons également observé une corrélation positive entre la gravité de la spasticité et les signes de douleur observés au cours des soins (mesurée avec la NCS-R). Cette observation souligne l'importance d'une prise en charge commune de la douleur et de la spasticité au sein de cette population. Enfin, nous avons identifié une corrélation positive entre la sévérité de la spasticité et le temps écoulé depuis l'accident, soulignant l'importance de traitements prolongés et adaptés lorsque les patients en sont à un stade chronique. Récemment, nous avons réalisé une étude contrôlée randomisée en simple aveugle visant à évaluer l'efficacité d'attelles souples sur la spasticité des membres supérieurs chez des patients en ECA chroniques. Un effet positif des attelles sur la spasticité des muscles intrinsèques de la main (évaluée par l'échelle d'Ashworth modifiée) et sur l'ouverture de la main a été observé après le port de celles-ci pendant 30 minutes. Ces résultats suggèrent que ces attelles souples, grâce à leur effet positif sur l'hypertonie musculaire, leur confort et leur application aisée, pourraient être efficaces et facilement implémentées à la prise en charge quotidienne des patients en ECA.

2. Traitements curatifs : zolpidem et stimulation transcranienne à courant continu

Le zolpidem, un médicament hypnotique à courte durée d'action, a déjà induit un effet paradoxal chez quelques rares cas de patients en ECM. En effet, certains patients ont récupéré, de manière transitoire, des signes comportementaux de conscience à la suite de la prise de ce médicament. En utilisant la tomographie à émission de positons (TEP), nous avons évalué les changements induits par le zolpidem sur le métabolisme cérébral régional chez trois patients en ECM qui répondaient à ce médicament (à la suite d'une prise de zolpidem, ces patients ont émergé temporairement de l'ECM). Le but de cette étude était de mieux comprendre les mécanismes neuronaux qui sous-tendent une telle évolution. Nos résultats

mettent en évidence une augmentation du métabolisme dans les régions préfrontales à la suite de la prise de zolpidem. Ce résultat corrobore le rôle clé du cortex préfrontal dans la récupération de la conscience.

La stimulation transcranienne à courant continu (tDCS – *transcranial direct current stimulation*) est une technique de stimulation cérébrale non invasive qui a montré un effet positif transitoire sur la mémoire de travail et l'attention de patients victimes d'un accident vasculaire cérébral ou souffrant de la maladie de Parkinson ou encore d'Alzheimer, à la suite d'une stimulation du cortex préfrontal dorsolatéral gauche (PFDL). Cependant, aucune étude n'a examiné sa capacité à promouvoir la récupération chez les patients en ECA. C'est pourquoi, nous avons effectué une étude pilote, randomisée en double aveugle contrôlée par placebo, chez des patients en ENR/EV et en ECM. Nous avons évalué l'effet sur le niveau de conscience (mesuré par la *Coma Recovery Scale Revised*; CRS-R) d'une stimulation anodique sur le cortex PFDL gauche. Cette étude a montré que la tDCS pouvait favoriser la récupération de signes comportementaux de conscience chez les patients en ECM. Cet effet n'a néanmoins pas été observé pour les patients en ENR/EV. Quarante-trois pour cent des patients en ECM (13/30) ont répondu à la stimulation, en montrant un nouveau signe de conscience (par exemple, une poursuite visuelle, une localisation ou reconnaissance d'objets, ou encore, une réponse à la commande) après la tDCS qui n'était pas présent avant, ni avant ou après la stimulation placebo. Sur ces 13 patients, cinq étaient dans cet état depuis plus d'un an. Cela suggère que la tDCS pourrait encore être efficace même après de longues périodes dans un ECM.

Afin d'améliorer notre compréhension des mécanismes qui sous-tendent les effets de la tDCS, nous avons investigué, rétrospectivement, les raisons pour lesquelles certains patients ont montré une amélioration suite à la tDCS (patients répondants), tandis que d'autres n'ont pas répondu à la stimulation (patients non-répondants). Des données de neuro-imagerie (TEP et Imagerie par Résonance Magnétique - IRM) acquises chez des patients en ECM chroniques ayant participé à l'étude pilote, nous ont permis de comparer le métabolisme cérébral et le volume de la matière grise des patients répondants par rapport aux non-répondants. Les résultats ont mis en évidence que la récupération transitoire de signes de conscience à la suite de la tDCS semble exiger une activité métabolique résiduelle et une préservation partielle de la matière grise au niveau de (i) la région stimulée (cortex PFDL gauche), (ii) d'aires corticales éloignées (précuneus), et (iii) de régions cérébrales sous-corticales (thalamus) ; celles-ci étant connues pour être impliquées dans l'éveil et la conscience. Ces résultats suggèrent que la tDCS pourrait être un traitement

efficace pour favoriser la récupération de nouveaux signes de conscience chez les patients en ECA. Cependant, certains patients semblent avoir une activité cérébrale plus adaptée pour bénéficier des effets de la tDCS que d'autres. Nous avons donc besoin d'approfondir notre compréhension des corrélats neuronaux qui sous-tendent les effets de la tDCS, surtout chez les patients atteints de lésions cérébrales sévères, afin de fournir des recommandations thérapeutiques claires pour les cliniciens prenant en charge des patients en ECA.

Ce travail nous a permis de mettre en évidence plusieurs traitements pharmacologiques et non-pharmacologiques qui pourraient potentiellement être efficaces pour améliorer la qualité de vie et la récupération des patients en ECA. Il serait donc intéressant de mener d'autres études sur le sujet afin de permettre l'établissement de recommandations thérapeutiques standardisées visant à perfectionner la prise en charge et le traitement des patients en ECA.

ABBREVIATIONS

DOC	Disorders of Consciousness
UWS/VS	Unresponsive Wakefulness Syndrome/Vegetative state
MCS	Minimally Conscious State
LIS	Locked-in Syndrome
FDG-PET	[18F]-fluorodeoxyglucose Positron Emission Tomography
EEG	Electroencephalography
fMRI	Functional Magnetic Resonance Imaging
VBM	Voxel Based Morphometry
CRS-R	Coma Recovery Scale Revised
NCS-R	Nociception Coma Scale Revised
TBI	Traumatic Brain Injury
MAS	Modified Ashworth Scale
MTS	Modified Tardieu Scale
ROM	Range of Motion
CNS	Central Nervous System
ACC	Anterior Cingulate Cortex
S1 & S2	Primary and Secondary Somatosensory Areas
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation
MEP	Motor Evoked Potential
GABA	γ-Aminobutyric Acid
NMDA	N-Methyl-D-aspartate

1. Introduction

"I have always loved the desert. One sits down on a desert sand dune, sees nothing, hears nothing. Yet through the silence something throbs, and gleams . . . "

Antoine de Saint-Exupery

Based on the following articles:

Thibaut A, Bruno MA, Chatelle C, Gosseries O, Vanhaudenhuyse A, Demertzi A, Schnakers C, Thonnard M, Charland-Verville V, Bernard C, Bahri M, Phillips C, Boly M, Hustinx R, Laureys S. Metabolic activity in external and internal awareness networks in severely brain-damaged patients. J Rehabil Med. 2012.

Thibaut A, Di Perri C, Bodart O, Laureys S. Behavioural Diagnosis of Disorders of Consciousness. In Clinical Neurophysiology in Disorders of Consciousness, Ed. Laureys S, Rossetti A.O., Paris, Springer-Verlag France. *In press.*

Thibaut A, Chatelle C, Stender J, Demertzi A, Bernard C, Hustinx R, Laureys S, Bruno MA. Positron emission tomography imaging in altered states of consciousness: Coma, sleep and hypnosis. In PET and SPECT in Neurology; Ed. Dierckx, R, Otte, A, Vries, E, Waarde, A, Leenders, K., Paris, Springer-Verlag France, 2014.

Bodart O, Thibaut A, Laureys S, Gosseries O. Disorders of consciousness. In Oxford Textbook of Neurocritical Care, Chap 25, Eds. M. Smith and F. Richardson. *In press.*

Di Perri C, Thibaut A, Heine L, Soddu A, Demertzi A, Laureys S. Measuring consciousness in coma and related states. World J Radiol. 2014.

Bruno MA, Vanhaudenhuyse A, Thibaut A, Moonen G, Laureys S. From unresponsive wakefulness to minimally conscious PLUS and functional locked-in syndromes: recent advances in our understanding of disorders of consciousness. J Neurol. 2011.

In the 1950s, the invention of the artificial respirator made surviving severe brain damage possible for many patients. Unfortunately, while the heart and the lungs can recover next to normal function after intensive cares, lesions in the brain cause various level of functional impairment and can lead to various states of impaired consciousness. These patients can stay in this state for years or, in some cases, recover spontaneously a certain degree of autonomy.

An accurate diagnosis is crucial since it influences the patient's cares, pain treatments, rehabilitation, outcome and end-of-life decisions. Therefore, it is of paramount importance to develop valid and sensitive behavioural scales to detect the presence of even the subtlest sign of consciousness. The clinical way to diagnose precisely these patients is to observe their spontaneous behaviours and their reactions to external stimuli. One of the most important limitation of these behavioural evaluations is their sensibility to patients' physical (e.g. motor impairment), mental (e.g. vigilance level) or language (e.g. aphasia) disabilities at the time of assessment. Missing signs of consciousness is not rare, and the diagnostic error rate can be as high as 40% (Schnakers, Vanhaudenhuyse et al. 2009). To circumvent those functional and cognitive impairments, neuroimaging (e.g. magnetic resonance imaging – MRI; Positron Emission Tomography – PET) are now available to help clinician in the assessment of consciousness levels in this challenging patient population.

In this chapter, we will first define the different states of consciousness following a severe brain injury, and the main existing scales developed for the assessment of consciousness at the bedside. We will then present the neuronal characteristics of patients with disorders of consciousness (DOC) and especially how MRI and PET-scan can complement standard examinations. Finally, we will present the objectives of this thesis.

1.1 Characteristics of patients with disorders of consciousness

Many definitions of consciousness have been proposed by scientists, neuroscientists or philosophers. If a commonly shared definition of consciousness does not exist, it is widely accepted that it is a multi-component term involving a series of cognitive processes such as attention and memory (Baars, Ramsoy et al. 2003, Zeman 2005). At the bedside, consciousness can be defined by reducing it to two components: (i) arousal (i.e. wakefulness), that we can call the level of consciousness and (ii) awareness (e.g. awareness of the environment and if the self), which is the content of consciousness (Laureys, Faymonville et al. 2002, Posner, Saper et al. 2007). For patients with DOC, both arousal level and the contents of consciousness can be affected.

From a pathological point of view, DOC can result from focal brain injuries that induce widespread functional changes, or from a diffuse brain injury. Specific brain areas and networks seem to be particularly important for the recovery of consciousness (i.e. lateral fronto-parietal network for external consciousness and medial network – mesiofrontal/anterior cingulate cortex and precuneus/posterior cingulate cortex – for internal consciousness). In these networks, the cortico-cortical connectivity and the cortico-thalamic connectivity also have a huge importance (Laureys, Antoine et al. 2002).

Clinically, after the period of coma, some patients will regain full consciousness, some will die, while other progress to a state of preserved wakefulness in the absence of awareness (i.e. unresponsive wakefulness syndrome/vegetative state – UWS/VS) or with minimal and fluctuating signs of awareness, not encompassing the ability to communicate consistently (i.e. minimally conscious state – MCS). These states can be described in terms of degree of arousal and awareness. Gradual recovery from coma is illustrated in figure 1.

1.1.1 Brain death

Brain death is classically caused by a severe brain lesion (e.g. massive traumatic injury, intracranial haemorrhage, or anoxia) that results in increased intracranial pressure. In brain death, critical functions such as respiration, blood circulation, neuroendocrine and homeostatic regulation are absent, the organism survive only thanks to medical assistance (Bernat 1998, Haupt and Rudolf 1999). The patient is apnoeic and unreactive to environmental stimulation. The diagnosis of brain death can be posed only after the demonstration of irreversible cessation of all clinical functions of the brain and brainstem, and can be made within 6–24 h post injury (1981). Patients in brain death show no residual brain activity on PET-scan. This syndrome is sometimes called the "black box syndrome", due to the complete loss of brain metabolism (figure 2a).

1.1.2 Coma

Coma is an acute state of non-responsiveness in which patients cannot be awakened even when intensively stimulated (Jennett and Plum 1972, Plum and Posner 1972, Posner, Saper et al. 2007), and only show some reflex behaviours (Teasdale and Jennett 1974). It is the result of diffuse cortical or white matter damage and/or an acute lesion in the brainstem. Brain-injured patients can stay in coma for several days or weeks, showing no arousal (i.e. eyes closed) and no awareness (i.e. no voluntary behavioural responses). This state rarely lasts longer than two to four weeks (Laureys 2007), thus the term "acute" in the definition.

Coma has numerous aetiologies, which can clinically be divided in two major categories - traumatic and non-traumatic. Traumatic brain injury (TBI) can induce coma because of the considerable initial brain damage (as in diffuse axonal injury or extensive bilateral hemisphere lesions) or the strategic location of the lesion (e.g. of the brainstem or bilateral lesion of the thalami). The underlying pathophysiology is the same for both aetiologies, consisting of interruption and/or global impairment of the arousal system. Even when the primary lesion does not itself result in coma, patients can suffer complications and secondary lesions, including brain swelling, haemorrhage or brain herniation, which can lead to loss of consciousness.

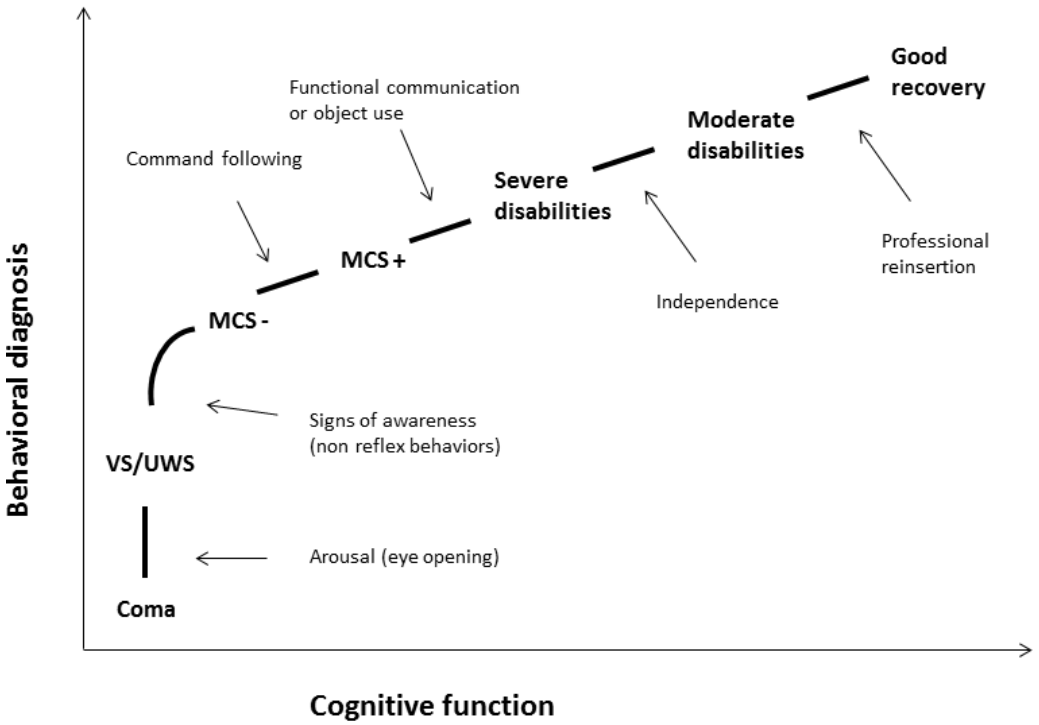


Figure 1. Different clinical entities encountered on the gradual recovery from coma, illustrated as a function of cognitive and motor capacity. Restoration of spontaneous or elicited eye-opening, in the absence of voluntary motor activity, marks the transition from coma to vegetative state/unresponsive wakefulness syndrome (UWS/VS). The passage from the UWS/VS to the minimally conscious state minus (MCS-) is marked by reproducible evidence of 'voluntary behaviour'. Simple command following characterizes the MCS plus (MCS+). Emergence from MCS is signalled by the return of functional communication or object use. Adapted from (Chatelle and Laureys 2011).

The prognosis for recovery is influenced by different factors such as aetiology, patient's general medical condition, and age. The outcome is known to be unfavourable if, during three consecutive days, there is an absence of pupillary or corneal reflexes, presence of stereotyped or absent motor responses to noxious stimulation, and presence of isoelectric or burst suppression electroencephalogram (EEG) patterns (Attia and Cook 1998). Recovery from coma may lead to an unresponsive wakefulness syndrome/vegetative state (UWS/VS), a minimally conscious state (MCS), and more rarely, to a locked-in syndrome (LIS).

1.1.3 Unresponsive wakefulness syndrome/vegetative state

This state was first named apallic syndrome (Kretschmer 1940) or coma vigil (Calvet and Coll 1959), and in 1972 it was called vegetative state (VS; Jennett and Plum 1972). A new terminology was proposed in 2010 - the unresponsive wakefulness syndrome (UWS) (Laureys, Celesia et al. 2010) - to avoid the strong negative connotation of the term "vegetative". Moreover, the term UWS allows a more accurate description of the clinical state, referring to patients that are unable to respond appropriately to external stimuli (hence unresponsive), while showing periods of time with eyes opened (hence wakefulness). This state implies the preservation of autonomic functions (e.g. cardiovascular regulation, thermoregulation) and period of eyes opening in the absence of awareness. Behaviourally, patients in UWS/VS open their eyes spontaneously or in response to stimulation, but they only show reflex behaviours, unrelated to the environment (The Multi-Society Task Force on PVS 1994). The UWS/VS may be transitory, chronic or permanent.

Although recovery of the sleep-wake cycle is part of the criteria of UWS/VS, recent studies have demonstrated an absence of electrophysiological characteristics of sleep (Landsness, Bruno et al. 2011, Cologan, Drouot et al. 2013) in UWS/VS. Brain metabolism is usually diminished by 40 to 50% with impaired cortico-thalamo-cortical circuits but relatively preserved brainstem functions (Gosseries, Bruno et al. 2011) (figure 2b).

In term of prognosis, increased length of time spent in an unconscious state, as well as the aetiology of the coma, also have major impacts on prognosis. Traumatic aetiology is usually associated with a better prognosis than non-traumatic causes of coma. Patients with an UWS/VS following TBI may continue to improve for up to 12 months after the original insult, whereas little improvement is often observed beyond 3 months in non-traumatic cases of coma (The Multi-Society Task Force on PVS 1994). Nevertheless, more recent studies have been challenging these temporal boundaries of irreversibility (e.g. Estraneo, Moretta et al. 2010).

1.1.4 Minimally conscious state

Since the formal definition of the minimally conscious state (MCS), there is a little more than 10 years ago (Giacino, Ashwal et al. 2002), a number of authors have questioned the usefulness of differentiating

UWS/VS from MCS patients considering both patient groups as hopelessly brain damaged (Bruno, Gosseries et al. 2011). Several studies have demonstrated the high interest of disentangling both clinical entities, as functional neuroimaging has shown differences in residual cerebral processing and hence, conscious perception (e.g. Boly, Faymonville et al. 2008, Coleman, Bekinschtein et al. 2009, Vanhaudenhuyse, Noirhomme et al. 2010). PET-scan showed that metabolic activity in MCS patients is usually reduced but cortico-cortical and thalamo-cortical connections are partly restored (figure 2c; Laureys, Faymonville et al. 2000). Furthermore, there is a differences in outcome between UWS/VS and MCS (e.g. Luaute, Maucort-Boulch et al. 2010, Bruno, Ledoux et al. 2012).

Behaviourally, patients in a MCS are awake and show fluctuating but reproducible signs of awareness (Giacino, Ashwal et al. 2002). These patients can manifest reproducible responses to verbal or written commands, visual pursuit, localisation to pain, intelligible verbalizations, intentional communication, and reaching/holding objects. However, these behaviours can fluctuate in time, which makes the detection of awareness a difficult endeavour. Recently, we have proposed to subcategorize the clinically heterogeneous "MCS entity" in minimally conscious PLUS (MCS+) and MINUS (MCS-) based on the level of complexity of observed behavioural responses (Bruno, Vanhaudenhuyse et al. 2011). MCS+ was defined by the presence of (i) command following, (ii) intelligible verbalization or (iii) gestural or verbal yes/no responses. In contrast, MCS- patients only show minimal levels of behavioural interaction characterized by the presence of non-reflex movements such as: (i) orientation of noxious stimuli, (ii) pursuit eye movements that occur in direct response to moving a salient stimuli, (iii) movements or affective behaviours that occur in contingent relation to relevant environmental stimuli (such as appropriate smiling or crying, vocalizations, objects localization or manipulation). This classification is supported by neuroanatomical data that demonstrate better preservation of language-related networks in patients in MCS+ as compared to MCS- (Bruno, Majerus et al. 2012). Emergence from MCS (i.e. EMCS) is defined by the recovery of functional communication and/or functional object use.

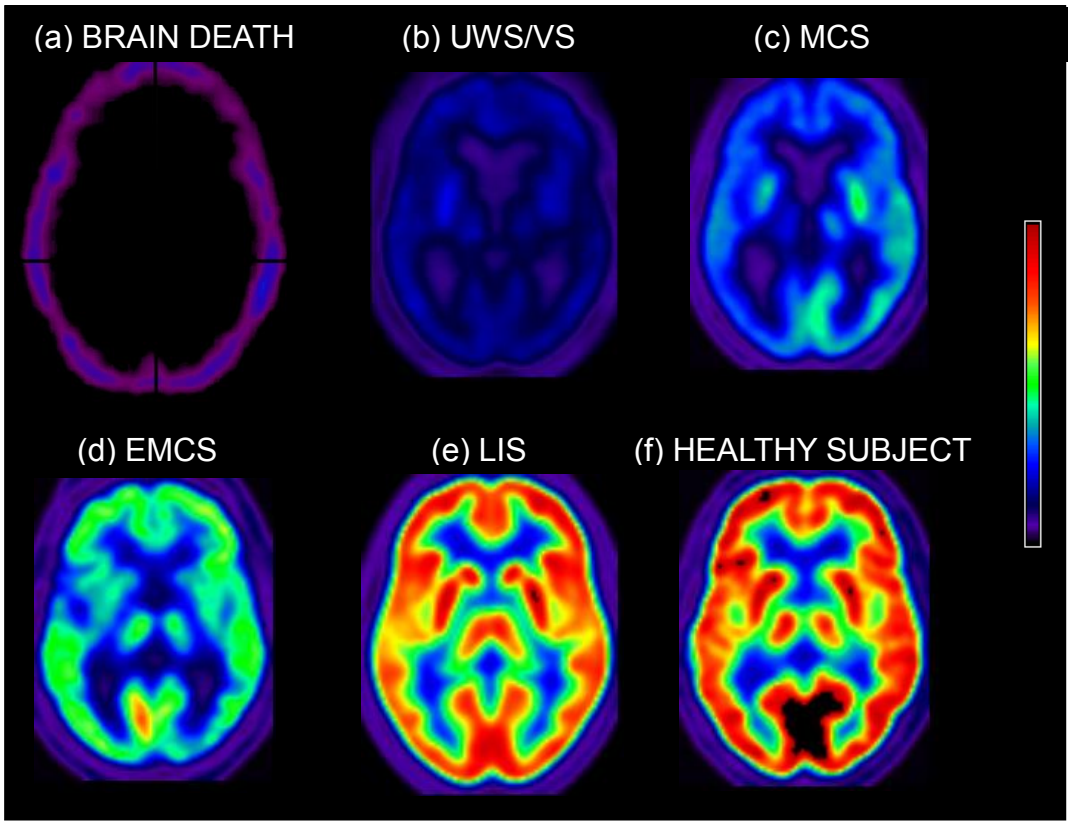


Figure 2: Cerebral brain metabolism acquired with ^{18}fluorodesoxyglucose-positron emission tomography (FDG-PET) in patients in brain death (a), unresponsive wakefulness syndrome/vegetative state (UWS/VS) (b), minimally conscious state (MCS) (c), emergence of the minimally conscious state (EMCS) (d), locked in syndrome (LIS) (e) and in healthy subjects (f). Adapted from (Laureys, Owen et al. 2004) and (Laureys 2005).

Concerning the prognosis of patients in MCS, a Belgian study demonstrated that half of patients in MCS diagnosed one month after a traumatic injury, emerged from this state at 12 months, whereas only 23 % of those in an UWS/VS (also of traumatic aetiology) improved either to MCS or emergence from this state (Ledoux, Bruno et al. 2008). However, there are reported cases of UWS/VS (Estraneo, Moretta et al. 2010) and MCS (Luaute, Maucort-Boulch et al. 2010) that have improved long after these intervals, albeit usually with a very poor functional outcome. In summary, UWS/VS of non-traumatic aetiology has the worst prognosis, with 90% of patients either dead or still unconscious at 12 months, whereas the MCS of traumatic origin has the best prognosis, with 50% emerging from that state after one year (Ledoux, Bruno et al. 2008).

Even if prognosis is better as compared to UWS/VS, some patients can remain in an MCS without fully recovering consciousness for a prolonged period of time (Giacino, Ashwal et al. 2002).

1.1.5 Locked-in syndrome

Disorders of consciousness must be differentiated from the locked-in syndrome (LIS), which usually results from lesions of the mid-pons and complete disruption of the pyramidal tracts and most of the cranial nerves (American Congress of Rehabilitation Medecine 1995). In this condition, usually following a period of coma, patients seem unresponsive. However, they are actually fully conscious and lack the ability to react to stimuli because they are completely paralyzed (except for eye movements and blinking; Gosseries, Bruno et al. 2009). LIS patients cannot move or talk due to quadriplegia but show preserved sensory and cognitive functions (Schnakers, Majerus et al. 2008), as well as normal supratentorial brain metabolism (Thibaut, Bruno et al. 2012 - figure 2e). It is vital to make the correct diagnosis, but this is unfortunately often delayed. The primary way of communication is through vertical eye movements or blinking (Gosseries, Bruno et al. 2009, Schnakers, Perrin et al. 2009). Through the recovery of distal movements (e.g. tip of a finger or head movement), chronic patients are often able to communicate with a computer and even, to control their wheelchair. Brain computer interfaces have also been tested to communicate with patients in LIS, by measuring electrical brain activity (Lule, Noirhomme et al. 2013) and pupil size (Stoll, Chatelle et al. 2013). We can divide LIS in 3 different categories based on the extent of motor impairment: (1) “classical LIS” (i.e. total immobility but preserved vertical eye movements and blinking); (2) “incomplete LIS” (i.e. remnant non-ocular voluntary motions – head or fingers movements); (3) “total LIS” (i.e. no residual mobility, no eye control). Finally, it is important to note that, according to a survey conducted in France, most chronic LIS patients report living a happy and meaningful life and the demand for euthanasia, albeit existing, is infrequent (Bruno, Bernheim et al. 2011).

1.2 Clinical diagnosis

To date, detection of signs of consciousness is mainly performed at the patient's bedside by searching for a response to command or non-reflexive behaviours in response to external stimulations. The detection of voluntary behaviours is nevertheless difficult and signs of consciousness can easily be missed due to sensory and motor disabilities, tracheostomy, or fluctuating arousal levels (Majerus, Gill-Thwaites et al. 2005). Currently, the diagnostic is extremely challenging leading to a diagnostic error rate up to 40% when it is not assessed with an appropriate standardised scale (Schnakers, Vanhaudenhuyse et al. 2009). Numerous behavioural rating scales have been developed and validated to assess levels of consciousness and to help establishing accurate diagnoses. In this section, we will review behavioural scales commonly used for the assessment of consciousness.

The Glasgow Coma Scale (GCS): The GCS was the first validated rating scale and remains the most widely used to assess to level of consciousness in acute-care settings. This scale is short to perform and can be easily incorporated into clinical daily cares. It includes three subscales that evaluate: (1) arousal level, (2) motor function, and (3) verbal abilities. The total score ranges from 3 to 15 and gives information about the conscious state of a person. The GCS has been widely investigated for its prognostic value (Jagger, Jane et al. 1983, Weir, Bradford et al. 2003, Bodart, Laureys et al. 2013, Kouloulas, Papadeas et al. 2013). Despite its extensive use, the GCS has been criticized for variable inter-rater agreement and problems in scoring patients with ocular trauma, tracheostomy, or ventilator support (Rowley and Fielding 1991, Moskopp, Stahle et al. 1995, Wijdicks, Kokmen et al. 1998). Moreover, it does not assess visual pursuit, which is one of the first sign of consciousness recovered, and which can diagnose a LIS (Giacino, Ashwal et al. 2002).

The Full Outline of UnResponsiveness Scale (FOUR): The FOUR was developed to replace the GCS to assess patients with severe TBI in intensive care (Wijdicks, Bamlet et al. 2005, Wijdicks 2006, Bruno, Ledoux et al. 2011). The scale has the advantage to be short and easy to perform and includes four subscales assessing motor and ocular responses, brainstem reflexes, and breathing. The total score ranges from 0 to 16. As compared to the GCS, the FOUR does not assess verbal functions since a high number of patients are intubated in intensive care. Moreover, it assesses visual functions and, therefore, is specifically designed to detect patients in a LIS by using oculomotor commands that detect vertical eye movements and eye blinks,

both being preserved in LIS. A score of 0 on the FOUR assumes the absence of brainstem reflexes and breathing and, consequently, helps to diagnose brain death.

The Wessex Head Injury Matrix (WHIM): The WHIM was developed to capture changes in patients in UWS/VS until emergence from post-traumatic amnesia (Shiel, Horn et al. 2000, Wilson, Elder et al. 2009). It permits to assess the cognitive evolution of the patient through items of increasing complexity. This tool is particularly sensitive to detecting small behavioural changes in patients in MCS not apprehended by traditional scales such as the GCS. The 62 items were ordered according to the mean sequence of recovery observed in 97 severely brain-injured patients who recovered from coma; this items assess arousal level and concentration, visual behaviours, communication, cognition (i.e. memory and spatiotemporal orientation), and social behaviours. The WHIM score represents the rank of the most complex behaviour observed (Wilson, Elder et al. 2009).

The JFK Coma Recovery Scale-Revised (CRS-R): The CRS was initially described by Giacino et al in 1991 (Giacino, Kezmarsky et al. 1991). The scale was revised in 2004 as the CRS-R (Giacino, Kalmar et al. 2004). It was created on the basis of the MCS's criteria and was shown to be significantly more effective than the GCS in detecting signs of awareness in non-communicating patients (Schnakers, Vanhaudenhuyse et al. 2009). Nowadays, the CRS-R seems the most appropriate scale for differentiating patients in UWS/VS and patients in MCS. The scale consists of 23 items classified in six subscales addressing auditory, visual, motor, verbal, communication, and arousal functions. Each CRS-R subscales are comprised of hierarchically arranged items associated with brain stem and cortical processes. The lowest item on each subscale represents a reflex response while the highest items represent a higher cognitive process. The purpose of the CRS-R is to assist the differential diagnosis, prognostic assessment, and treatment planning in patients with DOC. It should be administered by trained examiners and it produces stable scores over repeated assessments. Validity analyses have shown that the CRS-R is capable of discriminating patients in MCS from those in UWS/VS, which is of critical importance in establishing prognosis but also influence treatment interventions or even end of life decision (Schnakers, Giacino et al. 2006, Schnakers, Majerus et al. 2008).

However, all these scales are based on motor responses and language comprehension, which makes the diagnosis difficult since patients with DOC suffer from motor disabilities, aphasia (Majerus, Bruno et al.

2009) and fluctuation of vigilance (Giacino, Ashwal et al. 2002). It is therefore important to develop other neuroimaging tools to detect signs of consciousness when no response can be observed at the bedside.

1.3 Neural characteristics of patients with disorders of consciousness

As we discussed in the previous section, it is difficult to establish a diagnosis in patients with DOC based only on behavioural assessments. In this section, we will give an overview of the contribution that neuroimaging techniques (i.e. MRI and PET-scan) can provide in assessing non-communicative patients (for a complete review see Di Perri, Thibaut et al. 2014). Such techniques are expected to improve our understanding of brain function in states of unconsciousness and to lead to a more accurate evaluation of individual patients' cognitive abilities, providing both diagnostic and prognostic indicators. That noted, we will discuss neither electrophysiological (EEG) pattern of patients with DOC nor cortical connectivity assessed with transcranial magnetic stimulation coupled with EEG (TMS-EEG) in this section.

1.3.1 Magnetic Resonance Imaging

Structural MRI is the method of choice to detect brain oedema, contusion, haematomas, herniation, haemorrhage, hydrocephalus, or haemorrhagic shearing lesion due to diffuse axonal injuries common in post-traumatic patients (T2* sequences) (Kampfl, Schmutzhard et al. 1998, Giacino, Fins et al. 2014). However, these methods have failed to explain why some patients in an UWS/VS or in a MCS have no or minimal brain lesions. This highlights the lack of specificity and sensitivity of conventional MRI in DOC, which alone cannot be considered a reliable tool for assessing this patient category.

Functional Magnetic Resonance Imaging (fMRI)

Activation studies using visual, auditory and somatosensory stimuli have revealed high-level cortical activation encompassing the associative cortices in patients in MCS, similar to that observed in healthy controls (Di, Yu et al. 2007, Di, Boly et al. 2008). In contrast, only low-level cortical activation, limited to the primary sensory areas, was detected in UWS/VS. The minority of patients in UWS/VS with high level cortical activation often showed signs of recovery on the long term follow up (Owen, Coleman et al. 2005, Di, Yu et al. 2007). Besides the prognostic value of this technique, active fMRI paradigms have recently been performed to detect covert awareness in patients who are behaviourally unresponsive by investigating signs which are independent from motor command following, and in some cases even establishing "yes-no"

communication (Monti, Coleman et al. 2009, Monti, Vanhaudenhuyse et al. 2010, Bekinschtein, Manes et al. 2011).

Resting-state fMRI is a non-invasive technique used to investigate the spontaneous temporal coherence in BOLD (*blood-oxygen-level dependent)* fluctuations related to the amount of synchronized neural activity (i.e. functional connectivity) between distinct brain locations, in the absence of input or output tasks (Biswal, Van Kylen et al. 1997). This technique has been increasingly used in the analysis of patients with DOC, mainly because it is not invasive and it bypasses the requirement for motor output or language comprehension. To date, resting state fMRI studies suggest that activity of this default mode network (DMN – precuneus, bilateral temporo-parietal junctions and medial prefrontal cortex) decreases concurrently with the level of consciousness. It has been demonstrated, for example, that the connectivity of this network is correlated to the level of consciousness, ranging from patients in UWS/VS (low connectivity) to patients in MCS and to healthy controls (higher connectivity) (Vanhaudenhuyse, Noirhomme et al. 2010). Recently, more networks at resting state have been investigated in DOC, such as the bilateral fronto-parietal or executive control networks, salience, sensorimotor, auditory, visual systems, and the cerebellar network. It was found that, besides DMN, the bilateral executive control networks and the auditory system were also significantly less identifiable (in terms of spatial and neural properties) in patients with DOC compared to healthy controls, and showed consciousness-level dependent decreases in functional connectivity across the spectrum of DOC (Demertzi, Gomez et al. 2014).

1.3.2 Positron Emission Tomography

18-Fluorodesoxyglucose-PET (FDG-PET) studies were the first to demonstrate massive decrease in brain metabolism in patients with DOC (for a review see Thibaut, Chatelle et al. 2014). Using PET in resting state conditions, it was shown that patients in UWS/VS exhibit a decrease in brain metabolism of up to 40% of the normal value (Laureys 2005). Nevertheless, recovery from the UWS/VS does not coincide with the recovery of global metabolic levels. Instead it seems that some areas are more important to consciousness than others. In fact, patients suffering from DOC show decreased metabolism in a widespread network encompassing fronto-parietal areas, such as in the lateral prefrontal and posterior parietal regions as well

as midline anterior cingulate/mesiofrontal and posterior cingulate/precuneal associative cortices (Nakayama, Okumura et al. 2006, Silva, Alacoque et al. 2010). Importantly, recovery from the UWS/VS parallels connectivity restoration in these areas (cortico-cortical) and between these regions and the thalamus (thalamo-cortical; Laureys, Lemaire et al. 1999).

To better understand the metabolic characteristics of patients with DOC and how we can differentiate patients in UWS/VS and MCS, we realised a prospective study where we assessed brain metabolism in 70 patients in UWS/VS, MCS, EMCS and LIS (Thibaut, Bruno et al. 2012). Data were pre-processed and analysed using Statistical Parametric Mapping (SPM8). We identified areas of significant hypometabolism in UWS/VS, MCS, EMCS and LIS as compared to 39 healthy controls. We also identified brain regions showing a linear correlation with CRS-R total scores. Our results highlighted that UWS/VS patients showed metabolic dysfunction in both thalami and both extrinsic/lateral and intrinsic/medial networks, as compared to controls, while MCS patients showed metabolic dysfunction in both thalami but only in the intrinsic/medial network. EMCS patients showed hypometabolism in the posterior cingulate cortex, while LIS patients did not show metabolic dysfunction in the supra-tentorial regions (see figure 3). CRS-R total scores correlated both extrinsic/lateral network (i.e. bilateral posterior parietal and prefrontal areas) and part of the intrinsic/medial network (i.e. the precuneus and adjacent posterior cingulate cortex).

Our results in UWS/VS of different aetiologies show a widespread fronto-parietal cortical dysfunction, in agreement with previous studies (Laureys, Goldman et al. 1999, Juengling, Kassubek et al. 2005, Nakayama, Okumura et al. 2006, Bruno, Gosseries et al. 2011). We observed a hypometabolism in the external network (i.e. external awareness network, related to sensory awareness or awareness of the environment; (Vanhaudenhuyse, Demertzi et al. 2011, Demertzi, Soddu et al. 2013, Demertzi, Vanhaudenhuyse et al. 2013) encompassing left and right lateral parietal and lateral prefrontal cortices and in the internal network (i.e. intrinsic awareness network, related to internal awareness or self-related processes) encompassing midline precuneus/posterior cingulate and mesiofrontal/anterior cingulate cortices. While, in MCS patients it seems that the extrinsic/lateral network is less impaired than the intrinsic/medial network.

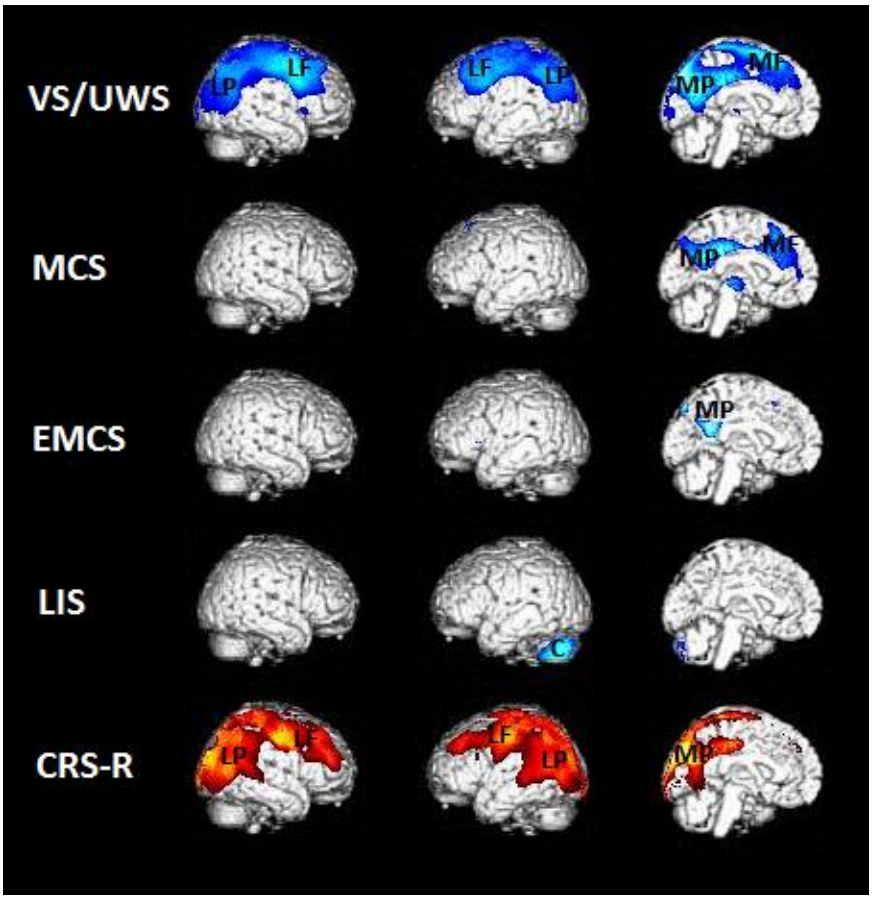


Figure 3: Areas showing metabolic impairment (shown in blue) in vegetative state/unresponsive wakefulness syndrome (UWS/VS, n=24), minimally conscious state (MCS, n=28), emergence from MCS (EMCS, n=10) and locked-in syndrome (LIS, n=8) as compared to age-matched controls (n=39) (thresholded at $p<0.01$ family wise correction for multiple comparisons). In red, areas showing a correlation with Coma Recovery Scale –Revised (CRS-R) scores are shown (thresholded at uncorrected $p<0.001$). Note that in UWS/VS there is a metabolic dysfunction in the thalamus (T), external network encompassing left and right lateral parietal (LP) and lateral prefrontal (LF) cortices, and in the internal network encompassing midline precuneus/posterior cingulate (MP) and mesiofrontal/anterior cingulate (MF) cortices. In MCS the thalamus (T) and intrinsic network are impaired (MP, MF). EMCS shows partly impaired intrinsic network activity (MP), and LIS fully preserved awareness networks, with only impairment in the cerebellum (C). The behavioural assessment scores correlate with activity in the extrinsic network (LP, LF) and part of the intrinsic network (MP). From (Thibaut, Bruno et al. 2012).

Our findings are consistent with the clinical finding that these patients show evidence of external/sensory awareness, known to depend upon the functional integrity of the extrinsic/lateral fronto-

parietal system (Bornhovd, Quante et al. 2002, Sergent and Dehaene 2004, Boly, Balteau et al. 2007, Rees 2007, Fuhrmann, Hein et al. 2008, Vanhaudenhuyse, Demertzi et al. 2011). The predominance of intrinsic/midline network impairment in MCS could reflect an impaired internal/self-awareness in these patients, difficult to quantify at the bedside. Indeed, CRS-R assessments only have one item possibly assessing some form of internal/self-awareness: visual pursuit in response to a moving mirror (Vanhaudenhuyse, Giacino et al. 2008). In summary, our data show, a progressive recovery of intrinsic network metabolic activity in severely brain-damaged patients ranging from UWS/VS, MCS, EMCS to LIS. MCS patients showed a cortical dysfunction of the intrinsic/internal awareness network more than of the extrinsic/external awareness networks. These findings indicate an impairment of a clinically barely measurable dysfunction of internal or self-awareness in MCS.

More recently, in a study in which it was compared to active fMRI, FDG-PET proved to be the more sensitive tool for identifying patients in MCS (Stender, Gosseries et al. 2014). This study included 41 patients in UWS/VS, 81 in MCS and 4 in LIS. They were assessed with repeated CRS–R, cerebral FDG-PET, and fMRI during mental activation tasks and we assessed outcome after 12 months with the Glasgow Outcome Scale–Extended. We found that FDG-PET had high sensitivity for identification of patients in MCS (93%) and high congruence (85%) with CRS–R scores. The active fMRI method was less sensitive at diagnosis of MCS (45%) and had lower overall congruence with CRS-R scores (63%). Moreover, FDG- PET correctly predicted outcome in 75 of 102 patients (74%), and fMRI in 36 of 65 patients (56%, 43–67). These findings suggest that FDG-PET is currently the most accurate neuroimaging tool for diagnosing patients in MCS.

1.4 Objectives of this work

The clinical management of patients with DOC remains very challenging, but technological advances in neuroimaging are now offering new ways to improve their diagnosis. A proper diagnosis is essential since it may contribute to premature withdrawal of life-sustaining care and lead to inappropriate medical management such as neglect of pain treatment (Giacino, Fins et al. 2014). The failure to detect sign of consciousness may also limit access to specialized neuro-rehabilitation centres, and therefore, limits patients' chances to recover. Moreover, patients in MCS are thought to have a better prognosis than UWS/VS (Luaute, Maucort-Boulch et al. 2010, Bruno, Ledoux et al. 2012) and some patients might be diagnosed in an UWS/VS or a MCS despite being in a LIS, where an accurate diagnosis would have a huge impact on the quality of life and rehabilitation of the patient. A lot of work has been done to try to diagnose patients and understand the neural correlates of consciousness. Nevertheless, only a few studies have investigated how to treat such patients in order to improve their quality of life (i.e. symptomatic treatments) and their rehabilitation (i.e. curative treatments). In this work, we investigated both parts of the problem in attempt to find ways to treat patients with DOC.

In the first part of this work, our primary concern was to improve our knowledge of clinical ways to detect signs of pain in non-communicative patients. In a recent review, we aimed to provide an overview of current knowledge on pain processing, assessment and management in patients with DOC (Thibaut, Chatelle et al. 2014). Previous studies have investigated pain processing in DOC using MRI and PET-scan, as these patients cannot communicate their feelings (Laureys, Faymonville et al. 2002, Boly, Faymonville et al. 2008). Nevertheless, in daily clinical practice it is not possible to perform such investigations to find out if a patient is in pain or not. In order to behaviourally assess pain in DOC, Schnakers and Chatelle developed a scale, the Nociception Coma Scale – Revised (NCS-R; Chatelle, Majerus et al. 2012). This validated scale aims to assess pain in patients with DOC in a way which is simple and usable for all caregivers. To further investigate the sensitivity of this scale, we assessed whether NCS-R scores could reflect nociceptive brain processing in this population of patients (Chatelle, Thibaut et al. 2014).

Secondly, we wanted to explore the prevalence and impact of spasticity in patients in UWS/VS and MCS. This motor disorder can induce pain and influence patients' quality of life (Thibaut, Chatelle et al.

2013). It is even more important to treat this motor disorder in patients with DOC since they cannot communicate, express their feelings and they are nearly always lying in bed without any voluntary or induced movements. This explains, hypothetically, why spasticity can arise more easily, be more severe and induce more serious side-effects in this specific population of patients. In addition, as we discussed above, there is a high incidence of misdiagnosis that can be put down to motor disorders (Schnakers, Vanhaudenhuyse et al. 2009). Several studies have shown that between 10 and 30% of patients behaviourally unconscious at the bedside, correctly activated the corresponding brain area following a simple command when bypassing the motor interface using fMRI or EEG (Monti, Vanhaudenhuyse et al. 2010, Cruse, Chennu et al. 2011). This considered, spasticity could be a disabling factor as it could reduce the patient's ability to behaviourally express a sign of consciousness. For these two reasons (patients' quality of life and detection of consciousness), we aimed to improve our knowledge of spasticity in patients with DOC. Our first step was to carry out a cross-sectional study evaluating spasticity in a cohort of patients in UWS/VS and MCS. We also evaluated the correlation between spasticity and potential factors of co-morbidity, frequency of physical therapy, time from insult, presence of pain, presence of tendon retraction, and aetiology and diagnosis. In another study, we tried to find a simpler way to reduce spasticity. Only passive treatments such as stretching are possible, and said treatment usually requires the presence of a physical therapist treating the patient for about 30 minutes per day. To overcome this problem, we compared the effect of soft-splints to conventional manual stretching on upper limb spasticity in chronic patients with DOC. The soft splints we used have the advantage of being easy to use, comfortable and they can be worn several hours per day without any risks or need of any supervision (Thibaut, Deltombe et al. in press).

The second part of this work focused on finding and understanding treatments to improve the recovery of severely brain-damaged patients. Another important way to enhance patients' quality of life is to stimulate their recovery and improve their interaction with their environment, with the main goal of establishing a communication. The recovery of communication, even using only a binary code, allows patients' to interact with their relatives and caregivers to tell them when and where they are in pain, how they feel, or what they want. Communication is a major improvement, first for patients, but also for clinicians and families.

There are no guidelines to date regarding the treatment of these patients and only one drug (i.e. Amantadine) showed positive results in a placebo-controlled study on a large cohort of patients with DOC (Giacino, Whyte et al. 2012). Another drug called Zolpidem has shown to be impressively efficient, inducing the recovery of communication or functional use of objects for patients in MCS (i.e. emergence from MCS). Nevertheless, its effects have been observed in only a very few patients and the mechanisms underlying its benefits have not yet been fully understood. We therefore analysed the PET-scan under both zolpidem and a placebo of three chronic post-anoxic MCS patients with known zolpidem response (Thibaut, Chatelle et al. 2014). Our objective was to identify which brain areas are activated following zolpidem intake. Moreover, the results would subsequently highlight the areas relevant to consciousness recovery.

We then turned our attention to trying to find a new way to improve patient recovery. Transcranial direct current stimulation (tDCS) has shown to improve cognitive functions in patients with neurological lesions (e.g. Parkinson's and Alzheimer disease, stroke and traumatic brain-injured patients; Thibaut, Chatelle et al. 2013). This technique has the advantage of being safe, inexpensive, easy to carry out and it does not induce seizure or severe side-effects like Amantadine or deep brain stimulation can. To find out if tDCS could be beneficial for patients with DOC we carried out a first double-blind sham controlled, randomized cross-over study (Thibaut, Bruno et al. 2014). We decided to stimulate the left prefrontal dorsolateral (DLPF) cortex as the stimulation of this area has already been shown to improve working memory and the attention of patients with neurological disorders. We enrolled patients in UWS/VS and MCS, acute and chronic, traumatic and non-traumatic- and assessed the effects using the CRS-R.

Following this first study, we wanted to then understand why some patients responded to tDCS while others did not. In order to answer this question, we investigated the relationship between tDCS responsiveness and neuroimaging data (i.e. MRI and PET) in MCS patients (Thibaut, Di Perri et al. submitted). Thanks to this study we were able to identify brain areas where a preservation of grey matter and a residual metabolic activity is needed to induce an improvement after a tDCS session. The results of this study helped us to define a subgroup of patients with DOC that can respond to this specific treatment. These results were very encouraging and we are currently carrying out further studies to determine whether the effects of tDCS can be amplified and made more durable, the likes of which would be necessary if tDCS were to be used in clinical practice.

2. Palliative treatments for patients with disorders of consciousness

"The world is a dangerous place to live; not because of the people who are evil, but because of the people who don't do anything about it."

Albert Einstein

Based on the following articles:

Thibaut A*, Chatelle C*, Whyte J, De Val MD, Laureys S, Schnakers C. Pain issues in disorders of consciousness. Brain Inj. 2014;28(9):1202-8. *Equally contributed

Thibaut A, Chatelle C, Ziegler E, Bruno MA, Laureys S, Gosseries O. Spasticity after stroke: physiology, assessment and treatment. Brain Inj. 2013;27(10):1093-105.

Thibaut A, Chatelle C, Wannez S, Deltombe T, Stender J, Schnakers C, Laureys S, Gosseries O. Spasticity in disorders of consciousness: A behavioural study. Eur J Phys Rehabil Med. 2014 [Epub ahead of print]

Thibaut A, Deltombe T, Wannez S, Gosseries O, Ziegler E, Dieni C, Deroy M, Laureys S. Impact of soft splints on upper limb spasticity in chronic patients with disorders of consciousness: A randomized, single-blind, controlled trial. Brain Inj. 2015 [Epub ahead of print].

Chatelle C, Thibaut A, Bruno MA, Boly M, Bernard C, Hustinx R, Schnakers C, Laureys S. Nociception coma scale-revised scores correlate with metabolism in the anterior cingulate cortex. Neurorehabil Neural Repair. 2014;28(2):149-52.

2.1 The challenge of managing pain in disorders of consciousness

Pain management in patients with DOC is an extremely challenging task, as assessment is limited by the absence of patients' communication and subjective report. It is, therefore, impossible for them to express their feelings or even use any usual scale (such as the Visual Analog Scale) to communicate the presence of pain and its subjective intensity (Huskisson 1982). Additionally, several conditions are likely to induce pain in acute as in chronic stages, such as polytraumatic injuries, open wounds, spasticity, tendon retraction or peripheral injuries, especially during care and mobilization. This is, however, one of the most important questions to address as it has obvious clinical and ethical implications (Demertzi, Racine et al. 2013). In this chapter, we will introduce the neuroimaging findings relative to pain perception in patients with DOC as well as the recent and current investigations performed to develop and validate behavioural protocols (such as the Nociception Coma Scale-Revised – NCS-R; Chatelle, Majerus et al. 2012) which will help clinicians assess and treat pain in these patients. We will also discuss the findings from our recent study investigating the neural correlate of the NCS-R.

2.1.1 Pain processing in patients with disorders of consciousness

Pain is defined as ‘‘an unpleasant sensory and emotional experience associated with real or potential tissue damage” (International Association for the Study of Pain 1979), whereas nociception is described as “an actually or potentially tissue damaging event transduced and encoded by nociceptors” (Loeser and Treede 2008), referring to the basic processing of a noxious stimulus. Nociception is necessary to pain perception but it will not necessarily lead to a conscious experience (Loeser and Treede 2008). On the contrary, pain is a conscious first-person experience, which has to be reported, verbally or non-verbally, to be correctly assessed. A distinction has been proposed between brain areas involved in nociception versus suffering as suffering is related to the conscious perception of pain. Neuroimaging studies suggest that pain is mediated by a widely distributed cerebral network. First of all, the stimulation of nociceptors (A-δ and C fibres) leads to the transmission of information via the spinothalamic and spinoreticular pathways to midbrain (i.e. periaqueductal matter) and the thalamus (which participates in the increase of arousal following a nociceptive stimulus). These two regions are thought to be involved in the modulation of reflex responses to nociceptive stimuli. Afterwards, nociceptive information may be transmitted to the cortex or not. In contrast, pain processing mainly involves cortical activation. The primary and secondary somatosensory cortices (S1 and S2 – lateral network) participate in the sensory–discriminative aspects of pain processing (Ploner, Schmitz et al. 1999, Ploner, Gross et al. 2002, Lockwood, Iannetti et al. 2012), whereas the cingulate, insula, and prefrontal cortices (medial network) are considered to be involved in the motivational-affective and cognitive-evaluative aspects of pain processing (Sikes and Vogt 1992, Peyron, Garcia-Larrea et al. 2000, Vogt 2005, Shackman, Salomons et al. 2011). The involvement of all these areas is what is called the “pain matrix” (Ingvar 1999). Even though recent studies support the idea that the “pain matrix” is not only related to pain but involved in multimodal processing of saliency (Mouraux, Diukova et al. 2011, Moulton, Pendse et al. 2012, Ronga, Valentini et al. 2013), the connectivity within these regions seems to play an important role in conscious perception of pain (Massimini, Boly et al. 2009, Owen, Schiff et al. 2009, Schnakers, Chatelle et al. 2012).

For patients with DOC, it is a real challenge to know whether consciousness is required for sensory perception, including nociception and pain. Using neuroimaging techniques, previous studies aimed to

objectively measure pain perception in this population of patients. Laureys et al. investigated central processing of pain stimuli by using H_2O PET imaging in post-comatose patients (Laureys, Faymonville et al. 2002). In response to an electrical stimulation applied in the median nerve at the wrist, they observed an increase of metabolism in midbrain, contralateral thalamus, and S1 in an UWS/VS (n=15). Nevertheless, the activated S1 was functionally disconnected from S2, bilateral posterior parietal, premotor, polysensory superior temporal and prefrontal cortices as compared to 15 healthy controls. For patients in an UWS/VS, the severely impaired functional connectivity in cortico–cortical pathways suggests that the activation of the primary cortex seems to be isolated from higher-order associative cortices, reducing the probability that painful stimuli are experienced in an integrated and conscious manner. Boly et al. studied the same processes for patients in MCS. They reported brain activation similar to controls in response to noxious stimuli encompassing not only midbrain, thalamus, and S1 but also S2, insular, posterior parietal and posterior part of the anterior cingulate cortex (ACC; Boly, Faymonville et al. 2008). The activation of these areas (and, particularly, ACC and insula) suggests that patients in a MCS may perceive the unpleasant aspect of painful stimuli (Bingel, Quante et al. 2002, Shackman, Salomons et al. 2011). Moreover, intact connectivity between primary and associative cortices has also been observed in these patients, suggesting the existence of an integrated and distributed neural processing which makes plausible the existence of conscious pain perception in this population.

It is important to stress that, despite these neuroimaging studies, it is still unclear whether all patients in an UWS/VS are unable to feel pain. Kassubek et al. scanned (H_2O PET) post anoxic patients in an UWS/VS during an electrical noxious stimulus. They reported atypical pain-induced activation in areas known to be involved in pain processing (i.e. posterior insula; Kassubek, Juengling et al. 2003). In parallel, two recent studies have found activation in ACC and/or insula – part of the affective pain network – in about 20 to 30% of patients in an UWS/VS in response to pain cries and noxious stimulation (Markl, Yu et al. 2013, Yu, Lang et al. 2013). This suggests that some patients diagnosed as being in an UWS/VS may have residual pain perception. As a certain number of patients diagnosed at the bedside as being in an UWS/VS, have previously shown brain activation in response to active cognitive tasks (Schnakers, Perrin et al. 2009, Monti, Vanhaudenhuyse et al. 2010, Cruse, Chennu et al. 2011), it is plausible to assume that a percentage of patients who do not show behavioural signs of consciousness may be able to perceive external stimuli such

as pain. This underlines the importance to consider potential pain experience in all patients with DOC, both MCS and UWS/VS, and to develop tools to appropriately assess and treat pain in those non-communicative patients.

2.1.2 Pain assessment in non-communicative patients

Recently, Schnakers et al. developed a new scale to assess nociception and pain in patients with DOC, the Nociceptive Coma Scale (NCS – Schnakers, Chatelle et al. 2010). The term "nociception" was chosen for two reasons. First, the NCS aimed to assess both patients in an UWS/VS and in a MCS and is therefore assessing responses underlying both high-level brain processing related to pain and low-level brain processing related to nociception. Second, as pain is a subjective experience, it is difficult to use this term when no self-report is available. The first version of the NCS (Schnakers, Chatelle et al. 2010) has been developed on the basis of pre-existent pain scales for non-communicative patients with advanced dementia (Hummel 2006) and new-borns (American Geriatrics Society 2002). It consisted of 4 subscales assessing motor, verbal and visual responses as well as facial expression to noxious stimuli. Its total score ranged from 0 to 12. This version of the NCS has been validated in patients from intensive care, neurology or neurosurgery units, rehabilitation centres and nursing homes.

Another study was performed in order to compare NCS scores observed at rest, in response to a non-noxious stimulus (i.e. tap on the shoulders) and in response to a noxious stimulus (i.e. nail bed pressure) in patients with DOC (Chatelle, Majerus et al. 2012). Results showed that the NCS total scores as well as the motor, verbal and facial sub-scores were significantly higher in response to the noxious stimulus than at rest or in response to a non-noxious stimulus, reflecting the good sensitivity of the scale. However, no difference could be observed between noxious and non-noxious conditions for the visual sub-scores. The authors therefore decided to propose a new version excluding the visual subscale, the Nociception Coma Scale – Revised (NCS-R – table 1; Chatelle, Majerus et al. 2012). Based on this version, a cut-off score of 4 has been defined as a potential clinical threshold for detecting pain or nociception for both patients in MCS and UWS/VS, with a sensitivity of 73%, a specificity of 97% and an accuracy of 85%.

MOTOR RESPONSES
3 - Localization to painful stimulation
2 - Flexion withdrawal
1 - Abnormal posturing
0 - None/Flaccid

VERBAL RESPONSES
3 – Groaning
2 - Verbalisation (intelligible)
1 – Vocalisation
0 – None
FACIAL RESPONSES
3 - Cry
2 - Grimace
1 - Oral reflexive movement/Startle response
0 - None

Table 1: the Nociception Coma Scale Revised. From (Chatelle, Majerus et al. 2012).

2.1.3 Correlation between the Nociception Coma Scale-Revised and pain matrix cortical activity

As described above, previous studies have shown that the NCS-R is a validated and sensitive tool to assess responses to noxious stimulation (Schnakers, Chatelle et al. 2010, Chatelle, Majerus et al. 2012). In the present study, we investigated whether the NCS-R was related to cortical processing of pain. Using FDG-PET scan, we looked at the correlation between NCS-R total scores and brain metabolism measured in areas known to be part of the pain matrix (Ingvar 1999, Peyron 2000, Boly, Faymonville et al. 2008).

FDG-PET was performed at rest after intravenous injection of 5 to 10 mCi (185-370 MBq) FDG on a Gemini Big Bore PET/CT scanner (Philips Medical Systems). Each patient was assessed with the NCS-R the day of FDG-PET imaging. We measured patients' responses following a noxious stimulation (i.e. applying 52±8 Newton pressure on the right and left middle fingers' nailbed) during 5 seconds (Schnakers, Chatelle et al. 2010, Chatelle, Majerus et al. 2012). Patients' diagnosis was based on the best score obtained from repeated CRS-R assessments (performed on day of scanning, two days before and two days after).

PET data were spatially normalized and smoothed using a 16 mm full width at a half maximum Gaussian kernel. Statistical analyses were performed using Statistical Parametric Mapping (SPM8; www.fil.ion.ucl.ac.uk/spm). T-contrasts identified positive and negative linear correlations between regional brain metabolism and NCS-R total scores. Differences in the level of consciousness (i.e. CRS-R total scores), diagnosis (i.e. UWS/VS versus MCS), aetiology (i.e. traumatic versus non traumatic) and interval since insult (i.e. acute/sub-acute vs longstanding; >1 year post-insult) were modelled as additional covariates in the design matrix. Global normalization was performed by proportional scaling. Results were considered significant at $p<0.05$ small-volume corrected for multiple comparisons, using 10 mm-radius spheres centred on a priori coordinates for areas previously identified in pain processing in DOC (i.e., thalamus, S1, S2, insula and ACC; Boly, Faymonville et al. 2008).

Forty-two patients were included (29 males, aged 42±17 years, 24 ± 32 months post-insult, 19 TBI; 26 MCS), 22 were in an acute/sub-acute stage (i.e. <1 year post-insult). We identified a significant positive correlation between metabolism and NCS-R total scores in the posterior ACC ($Z=2.43$; corrected $p=0.038$; Talairach coordinates x=12, y=2, z=42, see figure 4). Moreover, this area positively correlated with NCS-R

and not with CRS-R total scores (Z= 2.74; corrected p= 0.018; Talairach coordinates x=20, y=16, z=36 mm). We did not find a significant effect of level of consciousness, aetiology or chronicity. No negative correlations with NCS-R scores were observed.

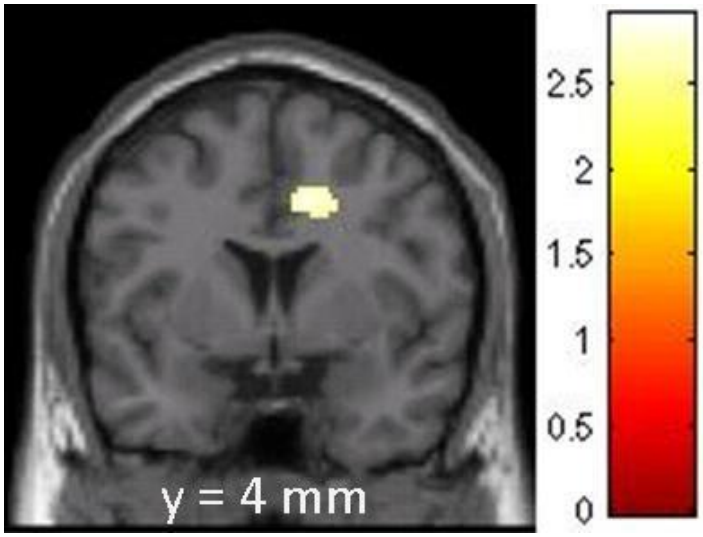


Figure 4. Metabolism in the posterior part of the anterior cingulate cortex (ACC) showed a positive correlation with Nociception Coma Scale-Revised total scores (NCS-R). Results were considered significant at p<0.05 small-volume corrected for multiple comparisons. From (Chatelle, Thibaut et al. 2014).

Summary and discussion

In this study, we investigated the correlation between regional brain metabolism and NCS-R total scores in patients with DOC and highlighted the posterior ACC. According to previous neuroimaging studies on pain perception, the posterior ACC is part of the network involved in the cognitive and affective aspects of pain processing (Peyron 2000). This region is a hub where information about negative reinforcers and pain can be linked to motor centres responsible for expressing affect and executing goal-directed behaviour (Shackman, Salomons et al. 2011). It should be noted that metabolism in the ACC correlated with NCS-R total scores and not with CRS-R total scores, suggesting that the NCS-R reflects nociception and pain rather than differences in patients' level of consciousness.

Near-normal regional cerebral blood flow increases in response to nociception in MCS patients, including the ACC was reported in a previous study (Boly, Faymonville et al. 2008). In contrast, a study on UWS/VS patients showed isolated S1 activation to noxious stimuli, disconnected from the rest of the brain (Laureys, Faymonville et al. 2002), while others have reported residual activation in the ACC in some patients behaviourally diagnose as being in an UWS/VS (Markl, Yu et al. 2013). In the present study, we did not find an effect of the clinical diagnosis (UWS/VS versus MCS) which support the idea that some behaviourally "unconscious" patients might actually show higher cortical processing, not detectable at the bedside. Given the high rate of misdiagnosis reported in this population (Monti, Vanhaudenhuyse et al. 2010, Cruse, Chennu et al. 2011), we think it is important to use a sensitive scale to manage possible pain in every patient, both UWS/VS and MCS (Schnakers, Chatelle et al. 2012).

Recently, some authors supported the idea that the network encompassing the pain matrix (including the ACC) is not specific to pain processing but rather be involved in multimodal processing (Menon, Ford et al. 1997, Iannetti, Hughes et al. 2008, Lui, Duzzi et al. 2008, Mouraux and Iannetti 2009, Mouraux, Diukova et al. 2011, Moulton, Pendse et al. 2012, Ronga, Valentini et al. 2012). Indeed, those studies have reported the activation of this network in other modalities such as auditory, visual or non-noxious stimulations. However, if not uniquely involved in pain processing, the key role of the posterior ACC in the cognitive evaluative dimension of pain (e.g. avoidance behaviour, attention shifting) has been supported by numerous studies (Peyron 1999, Vogt 2005, Shackman, Salomons et al. 2011). Lesion studies reported that ACC damages in humans and animals induced a decrease in pain affect associated with a preserved sensory

discriminative ability (Foltz and White 1962, Vaccarino and Melzack 1989, Sikes and Vogt 1992). Moreover, previous studies showed that an increased activity in the posterior ACC prior to painful stimulation was linearly associated with increased painfulness in conscious healthy volunteers (Boly 2007), and that this area showed a reduced activity during hypnosis-induced analgesia (Rainville 1997, Vanhaudenhuyse, Boly et al. 2009). Altogether, those results suggest that posterior ACC is a key area for the cognitive integration of pain (Peyron 2000, Shackman, Salomons et al. 2011).

In conclusion, our results support the hypothesis that the NCS-R is related to cortical processing of nociception and hence may constitute an appropriate behavioural tool to assess pain perception in non-communicative patients with severe DOC. Future studies using event-related fMRI should investigate the correlation between NCS-R scores and brain activation and connectivity in response to noxious stimulation at the single-subject level in this challenging patient population.

2.2 Spasticity in disorders of consciousness

Spasticity is a very common motor disorder that can affect patients after a stroke or acquired brain damage. The mechanisms underlying this disorder are not yet completely understood. Spasticity can induce pain and decrease the potential recovery of patients (Thibaut, Chatelle et al. 2014). It is, therefore, very important to take this into account when discussing the treatment of patients with DOC as (i) they are unable to communicate their sensations, (ii) they are more likely to be misdiagnosed (i.e. being considered unconscious, whilst actually being conscious, because of limited motor abilities), (iii) their participation in active rehabilitation programs is even more limited in the case of spasticity.

In this chapter we will provide an overview regarding assessments and treatments (pharmacological and physiotherapeutic) of spasticity for brain-injured patients. We will then discuss the findings from our study assessing the occurrence and clinical impact of spasticity in patients with DOC. Finally, we will report the results of our single-blind randomized sham-controlled trial study which aims to assess the efficacy of soft splints on upper limb spasticity of chronic patients with DOC.

2.2.1 Spasticity: Principles

Definition

Spasticity was first described by Lance in 1980 as "*a motor disorder characterized by a velocity dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyperexcitability of the stretch reflex, as one component of the upper motor neuron syndrome*" (Lance 1980). This description characterizes spasticity during passive movement, but does not take into account its effects on voluntary gestures. In 1994, Young added neurophysiological elements to define spasticity independently of the type of movement: "*a motor disorder characterized by a velocity-dependent increase in tonic stretch reflexes that results from abnormal intra-spinal processing of primary afferent input*" (Young 1994). There is, however, still no consensus for the definition of spasticity, and this reflects the complexity and the diversity of the phenomena. This is especially true for motor disorders occurring after a brain damage, which can give rise to a considerable variety of symptoms (e.g. clonus, dystonia, muscle weakness, abnormal reflex responses). The name "*upper motor neuron syndrome"* defines spasticity as one of the motor disturbances appearing after a brain lesion, and merges it with other motor symptoms which occur after lesions in the descending corticospinal system (Mayer and Esquenazi 2003). According to this definition, spasticity is one of the positive signs of the upper motor neuron syndrome (see table 2).

Positive signs	
Increased tendon reflexes	Result from hyper-excitability of the stretch reflex
Clonus	Series of involuntary, rhythmic, muscular contractions and relaxations due to a self-re-excitation of hyperactive stretch reflexes in the affected muscle
Positive Babinski sign	Extension of the big toe, while the other toes fan outwardly in response to rubbing of the sole of the foot. It indicates a lesion of the corticospinal tract
Spasticity	Muscle hypertonia, dependent upon velocity of muscle stretch
Extensor/flexor spasms	Spasms occur spontaneously or in response to stimulation (movement of the leg, change of position). The commonest pattern of flexor spasm is flexion of the hip, knee and ankle
Spastic co-contraction (during movement)	Agonist and antagonist muscles co-contract simultaneously inappropriately and thus disrupt normal limb movement. This is due to the perturbation of the spinal reflexes that contribute to reciprocal innervation
Associated reactions and other dyssynergic stereotypical spastic dystonia	Remote form of synkinesis due to a failure to inhibit spread of motor activity (e.g. flexion of the elbow simultaneously to flexion of the hip during walking)
Negative signs	
Muscle weakness	Muscles have lower strength due to the loss of corticospinal drive
Loss of dexterity	Loss of hand precise movements, such as opposition of the thumb due to a weakness of the intrinsic and extrinsic hand muscles
Fatigability	Greater effort required to perform a movement leading to tiredness

Table 2: Description of positive and negative signs observed in case of upper motor neuron syndrome due to stroke (Sheean 2002).

Spasticity is a serious complication to brain injury, often accompanied by dyskinesia, spasms or muscle flaccidity (Ward 2012). This disorder results from impaired reflex functions and pathological changes in rheologic muscle properties such as atrophy, stiffness and fibrosis (Dietz and Sinkjaer 2007). In addition to hyper-excitability of the stretch reflex, patients may suffer from spastic dystonia (i.e. muscle constriction in the absence of voluntary movement), and/or spastic co-contraction (i.e. contraction of both agonist and antagonist muscles) (McComas 1994, Sommerfeld, Eek et al. 2004, Gracies, Bayle et al. 2010). These modifications can induce pain and reduce functional autonomy (Doan, Brashear et al. 2012). Spasticity has

also been reported to be associated with tendon retraction, fixed equinovarus feet and pain in patients suffering from multiple sclerosis (Svensson, Borg et al. 2014) or stroke (Ada, O'Dwyer et al. 2006, Malhotra, Pandyan et al. 2011, Brainin 2013). All these complications increase the clinical impact of spasticity on recovery by impeding the patient's ability to perform activities of daily living and by increasing the cost of treatment (Brainin 2013, Zorowitz, Gillard et al. 2013, Svensson, Borg et al. 2014). Spasticity occurs in approximately 25 to 42% of patients with stroke (Urban, Wolf et al. 2010, Wissel, Schelosky et al. 2010). Although the onset is usually within the first few days or weeks post-insult (Mayer and Esquenazi 2003), spasticity may appear in the short-, medium-, or long-term period post-insult (Ward 2012).

A study by Wissel et al. (2010) showed that 25% of stroke patients suffer from spasticity within the first six weeks of the event. They also observed that spasticity primarily affects the elbow (79% of patients), the wrist (66%), and the ankle (66%) (Wissel, Schelosky et al. 2010). In the upper limbs, the most frequent pattern of arm spasticity is internal rotation and adduction of the shoulder coupled with flexion at the elbow and neutral positioning of the forearm and wrist (Marciniak 2011, Hefter, Jost et al. 2012). A high degree of paresis and hypoesthesia at stroke onset has been suggested as one of the predictors for the development of spasticity (Urban, Wolf et al. 2010, Coupar, Pollock et al. 2012). Other risk factors were also identified for the development of permanent spasticity after a stroke: (i) any paresis in affected limb, (ii) more severe paresis at 16 weeks compared to the first week, (iii) Modified Ashworth Scale (MAS) ≥ 2 in at least one joint within 6 weeks after stroke, (iv) more than two joints affected by increased muscle tone, (v) hemispasticity within 6 weeks after stroke, and (vi) lower Barthel Index at baseline (Wissel, Schelosky et al. 2010). Variables that can reliably predict recovery have yet to be identified. But before this can happen, we need to properly assess spasticity and various scales are being used in this context.

How to assess spasticity? Review of the most commonly used scales

Several scales have been developed and validated to assess spasticity in brain-injured patients. The two most commonly used are the Modified Ashworth Scale (Bohannon and Smith 1987) and the Modified Tardieu Scale (Tardieu, Shentoub et al. 1954, Held and Pierrot-Deseilligny 1969). These scales assess the degree and angle of muscle contraction, and in the case of retraction, the amplitude of the permitted movement.

The Modified Ashworth Scale (MAS): this scale measures the level of resistance to passive movement, but does not evaluate the velocity of passive joint movement, the angle of contraction outbreak, or potential tendon retraction (Pandyan, Johnson et al. 1999) (table 3). The MAS is effective in clinical practice because of its ease and speed of use. Moreover, this scale is widely used in research and has been highly investigated in many studies (e.g. to objectively measure the effect of a treatment; Gracies, Fitzpatrick et al. 1997, Brashear, Gordon et al. 2002, Brashear, Zafonte et al. 2002, Sommerfeld, Eek et al. 2004). Unfortunately, validation studies only showed "moderate" to "good" intra-rater reliability and "poor" to "moderate" inter-rater reliability (Lee, Carson et al. 1989, Brashear, Zafonte et al. 2002, Ghotbi, Nakhostin Ansari et al. 2011). Even if this scale seems to measure the resistance adequately, the reduced range of joint motion due to contractures might also limit its reliability (Mehrholz, Major et al. 2005). It is now established that the MAS evaluates a combination of soft tissue contracture and spastic dystonia, in addition to spasticity itself (Thakker and Rubin 2004, Gracies, Burke et al. 2010). Furthermore, it is not velocity dependent, as Lance's definition of spasticity specified.

The Modified Tardieu Scale (MTS): in comparison with the MAS, the MTS does take into account the velocity of passive joint movement, the angle of contraction outbreak, and the potential tendon retraction. In this scale, spasticity is assessed with three velocities (low, normal, and fast; see table 3), and the snap angle is reported as the angle of retraction (Held and Pierrot-Deseilligny 1969, Boyd and Graham 1999, Mehrholz, Wagner et al. 2005). The MTS tends to be more sensitive in the detection of post-treatment changes because it measures muscle resistance as well as the velocity of the movement that induces muscular contraction (Katz, Rovai et al. 1992, Gracies, Marosszeky et al. 2000, Mehrholz, Wagner et al. 2005). When comparing the two scales, the distribution of the mean scores correlates poorly (Mehrholz, Wagner et al. 2005). This may be explained by the fact that these two scales measure two different dimensions (Mehrholz, Wagner et al. 2005). The MTS is closer to Lance's definition, as it assesses spasticity at three different velocities. Considering its good inter- and intra-subject reliability (Brashear, Zafonte et al. 2002), the MTS might be a more appropriate instrument for the measurement of spasticity than the MAS. Although this scale seems more accurate than the MAS, its validity still needs to be assessed (Yelnik, Simon et al. 2010). In theory, this scale demonstrates several advantages over the MAS, as it uses both a fast and slow speed of movement, and incorporates an interval level measure (range of movement; ROM), as well

as a subjective rating scale. Further studies need to be performed on a larger population to confirm the reliability and the specificity of this scale for assessing spasticity.

Modified Ashworth Scale	
0	No increase in muscle tone
1	Slight increase in muscle tone, manifested by a catch or by minimal resistance at the end of the range of motion (ROM) when the affected part(s) is (are) moved in flexion or extension
1+	Slight increase in muscle tone, manifested by a catch, followed by minimal resistance throughout the remainder (less than half) of the ROM
2	More marked increase in muscle tone through most of the ROM, but affected part(s) easily moved
3	Considerable increase in muscle tone, passive movement difficult
4	Affected part(s) rigid in flexion or extension
Modified Tardieu Scale	
X: Quality of movement mobilization	
0	No resistance throughout the course of the passive movement
1	Slight resistance throughout the course of passive movement, no clear catch at a precise angle
2	Clear catch at a precise angle, interrupting the passive movement, followed by release
3	Fatigable clonus with less than 10 seconds when maintaining the pressure and appearing at the precise angle
4	Unfatigable clonus with more than 10 seconds when maintaining the pressure and appearing at a precise angle
5	Joint is fixed
V : measurements take place in 3 different velocities	
V1	As slow as possible
V2	Speed of limb segment falling under gravity
V3	As fast as possible
Y: Angle of catching (muscle reaction)	

Table 3: The Modified Ashworth Scale (MAS; Bohannon and Smith 1987) and the Modified Tardieu Scale (MTS; Held and Pierrot-Deseilligny 1969).

Treatments: current knowledge

Pharmacological treatments: treatment to reduce spasticity should be introduced when the patient suffers from motor disability due to spasticity. Motor hyperactivity is the only motor disorder that can benefit from drug treatment. Medication should be tailored according to the lesion area (e.g. cortical, spinal cord) and the intended effects. Pharmacological treatments either act on the central nervous system (CNS) or directly on the muscle. Several treatments aim to reduce muscle tone, either through oral or injectable administrations, or through an intrathecal pump. Their efficacy, however, remains controversial and not well understood. A summary of the most commonly used treatments are recapitulated in table 4; for a complete review see (Thibaut, Chatelle et al. 2013). Unfortunately only a few studies are double-blind sham controlled and the posology of each treatment can vary considerably from country to country, or even from a hospital to another one.

Drug	Dose (admin)	Mechanism of action	Side effects
Diazepam	5-20 mg 3 times daily (oral)	Increases the affinity of GABA for the $GABA_a$ receptor complex leading to an increase in presynaptic inhibition and reduction of synaptic reflexes	Sedation, weakness, hypotension, adverse gastrointestinal effects, memory trouble, confusion, depression, and ataxia
Clonazepam	0.5 to 1.0 mg once daily (oral)	Idem	Weakness, hypotension, ataxia, dyscoordination, sedation, depression, and memory impairment. Prolonged use could increase the risk of addiction
Gabapentin	240-360 mg daily (oral)	Structurally similar to the GABA. Increases the brain level of GABA	Fainting, somnolence, nystagmus, ataxia, headache, tremor
Baclofen	5-20 mg 3-4 times daily (oral)	Centrally acting GABA analogue. Binds to $GABA_b$ receptor at the presynaptic terminal and inhibits the muscle stretch reflex	Sedation, dizziness, weakness, fatigue, nausea. Lowers seizure threshold

Tizanidine	4-36 mg daily (oral)	Imidazole derivative, with agonist action on alpha-2 adrenergic receptors in CNS	Sedation, dizziness, mild hypotension, weakness, hepatotoxicity
Dantrolene	25-100 mg 4 times daily (oral)	Interferes with the release of calcium from the sarcoplasmic reticulum of the muscle	Generalized muscle weakness, mild sedation, dizziness, nausea, diarrhoea, hepatotoxicity
Phenol/ alcohol	30 mg/kg (injectable)	Chemical denervation of the muscles	Burning and dysesthesias. Damage of the sensory nerves and pain
Botulinum toxin	10-15 units/kg (injectable)	Inhibit the release of acetylcholine at the neuromuscular junction	Pain (during injection) Local weakness Swallowing trouble for patients with respiratory and swallowing disorders
Intrathecal baclofen	25-1000 µg daily (IP)	Binds to GABAB receptor at the presynaptic terminal and inhibits the muscle stretch reflex	Decreased ambulation speed and muscle weakness

Table 4: Dosing, mechanism of action and side effects of pharmacological treatment of spasticity. Adapted from (Satkunam 2003, Yelnik, Simon et al. 2009, Lapeyre, Kuks et al. 2010). IP= intrathecal pump.

Physical therapy: The basic treatment for all patients with spasticity is physical therapy (Gracies 2001, Watanabe 2004). Limiting muscle contractures and reducing hyperactivity for at least a short period of time can be helpful for patients. The aim of stretching is to improve the viscoelastic properties of the muscle-tendon unit and to increase its extensibility. Other structures can also be put under tension, such as tendons or connective, vascular, dermal, or neural tissue (Harvey, Herbert et al. 2002, Bovend'Eerdt, Newman et al. 2008). There is, however, no consensus about the optimal frequency, intensity, velocity, and duration of stretching. A recent systematic review of the effectiveness of stretching to treat and prevent contracture in patients with brain injuries concluded that stretching does not induce significant changes in joint mobility, pain, spasticity, or activity limitation (Katalinic, Harvey et al. 2010). Another method, casting, immobilizes the limb in a stretch position and induces prolonged muscle stretching. This technique allows

improving muscle length, increasing joint range of motion, and reducing contracture, pain, and spasticity (Watt, Sims et al. 1986). However, there are no guidelines yet, nor any scientific evidence that this method can reduce spasticity caused by neurological disorders (Lannin, Novak et al. 2007). Beside muscle stretching, muscle strength training is also used to recover functional motricity (Morris, Dodd et al. 2004). One of the most widely used approaches is the Progressive Resistance Strength Training, although, at this time, there is no gold standard for strengthening protocols (Sunnerhagen, Olver et al. 2013). Other physical therapies are used to decrease spasticity and improve motor function, such as Bobath technique, which is based on the reduction of spasticity and postural reflexes prior to facilitating voluntary activity in paretic muscles through attention to trunk posture as well as controlled muscle stretch at the limbs (Bobath 1979). Nonetheless, only few studies showed that this technique is efficient to reduce spasticity in patients suffering from a stroke (Ansari and Naghdi 2007). Additional therapies, like hydrotherapy, cryotherapy, thermotherapy, vibratory stimuli or neurodevelopmental inhibitory techniques, and robotic are used to relax muscles and to reduce the intensity of spasticity. Future studies should also be investigated to determine their effectiveness (Sunnerhagen, Olver et al. 2013).

Splints: these devices are frequently used in complement with physiotherapy sessions. Several types of splints exist but, as with physiotherapy, no practical guidelines have been defined so far. The aims of splinting are reductions in spasticity and pain, improvement of function, compensation for protective sensation, and prevention of contracture and deformity (Neuhaus, Ascher et al. 1981). The principal advantage of splints is the duration of their effectiveness, because they can be placed and left for several hours without the presence of a physiotherapist or nurse. Nevertheless, their efficacy is not yet proven by any double-blind studies. However, selective groups of patients seem to have benefited from some types of splints (Flinn and Craven 2014).

Summary

Spasticity seems to affect more than one out of four patients following a brain injury (Wissel, Schelosky et al. 2010). The complexity and the diversity of spasticity make the identification of its underlying mechanisms and predisposing factors complex. Understanding of the spasticity phenomenon is essential so that drugs and therapeutic strategies can be developed to efficiently treat causes rather than symptoms. Failures in motor neuron activation and alterations in spinal motor neurons appear to be two major

components of the pathophysiology behind paresis following a brain lesion. However, motor impairment due to paresis is greatly exacerbated by the muscles, the joint contracture, and the changes in muscle contractile properties caused by immobilization. In addition, chronic disuse causes an alteration of CNS function that further reduces the ability to voluntarily recruit motor units (Gracies 2005).

In terms of treatment, physical therapy and pharmacological treatment are essential for avoiding retraction and joint fixation, but to date, except for Botulinum toxin on the upper limb, there are no scientific guidelines for the application of different therapies in patients suffering from spasticity. For this reason, clear guidelines need to be developed regarding the revalidation of patients with spasticity through physical therapy, pharmacological treatment and other techniques. To do so, double-blind randomized controlled studies on pharmacological and non-pharmacological treatments should be performed to improve our insight into spasticity. However, no study investigating this motor disorder in a large cohort of patients with DOC has yet been performed. Firstly, therefore, we need to characterize spasticity in this specific population of patients.

2.2.2 Influence of spasticity on patients with disorders of consciousness

The occurrence of spasticity in severe brain-damaged patients with DOC has been poorly explored. As mentioned earlier, these patients are unable to express their feelings and cannot communicate about potential discomfort or pain (Schnakers and Zasler 2007, Chatelle, Thibaut et al. 2014). To our knowledge, only a few studies of small sample size have described motor patterns in this population. These studies reported the presence of abnormal primitive reflexes (Lapitskaya, Moerk et al. 2013), altered tonus, considerable posturing and varied degrees of reduced range of joint motion (Pilon and Sullivan 1996, Leong 2002) as well as abnormal cortical excitability of the motor cortex (Lapitskaya, Gosseries et al. 2013). In addition, due to severe motor impairments, which accompany spasticity, some patients fail to show clinical signs of consciousness, thus leaving them vulnerable to misdiagnosis (Bekinschtein, Coleman et al. 2008, Monti, Vanhaudenhuyse et al. 2010, Cruse, Chennu et al. 2011, Cruse, Chennu et al. 2012, Habbal, Gosseries et al. 2014, Stender, Gosseries et al. 2014). Thus the need to understand and prevent spasticity in this population is urgent.

The aim of our first study was to measure the occurrence and clinical impact of spasticity in patients with DOC. We assessed the presence of spasticity in a cohort of chronic patients in UWS/VS or MCS. We also investigated the correlation between the degree of spasticity and the level of consciousness, aetiology, potential factors of co-morbidity (i.e. tendon retraction in the upper extremities and fixed equinovarus feet), level of treatment (i.e. application of anti-spastic medication and physiotherapy), time since insult and presence of pain.

In this study, we included 65 patients (22 women; mean age: 44±14 years; 40 traumatic aetiology; time since insult: 39±37 months). Patients were diagnosed as being in UWS/VS (n=25) or in MCS (n=40) based on repetitive assessments using the CRS-R (Giacino, Kalmar et al. 2004, Schnakers, Majerus et al. 2008). Anti-spastic medication was classified as oral treatments (baclofen, clonazepam, tizanidine) or intrathecal baclofen therapy. Inclusion criteria were: 1) a diagnosis of UWS/VS or MCS, 2) time since onset of condition more than 3 months, and 3) age 16 years and over. Exclusion criteria were: 1) documented neurological disorders previous to the acquired brain damage, and 2) presence of skin or musculoskeletal lesions (e.g. bedsores, fractures, wounds). All patients were examined by the same physiotherapist with the

MAS (Mehrholz, Wagner et al. 2005) to minimize inter-rater variability. The score of the most affected upper limb muscle group was used for our correlation analyses. Among the 65 studied patients, 48 were also assessed with the NCS-R to assess behavioural signs of pain (Chatelle, Majerus et al. 2012).

MAS data were evaluated on a scale ranging from 0 to 5, assigning the 1+ a value of 2, the 2 a value of 3, and so on. We used the Mann-Whitney U tests to investigate the difference of MAS scores according to the level of consciousness (i.e. UWS/VS versus. MCS), joint deformities (i.e. presence vs. absence of upper limb tendon retraction and equinovarus feet), and medication (i.e. presence vs. absence of pharmacological treatment) (Hart 2001). We used the Wilcoxon test to assess differences in MAS scores between upper and lower extremities. Correlations between MAS scores and NCS-R total scores, time since insult, and frequency of physical therapy were assessed with Kendall's Tau tests (Kendall 1938, Brown and Hayden 1985). Differences in MAS scores according to the aetiology (i.e. anoxic, haemorrhagic, traumatic and mixt) were assessed with Kruskal-Wallis ANOVA. Statistical analysis was performed using Statistica 10.0 with statistical significance set at the 5% level.

We found that, out of 65 patients, 58 showed signs of spasticity (89%; MAS ≥ 1). Out of these 65 patients, 40 suffered from severe spasticity (62%; MAS ≥ 4) (see table 5). Seven patients (11%) showed no signs of spasticity (MAS=0) including five patients (8%) who were flaccid. Six patients (9%) had a maximal score of 2, 12 (18.5%) had a maximal score of 3, 12 (18.5%) had a maximal score of 4 and 28 (43%) had a maximal score of 5. Spasticity was more important for upper limbs than lower limbs (T=446.5; Z=2.55; p=0.01, see table 6).

A negative correlation was found between MAS scores and the frequency of physical therapy for both the upper limbs (tau=-0.20, Z=-2.37; p=0.018) and the lower limbs (tau=-0.20; Z=-2.41; p=0.016). On the other hand, a positive correlation was found between MAS scores and time since insult for both upper limbs (tau=0.23; Z=2.71; p=0.007) and lower limbs (tau=0.21; Z=2.46; p=0.014), and between MAS scores and NCS-R total scores for the upper limbs only (upper limbs, tau= 0.31, Z=3.11; p=0.001; lower limbs: tau=0.18; Z=1.80; p=0.072).

Presence of	% of patients – IC 95% (n=65)
Spasticity	89 ± 12%
Severe spasticity (MAS ≥ 4)	62 ± 11.8%
Upper extremity tendon retraction	42 ± 10.8%
Fixed equinovarus feet	57 ± 11.7%
Medication	67 ± 12%

Table 5: Percentage of motor disabilities and medication.

Twenty-seven patients had tendon retraction in the upper limbs (i.e. metacarporphalangean joint, wrist and elbow) and 37 fixed equinovarus feet (see table 5). The presence of retraction was associated with higher MAS score for the upper limbs ($U=155$; $Z=4.71$; $p<0.001$) and equinovarus feet were associated with higher MAS scores of the lower limbs ($U=139.5$; $Z=4.89$; $p<0.001$).

Regarding medication, 39 out of 58 patients who showed sign of spasticity received oral anti-spastic treatment (34 baclofen, 3 tizanidine, 2 clonazepam), 4 patients received intrathecal baclofen therapy and 15 patients did not receive any pharmacological treatment. Patients on anti-spastic medication (n=43, 74%) showed more spasticity than patients without anti spastic treatment for the lower limbs ($U=209.5$; $Z=2.52$; $p=0.01$) but not for the upper limbs ($U=260$; $Z=1.67$; $p=0.09$, see table 6).

Upper and lower limbs MAS scores did not differ according to the aetiology ($U=1.75$; $p=0.63$ and $U=0.76$; $p=0.86$, respectively). No difference was found between MAS score and the level of consciousness (upper limbs: $U=459$; $Z=0.55$; $p=0.59$; lower limbs: $U=477.5$; $Z=-0.30$; $p=0.76$, see table 6).

Comparison	Limbs		MAS median(IQR)	p value
UL and LL	UL		4 (1)	p=0.01*
	LL		3 (3)	
Treatment	UL	Medicated	3.5 (1)	p=0.09
		Unmedicated	3 (2)	
	LL	Medicated	3 (2)	p=0.01*
		Unmedicated	2 (1)	
Aetiology	UL	Trauma	4 (1)	p=0.56
		Anoxia	4 (5)	
		Subarachnoid hemorr.	4 (1.5)	
		Mixed	3.5 (3.5)	
	LL	Trauma	4 (1)	p=0.87
		Anoxia	2 (4)	
		Subarachnoid hemorr.	2 (1.5)	
		Mixed	4.5 (2.5)	
Diagnosis	UL	UWS/VS	3.5 (2)	p=0.59
		MCS	3 (2)	
	LL	UWS/VS	2 (3.5)	p=0.76
		MCS	2.5 (2)	

Table 6: Results of group comparisons with median and interquartile range (IQR) of the MAS and p value. Abbreviations: MAS= Modified Ashworth Scale; UL= upper limbs; LL= lower limbs; UWS/VS= vegetative state/unresponsive wakefulness syndrome; MCS= minimally conscious state. * indicated a significant result (p>0.05).

Summary and discussion

Current literature reports the presence of spasticity in 25 to 42% of patients after stroke or traumatic brain injury (Elovic, Simone et al. 2004, Urban, Wolf et al. 2010, Wissel, Schelosky et al. 2010). In our cohort of 65 chronic patients with DOC, 89% showed spasticity, 62% of which to a severe degree. This result suggests that spasticity is more frequent in this population of patients, than in patients with milder brain injuries. This high rate of spasticity supports previous results from a pilot study conducted by Pilon and collaborators, reporting important motor and posturing impairments in 12 patients with DOC (Pilon and Sullivan 1996). Extensive brain lesions, prolonged immobility, as well as weakness, disuse, and absence of

muscle movement in contracted positions are, since they are known to increase spasticity and contracture, likely to be causative factors, (Gracies 2005). Our analyses reported a negative correlation between the degree of spasticity and the frequency of physical therapy. This result suggests that frequent physiotherapy may have a positive effect on patients' spasticity. One could argue, however, that patients with less spasticity might receive more physical therapy as our result is based on a correlation. It is our view, however, that this is less likely to be the case as the amount of physical therapy is not determined by the severity of spasticity, but rather the health system of the country and insurance reimbursement. In fact, it is possible that patients showing more spasticity may actually receive less physical therapy, especially at chronic stage. Some patients may show signs of pain (e.g. grimace or other facial expressions) during stretching, which may make the physical therapist reduce the time of stretching or even stop it altogether. Another reason why patients could receive less therapy is that high levels of tendon retraction or joint fixation makes stretching very limited and, this, physical therapy is less effective. Overall, our results do not allow us to claim that less spasticity is the result of more physical therapy.

In the present study, spasticity appeared to increase over time. This result highlights problems of patient management (e.g. mobilization, stretching) associated with immobility. Spasticity and immobilization induce adaptive anatomical muscle change and reflex modifications (e.g. muscle atrophy, loss of sarcomeres and accumulation of connective tissue and fat; Gracies 2005), thus constituting a self-reinforcing negative effect. A positive correlation between upper limb spasticity and pain was previously observed in other patients with neurological disease (e.g. multiple sclerosis; Kheder and Nair 2012). This finding is critical as patients with DOC are, by definition, unable to communicate potential discomfort (Chatelle, Thibaut et al. 2014). Interventions to alleviate potential pain are, therefore, mandatory in this population of patients. Concerning side-effects, about half of our sample suffered tendon retraction, the presence of which was associated with higher levels of spasticity. This supports the notion that spasticity increases the risk of tendon retraction (Ada, O'Dwyer et al. 2006, Malhotra, Pandyan et al. 2011). Joint immobilization could also be a driving factor in this regard (for a review see Gracies 2005).

Surprisingly, patients not administered anti-spastic medication had lower MAS scores, for lower limbs specifically, than medicated patients. This probably indicates that patients who do not show signs of spasticity do not need anti-spastic medication, whilst patients who do suffer spasticity need it to decrease

spasticity severity. This treatment, however, may not be sufficient to completely abolish it. Currently available treatments can reduce spasticity by inhibiting excitatory pathways (e.g. baclofen), by stimulating inhibitory pathways (e.g. diazepam) or by inducing local muscle paralysis (e.g. botulinum toxin). To date, no standard treatment is known to totally suppress spasticity (Thibaut, Chatelle et al. 2013).

It should be noted that this study has some limitations. The first one is the single assessment of spasticity. Future longitudinal studies should assess spasticity several times in the same patients as spasticity may fluctuate over time. Moreover, controlled trials should be performed to provide more easily interpretable results, such as those regarding the correlation between physiotherapy and spasticity. Furthermore, our population was heterogeneous, involved various aetiologies and time from insult varied. Studies including a larger number of patients could enable us to compare the occurrence of spasticity with regard to different aetiologies.

Clinical advices

In line with recommendations for stroke patients (Hesse, Mach et al. 2012), our findings reinforce the fact that muscle hyperactivity should be treated early to minimize the risk of spasticity and joint fixation, thus improving the prospect of functional recovery. Subsequently, in clinical practice, even at acute stage, daily comprehensive stretching is highly recommended for all patients. Initial treatment of severely brain-injured patients tends to focus on cerebral and cardiopulmonary functions whilst muscular and motor functions, since they are not important for vital prognosis, are down-prioritized. Anti-spastic therapy is usually implemented at the sub-acute stage, even though we know that spasticity can occur earlier. Critical care physicians and medical doctors should therefore administer anti-spastic drugs as soon as muscle hypertonicity is detected, and physiotherapy sessions should be increased so that respiratory deficiency and movement disorders can be managed as early as possible. At chronic stage, when patients leave the rehabilitation unit, they should continue to receive adapted care management to minimize the adverse effects of spasticity and immobility. Such care includes daily mobilizations, several hours on a chair, raising and braces and appropriate, regularly adapted pharmacological treatment. Further studies need to be carried out to find spasticity-reducing treatments that are easily applicable in clinical practice.

2.2.3 How to manage spasticity in chronic severe brain-injured non-communicative patients

Patients in UWS/VS and MCS are unable to actively participate in rehabilitation. Therefore, passive techniques are mainly used to treat spasticity. As already mentioned, the mainstay of physical treatment is muscle stretching, which should be started as early as possible to prevent muscle shortening. The disadvantage of manual stretching is the required participation of a physiotherapist, which limits the frequency at which it is applied. In addition, severe contractures necessitate prolonged stretching that can only be achieved through the use of postures, splints, or casts (Dean, Mackey et al. 2000). Rigid splints are often used as orthopaedic devices in patients in UWS/VS and MCS during acute and chronic stages. Nevertheless, their effectiveness remains controversial, as they seem to be of little use if they are implemented when the patient already presents severe contractures and significant spasticity (Lannin, Cusick et al. 2007, Lannin, Novak et al. 2007, Shah 2007, Dockery, Hueckel-Weng et al. 2009, Basaran, Emre et al. 2012). Moreover, rigid splints can be harmful for patients with severe spasticity, because they may induce bedsores, oedema, or circulatory troubles (Feldman 1990). It is even truer for patients with DOC since they cannot move or remove the splint or communicate to their caregivers when they are uncomfortable. Soft splints are a potential alternative, but at present they are still rarely used. They may avoid these negative effects due to their softness and still decrease spasticity and improve hand opening by relaxing the patient and maintain their hands in an open position.

The aim of the present study was to compare the effectiveness of a hand rolled soft splint on the upper limb spasticity of patients with DOC, compared to conventional manual stretching. We hypothesized that: (i) soft splints decrease spasticity of the finger flexor muscles, as assessed with the MAS and the MTS; (ii) soft splints increase hand opening of patients, and (iii) manual stretching could have higher impact on elbow spasticity than splints.

For this study we used a soft splint with a form of roller that fits in the palm of the hand (see figure 5). We compare the soft splint (wore for 30 minutes) with conventional manual stretching (started from distal, fingers and wrist, to proximal joints, elbow and shoulder).

Figure 5: Soft splint placed in patient's hand. From (Thibaut, Deltombe et al. in press).

Spasticity was evaluated by means of the MAS (Bohannon and Smith 1987) (grades 0; 1; 1+; 2; 3; 4) and MTS (Held and Pierrot-Deseilligny 1969) (grades 0 to 5) over the fingers, wrist and elbow flexors. The ROM was measured at the level of the metacarpophalangeal joints, the wrist and the elbow, using standard and digital goniometers. Hand opening was assessed by measuring the passive major-palm distance. Assessments were always performed from distal to proximal, starting at the metacarpophalangeal joints and continuing up to the shoulder. All assessments were made while patients were lying in bed.

The inclusion criteria were: (i) age over 18 years, (ii) be in an UWS/VS or a MCS after a severe acquired brain injury according to published diagnostic criteria (Plum and Posner 1972, Giacino, Ashwal et al. 2002), (iii) be in an UWS/VS or a MCS for at least three months, (iv) have stable vital signs, and (v) have a spastic pattern in flexion bending in both upper limbs (MAS ≥ 1). Exclusion criteria were: (i) have cutaneous or joint pathologic states in the upper limb (e.g. wound, bedsore or fracture), (ii) have a spasticity pattern in extension, and (iii) demonstrate a hypersensitivity to polyurethane.

Patients received different consecutive treatments on each upper limb for 30 minutes, with a 60-minutes break between each. Assessments were performed before and directly after each treatment, and once again 60 minutes later. The treatments were manual stretching, splint, and a controlled condition with no treatment. The order of treatments was randomized. Since the patient population was small, two treatments were applied to the left upper limb and two to the right in order to get more measurements.

For example, a patient received splint then stretching 60 minutes later on the left upper limb, and no treatment then splint 60 minutes later on the right upper limb. The protocol was performed by two physiotherapists and lasted 4 hours and 30 minutes (figure 6). The first physiotherapist, the assessor, handled pre- (T1), post- (T2) and 60-minute post-treatment (T3) assessments alone in the patient's room and left the room after the assessments. The second physiotherapist, the experimenter, then entered the room and applied the stretching, the splint or the control condition to the patient (a different treatment on each upper limb) based on a list established by a classifier. The assessor physiotherapist stayed outside the patient's room during experimentation and was blinded to the treatment used prior to the assessment.

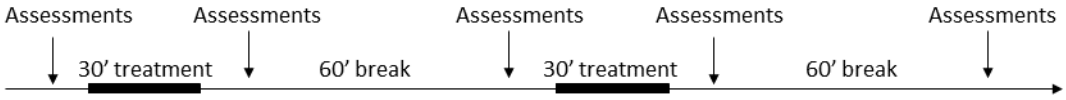


Figure 6: Experimental protocol. Treatments: splint, manual stretching, or no treatment. Assessments were performed by physiotherapist 1; treatments were performed by physiotherapist 2. From (Thibaut, Deltombe et al. in press).

Three groups were formed based on treatments received on one upper limb (left or right; the two upper limbs were considered independent): 1) splint and manual stretching (n=14), 2) splint and controlled condition (n=12), and 3) manual stretching and controlled condition (n=8). For parametric data (major-palm distances and ROM), differences between changes occurring after the treatment, and 60 minutes later were analysed by ANOVA with repeated measures (pre, post, and 60 min. post). When a significant time effect was observed, a post-hoc analysis was performed using a t-test for paired sample. Non-parametric data (MAS and MTS) were analysed using Wilcoxon's signed rank test. We compared the difference between T1 and T2; and T1 and T3. Statistical analysis was performed using Statistica 10.0 with statistical significance set at the 5% level, Bonferroni-corrected for multiple comparisons ($p<0.025$). We further evaluated the possible impact of treatment, diagnosis, and aetiology on major-palm distance and ROM using a repeated-measures analysis of variance with one independent variable. The independent variable represented whether patients were medicated or not, UWS/VS or MCS, or if their injury had traumatic or non-traumatic origin. A Mann-Whitney U test was used for the non-parametric measures (i.e. MAS or MTS).

We enrolled a total of 26 chronic patients; seven patients suffered from a spasticity pattern in extension and two patients had no spasticity (MAS = 0). Seventeen patients who fulfilled the selection criteria were included in this randomized, single blind and controlled study (5 UWS/VS, 12 MCS; mean age: 42±12 years; time since insult: 35±31 months; 7 women). The results showed significant changes at the level of the metacarpophalangean joints. No significant results were observed for the wrist and elbow. No differences were observed between treatment, diagnosis, or aetiology.

Splint-stretching group (n=14): Regarding the finger flexor muscles, the MAS score decreased significantly at T2 (post-technique) compared to T1 (pre-technique) after splint application (p=0.014; median (IQR) from 3.5(1.25) to 2.5(2.25)) and after the manual stretching (p=0.022; from 2.5(3) to 2(2)). At T3 (60 min post-treatment), however, no significant improvement was observed for either technique (p=0.093; 3(1.25) and p=1; 2.5(2.25), respectively; see figure 7). Hand opening (i.e. major-palm distance) increased significantly at T2 for the splinting (p=0.005; from 5.07±4.32 to 7.46±3.51), but not for the manual stretching (p=0.249; from 5.79±4.49 to 6.11±4.58). At T3, the values returned to baseline for the splint group (4.86±3.79). No significant changes were observed for the other variables (MTS, ROM).

Splint-no treatment group (n=12): When wearing the splint, the MAS score of finger flexor muscles decreased significantly between T1 and T2 (p=0.014; from 2.5(2) to 1.5(2)) and this reduction was maintained at T3 (p=0.022; 2(1.5)). Hand opening (i.e. major-palm distance) increased significantly in T2 (p=0.009; from 3.54±3.49 to 6.17±3.47), but the effect was not maintained at T3 (p=0.486; 3.88±3.29). Other variables (MTS, ROM) did not change significantly. No significant changes were found when the patients were not treated.

Stretching-no treatment group (n=8): We did not find any significant changes for all the variables of all tested joints neither after stretching nor after the absence of treatment.

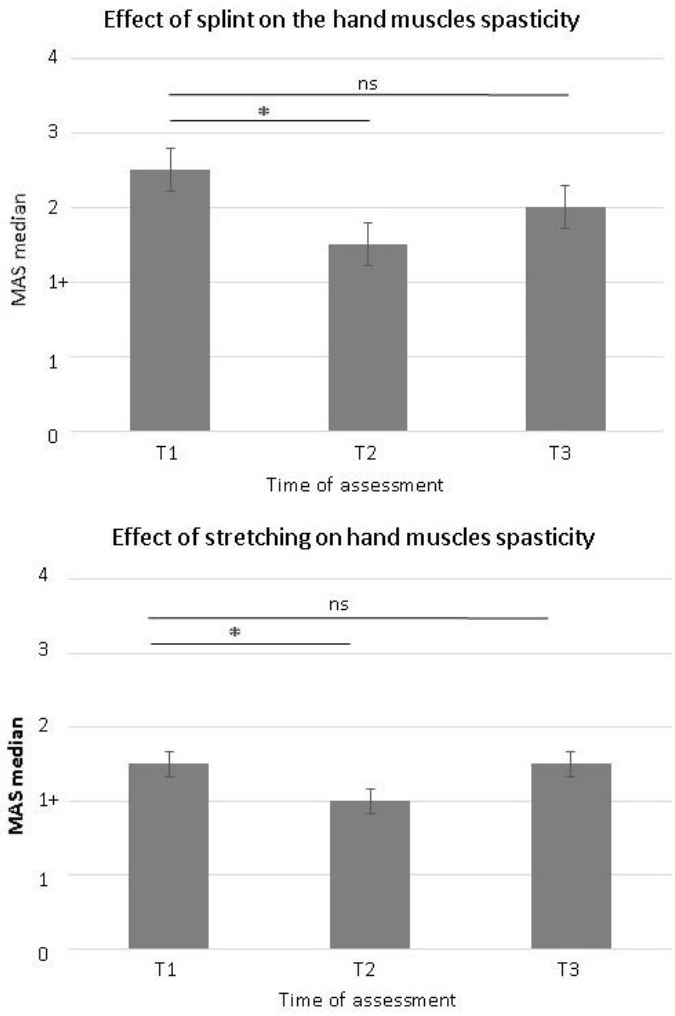


Figure 7: Median and interquartile range of Modified Ashworth Scale (MAS) scores at baseline (T1), after 30 minutes of treatment - splint or stretching (T2), and 60 minutes later (T3), for the group "splint-stretching". An asterisk (*) denotes statistical significance at $p < 0.05$ and NS stands for Non-Significant.

Summary and discussion

Our main results showed that the soft splint and manual stretching temporarily reduced spasticity of finger flexors muscles in patients with DOC. A decrease in spasticity was observed after 30 minutes of soft splinting and after 30 minutes of manual stretching. A longer lasting effect of the soft splint was observed in the group "splint-no treatment". The results of manual stretching are in line with previous studies which report the transient effect of manual stretching in children with DOC (Leong 2002). Other studies also showed the direct effect of manual stretching in reducing spasticity in post-stroke patients (Al-Zamil, Hassan et al. 1995), children with cerebral palsy (Tremblay, Malouin et al. 1990), and traumatic brain injury (Hale, Fritz et al. 1990). However, its effect fades after about 30 minutes. Gracies et al. (2000) tested the effectiveness of wearing a soft splint (a glove in Lycra) for 3 hours in 16 acute patients with stroke. They showed that this glove provided comfort to patients by reducing spasticity (MAS) in flexion of the wrist and fingers and improving wrist posture. The lasting effect of the soft splint in “splint-no treatment" group may be attributed to the fact that the splint is comfortable and could, as a result, be relaxing. Unlike manual stretching which involves mobilizing the upper limb, the use of a soft splint is less likely to cause pain, the likes of which could induce a spastic reaction (Katalinic, Harvey et al. 2010). Thus, the patient is relaxed when the splint is removed, and this relaxation effect can last longer.

The soft splint failed to show an improvement in spasticity and ROM of the wrist and elbow. This is logical as the splint extends the fingers, but has no direct action on the wrist or elbow. Nevertheless, manual stretching also failed to decrease spasticity of the wrist and elbow joints, even though all the joints were maximally elongated. This could be due to the joint assessment order; the physiotherapist performing the evaluations always started by assessing the fingers, then the wrist and then the elbow. This order could increase the likelihood of observing an effect on the fingers’ flexor muscles. Moreover, manual stretching can generate pain and increase spasticity. Indeed, the physiotherapists in our study often had to decrease the stretching due to signs of pain (e.g. grimaces or restlessness).

In both the “splint-stretching” and “splint-no treatment” groups, soft splinting improved hand opening (increased major-palm distance). The thickness of the splint allowed the metacarpophalangeal and interphalangeal joints to be positioned near their maximum stretching position. In patients with DOC, it is imperative to maintain good hand opening. Firstly, it is important from a point of view of hygiene as it

prevents problems such as maceration, pressure sores, or injury caused by driving nails into the palm. Secondly, it facilitates nursing. In addition, if patients recover cognitively, it is important that they are able to mobilize their fingers so they can grasp objects and maximize their autonomy.

One of the limitations of this study is the small number of patients and the fact that we considered the two upper limbs (left and right) independently. We must, therefore, interpret our results with caution. Further studies should assess the effect of soft splints in a larger group of patients, in both acute and chronic stages.

Clinical advices

The advantage of soft splints is that they are easy to place, comfortable, flexible, and they allow contraction (e.g. grasping reflex or muscle contraction) without resistance. In addition, the risk of injury and bedsores is less than with rigid splints. Rigid splints are often difficult to place, especially in severe patients with spastic and tendon retraction where hand opening and access to the palm is frequently difficult. Soft splints can be worn for hours without pain and without inducing a grasping reflex or muscle cramps. The use of a soft, comfortable splint should be recommended for patients with DOC since they are not able to communicate pain. Moreover, in cases of abrupt muscle contraction, a soft splint can adapt itself to the increase of patient activity. Soft splints, therefore, seem to be a really good alternative for reducing hand spasticity in chronic patients with DOC.

2.3 Conclusion and future directions

These studies aimed at developing tools that can help clinicians manage pain and spasticity in patients with DOC. As mentioned before, taking care of patients' pain is an ethical responsibility of clinicians and it can influence treatment decisions (Demertzi, Racine et al. 2013).

We first reviewed studies showing that the NCS-R is a validated and sensitive tool for assessing nociception in this population (Thibaut, Chatelle et al. 2014). Our study on the correlation between NCS-R and brain metabolism showed that its total scores positively correlate with the posterior part of the ACC, a key area for the cognitive integration of pain (Shackman, Salomons et al. 2011). These results suggest that the NCS-R is at least partially related to cortical pain processing and, hence, may constitute a suitable behavioural tool for assessing, monitoring and treating nociception and pain in non-communicative patients with DOC. Nevertheless, further studies should investigate whether or not the scale is related to the connectivity of the ACC with the rest of the pain matrix as it has been shown that cortico-cortical connectivity is related to consciousness and, hence, to conscious pain perception (see above "Pain processing in patients with DOC"). Overall, our research supports the use of the NCS-R for the assessment of nociception and pain in patients with DOC. The next step would be to validate the NCS-R for clinical use by evaluating its sensitivity in detecting the efficacy of an analgesic treatment. It would then be interesting to develop tools, behavioural scales or physiological monitoring, to indicate the presence of pain or nociception in patients as soon as possible.

It is a known fact that spasticity can influence a patient's quality of life and interfere with their rehabilitation. There is still, however, a significant lack of clear guidelines for its treatment. We first studied the impact of this motor disorder on patients with DOC. Our study shows an alarmingly high incidence of spasticity in this population of patients. Since these patients are already limited in their range of movement, spasticity constitutes one of the most important disabling factors to be treated.

Due to paresis and the lack of controlled voluntary movements, patients with DOC are almost constantly immobile. This chronic inactivity may enhance the severity of spasticity and the various other side-effects (e.g. ankylose, tendon retraction or joint fixation). This hypothesis is in line with our finding where we noticed a positive correlation between severity of spasticity and time from brain injury (Thibaut,

Chatelle et al. 2014). Moreover, disuse and paresis result in a decrease of cortical excitability of the motor cortical areas coupled with a decrease in motor representation of the immobilized body parts (Kaneko, Murakami et al. 2003). Besides, joint immobilization in a shortened position also aggravates the disuse (Talmadge, Roy et al. 1995). In addition to spastic paresis, patients suffer from muscle atrophy, loss of sarcomeres and accumulation of connective tissue and fat (for a complete review see; Gracies 2005). Previous studies have already highlighted the correlation between the degree of spasticity and the duration of immobilization and disuse (Harlaar, Becher et al. 2000). In conclusion, paresis and spasticity form a vicious circle which is exacerbated by prolonged immobilization. It is of upmost importance to break this vicious circle by managing these motor disorders from the time the first signs appear (a few days or weeks after the brain lesion) and by ensuring intensive mobilization of the patient' limb, even if no active movement is possible. In other words, efforts should be made to increase the intensity of passive treatments on the upper and lower limbs using, for example, a motorized movement trainer as soon as possible following brain injury.

In addition, managing spasticity could help this population to initiate and execute movements and may facilitate voluntary gestures, enabling, for example, a response to command. Complications such as pain or pathological tendon retraction impair these patients' quality of life and functional recovery. Further research should use neurophysiology testing and neuroimaging methods to examine the association between the brain lesion location and the presence of spasticity. Additionally, further studies should assess the impact of specific and combined treatments on spasticity or tendon retraction and behavioural signs of pain. The correlation between spasticity and pain highlights the detrimental effect of spasticity on quality of life and the importance of rapid action to address this complication. Moreover, as motor impairments have been shown to prevent the expression of signs of consciousness at the bedside (Monti, Vanhaudenhuyse et al. 2010, Cruse, Chennu et al. 2011), it is of critical importance to improve the quality of care and rehabilitation of this population. We are currently acquiring more data on spasticity in patients with DOC in order to classify patients according to their specific aetiology, brain lesion, rehabilitation and time since insult.

Finally, we tried to find a way to reduce spasticity and increase patients' comfort by using a simple technique; soft splints. As mentioned above, these splints have the advantage of being easy to use, they

can be worn several hours a day, do not require any supervision, are comfortable for the patient and they present no risk of injury. We found that the soft splints and manual stretching both transiently reduced spasticity of the flexor finger muscles. The soft splint, however, was the only treatment that improved hand opening. They are comfortable and simple to apply, so they could easily be applied in patient's daily care. Further studies of the long-term effects of wearing these splints are required prior to their being used in daily clinical practice.

3. Curative treatments for patients with disorders of consciousness

"You cannot teach a man anything, you can only help him to find it within himself."

Galileo Galilei

Based on the following articles:

Thibaut A*, Chatelle C*, Gosseries O, Bruno MA, Demertzi A, Bernard C, Hustinx R, Tshibanda L, Bahri MA, Laureys S. Changes in cerebral metabolism in patients with a minimally conscious state responding to zolpidem. Front Hum Neurosci. 2014;8:917. *equally contributed

Thibaut A, Chatelle C, Gosseries O, Laureys S, Bruno MA. Transcranial direct current stimulation: a new tool for neurostimulation. Rev Neurol (Paris). 2013;169(2):108-20.

Thibaut A, Bodart O, Laureys S. Brain stimulation for patients with disorders of consciousness. In Surgical Principles of Therapeutic Cortical Stimulation. Edited by S. Canavero, De Gruyter. 2015

Thibaut A, Bruno MA, Ledoux D, Demertzi A, Laureys S. tDCS in patients with disorders of consciousness: sham-controlled randomized double-blind study. Neurology. 2014; 82(13):1112-8.

Thibaut A,* Di Perri C*, C. Chatelle, M. A. Bruno, S. Wannez, C. Bernard, R. Hustinx and S. Laureys. Clinical response to tDCS depends on residual brain metabolism and grey matter integrity in patients with minimally conscious state. *Submitted;* *equally contributed

3.1 Problem statement

Patients in UWS/VS and MCS represent a challenging clinical entity which is prone to misdiagnosis (Schnakers, Vanhaudenhuyse et al. 2009, Stender, Gosseries et al. 2014) and lacks effective treatment options (Giacino, Fins et al. 2014). At present, there are no evidence-based guidelines regarding the treatment of patients with DOC (Bernat 2006, Giacino, Fins et al. 2014). Until recently, the medical community has viewed patients in UWS/VS and MCS with great pessimism regarding both prognosis and effective treatments. Unfortunately, this pessimism results in the neglect of patients in terms of health care as no improvement is expected. Nevertheless, in the past 10 years a number of studies have reported that some patients in MCS could improve even several years after the insult (Voss, Uluc et al. 2006, Estraneo, Moretta et al. 2010, Bruno, Ledoux et al. 2012) and several treatments can enhance signs of consciousness (Schiff, Giacino et al. 2007, Giacino, Whyte et al. 2012, Thonnard, Gosseries et al. 2014, Whyte, Rajan et al. 2014). Indeed, research on treatments improving cognitive abilities in patients with DOC has shown that deep brain stimulation (stimulation of the intralaminar nuclei of the thalamus; Schiff, Giacino et al. 2007) and some pharmacological agents such as amantadine (Schnakers, Hustinx et al. 2008, Giacino, Whyte et al. 2012), apomorphine (Fridman, Calvar et al. 2009), intrathecal baclofen (Sara, Sacco et al. 2007) and zolpidem (Whyte and Myers 2009, Thonnard, Gosseries et al. 2014) can sometimes improve behavioural signs of consciousness in patients with DOC (see table 7). So far, only amantadine has been shown to increase signs of consciousness in a large cohort of acute and sub-acute patients with DOC in a placebo-controlled trial (Giacino, Whyte et al. 2012). However, one of the most common adverse side-effects of this drug is the occurrence of epileptic seizures, the likes of which can be extremely frequent and can significantly affect the cognitive state of these patients (Bagnato, Boccagni et al. 2013). Moreover, the mechanisms underlying the recovery of behavioural signs of consciousness observed in some patients with DOC following the administration of these drugs are still poorly understood. Our next challenge is to better understand the mechanism of action of these drugs on the clinical improvement of patients with DOC and how to possibly improve treatment options for this category of patients.

Authors	Drug	Design	N (aetiology)	Time since injury	Results
Giacino et al., (2012)	Amantadine (antiviral and an anti-parkinsonian; NMDA antagonist and indirect dopamine agonist)	Prospective, multicentric, randomized, double-blind, placebo-controlled	184 (TBI)	1 to 3 months	Amantadine group: faster recovery; decrease of DRS scores and increase of behavioural bench markers on the CRS-R
Fridman et al., (2010)	Apomorphine (dopamine agonist used in Parkinson disease)	Prospective case series	8 (TBI)	1 to 4 months	Functional recovery with decrease of the CNC, DRS and increase of GOS scores
Whyte & Myers (2009)	Zolpidem (nonbenzodiaze-pine GABA agonist hypnotic used to treat insomnia)	Mutlicentric, double-blind, randomized study	15 (8 TBI)	3 months to 23 years	1 responder (UWS/VS to MCS+); increase in CRS-R score, visual pursuit, response to command
Thonnard et al., (2014)	Zolpidem	Open label study	60 (31 TBI)	2 months to 26 years	12 patients showed improvement in CRS-R scores. Change of diagnosis in 1 patient (from MCS+ to EMCS)
Sara et al., (2009)	Baclofen (GABA agonist used to decrease spasticity)	Case report	5 (2 TBI)	6 to 10 months	Clinical improvement in all patients after 2 weeks (increase in CRS-R scores)

Table 7: Main studies using amantadine, apomorphin, baclofen or zolpidem treatment in patients with disorders of consciousness. DRS: disability rating scale; CRS-R: Coma Recovery Scale; CNC: Coma/Near-Coma Scale; GOS: Glasgow Coma Scale; NMDA: N-methyl-D-aspartate; GABA: γ-Aminobutyric acid; TBI: traumatic brain injury; UWS/VS: Unresponsive wakefulness Syndrome/Vegetative State; MCS: Minimally Conscious State; EMCS: emergence from MCS.

In this chapter, we will first present the findings of our study investigating brain metabolism in patients who clinically responded to zolpidem, trying to better understand the mechanism of action of this drug. We will then explore the potential effects of a non-invasive brain stimulation technique, transcranial direct current stimulation (tDCS), on the improvement of signs of consciousness in patients with DOC. We will talk about what is currently known about this technique and I will present the results of our pilot double-blind, sham controlled, randomised cross-over study on a single session of tDCS over the left dorsolateral

prefrontal (DLPF) cortex in acute and chronic patients in UWS/VS and MCS. Finally, we will try to characterize the brain metabolism and morphology of the patients who clinically improved upon tDCS.

3.2 Pharmacological treatment: an example with zolpidem

Zolpidem, a short-acting non-benzodiazepine agent from the imidazopyridine class, GABA-A agonist, usually used to treat insomnia (Langtry and Benfield 1990, Sanger 2004), has been shown to induce paradoxical responses in some patients with DOC, leading improvement of arousal and cognitive abilities. Several case-studies showed that zolpidem can lead to very impressive recoveries in some severely brain-damaged patients of various aetiologies (Clauss, Guldenpfennig et al. 2000, Cohen, Chaaban et al. 2004, Clauss and Nel 2006, Brefel-Courbon, Payoux et al. 2007, Shames and Ring 2008, Williams, Conte et al. 2013). However, this effect remains rare (i.e. around 5-7% responders; Whyte and Myers 2009, Thonnard, Gosseries et al. 2014, Whyte, Rajan et al. 2014) and so far unpredictable.

In this study, we assessed zolpidem-induced changes in regional brain metabolism in a case-series of three patients with known zolpidem response after chronic post-anoxic MCS. According to the mesocircuit model for the recovery of consciousness (Schiff 2010), zolpidem is suggested to disinhibit the globus pallidus interna (GPi) and consequentially increase the thalamic excitatory role on prefrontal cortices (see figure 8). Based on this model, we hypothesized that an impaired brain metabolism in the thalamus, the striatum and the prefrontal cortex would be observed after placebo, whilst a recovery of brain metabolism would occur following zolpidem intake.

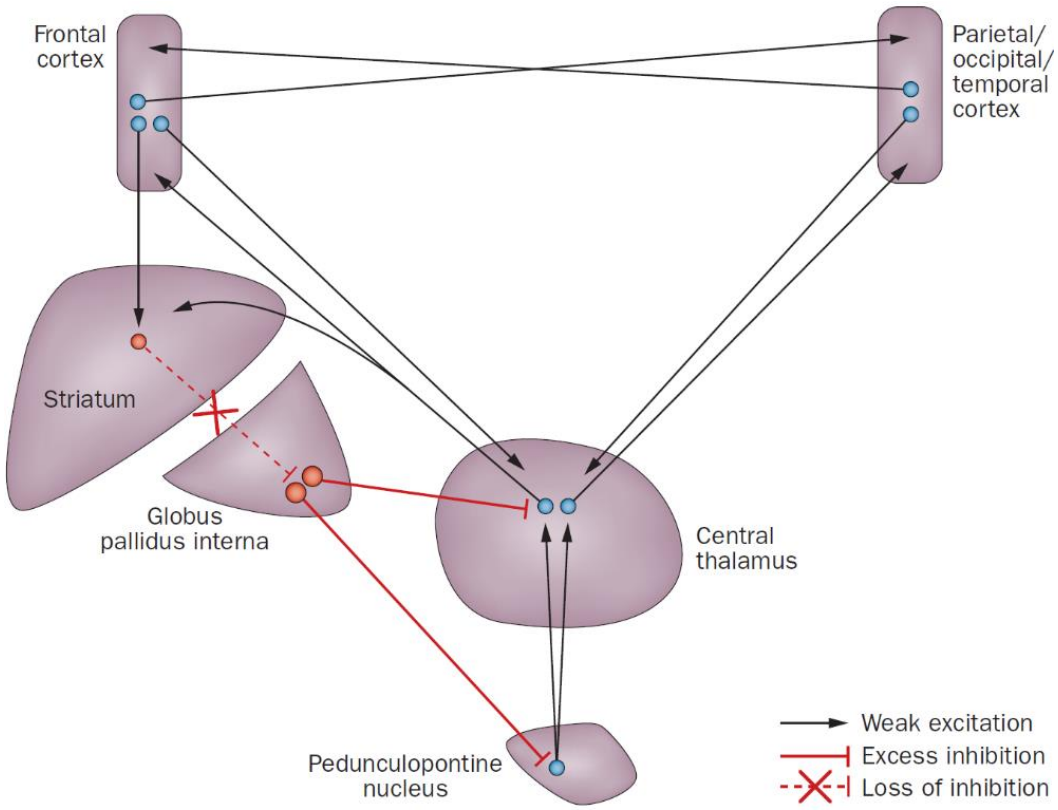


Figure 8. The mesocircuit model underlying forebrain dysfunction and interventions in severe brain injuries. Reduction of thalamocortical and thalamostriatal outflow following deafferentation and neuronal loss from the central thalamus withdraws the afferent drive to the striatum, which may then fail to reach firing threshold because of their requirement for high levels of synaptic background activity. Loss of active inhibition from the striatum allows neurons of the globus pallidus interna to tonically fire and provide active inhibition to their synaptic targets, including relay neurons of the already disfacilitated central thalamus, and possibly also the projection neurons of the pedunculopontine nucleus. Since the GABAA a-1 subunit is normally expressed in large quantities in the globus pallidus interna, zolpidem could inhibit the latter, substituting its normal inhibition from the striatum, and hence induce an increase of the thalamic excitatory influence on prefrontal cortices. From(Giacino, Fins et al. 2014).

In this study we investigated 3 patients in MCS who responded to zolpidem (i.e. who emerged from MCS after zolpidem intake, see table 8).

The first patient was a 37-year-old female, assessed 18 months post anoxia (cardio-respiratory arrest). Within one month post-insult, she evolved into a MCS plus. MRI showed ischemic brain lesions in the basal ganglia, in the left occipital and bilateral posterior parietal cortices. After zolpidem administration,

she could systematically follow simple commands, recognize different objects and use them adequately, as well as functionally communicate (i.e. emerged from MCS)

The second patient was a 38-year-old male, assessed 12 years and 7 months post-anoxia (cardio-respiratory arrest). The patient was diagnosed as MCS minus (e.g. visual pursuit and automatic motor reactions but no command following). The MRI showed lesions in the brainstem and thalami, and diffuse periventricular white matter damage, more pronounced in the left hemisphere and in the occipital regions bilaterally. After zolpidem administration, this patient was able to systematically follow simple commands, recognize objects and use them consistently, and functionally communicate. He was diagnosed as emerged from MCS.

The third patient was a 50-year-old female, assessed 7 years post anoxia (cardio-respiratory arrest). Structural MRI did not show focal abnormalities (PET glucose and activation blood-flow data have been reported elsewhere; Brefel-Courbon, Payoux et al. 2007). She showed automatic motor reactions, reproducible but not consistent command following, localization of objects and intentional communication (i.e. MCS plus). Following zolpidem intake, she showed consistent command following, functional use of objects and functional communication (i.e. emerged from MCS).

	MCS 1		MCS 2		MCS 3	
	P	Z	P	Z	P	Z
AUDITORY FUNCTION						
4 – Consistent Command Following*		X		X		X
3 – Reproducible Command Following*	X				X	
2 – Localization to Sound						
1 – Auditory Startle						
0 – None			X			
VISUAL FUNCTION SCALE						
5 – Object Recognition*		X		X		X
4 – Object Localization: Reaching*					X	
3 – Pursuit Eye Movements*	X		X			
2 – Fixation*						
1 – Visual Startle						
0 – None						
MOTOR FUNCTION SCALE						
6 – Functional Object Use †		X		X		X
5 – Automatic Motor Response *			X		X	
4 – Object Manipulation*						
3 – Localization to Noxious Stimulation*						
2 – Flexion Withdrawal	X					
1 – Abnormal Posturing						
0 – None/Flaccid						
VERBAL FUNCTION SCALE						
3 – Intelligible Verbalization*		X		X		X
2 – Vocalization/Oral Movement	X					
1 – Oral Reflexive Movement			X			
0 – None					X	
COMMUNICATION SCALE						
2 – Functional: Accurate †		X		X		X
1 – Non-Functional: intentional*	X				X	
0 – None			X			
AROUSAL SCALE						
3 – Attention				X		X
2 – Eye Opening w/o Stimulation	X		X			
1 – Eye Opening with Stimulation		X			X	
0 – Unarousable						
DIAGNOSIS	MCS+	EMCS	MCS-	EMCS	MCS+	EMCS

Table 8. Behavioural assessments after placebo and zolpidem intake (based on the Coma Recovery Scale-Revised). MCS-: minimally conscious state minus (i.e. non-reflex movements without response to command); MCS+: minimally conscious state plus (i.e. presence of response to command); EMCS: emergence from MCS (i.e. functional communication or object use). *indicates clinical signs compatible with MCS. † indicates EMCS. P: placebo; Z: zolpidem. From (Chatelle, Thibaut et al. 2014).

Zolpidem intake was stopped at least 12 hours prior to the research protocol and 10 mg of zolpidem or placebo (water) were administered via gastrostomy in a randomised order, in a double blind two-day design. All other treatments remained unchanged throughout the study. Standardized clinical assessment using the CRS-R (Giacino, Kalmar et al. 2004, Schnakers, Majerus et al. 2008) was performed 30 minutes after administration of zolpidem or placebo. FDG-PET cerebral metabolism data were acquired 90 minutes after zolpidem or placebo intake according to a standard clinical protocol. An intravenous injection of 300 MBq fluorodeoxyglucose was administered 30 minutes before the FDG-PET. PET data of patients were compared to an age-matched group of 39 healthy participants (mean age 45±16 years; 18 men).

Pre-processing of the PET data included spatial normalization and smoothing (using a 14 mm full width at a half maximum Gaussian kernel), implemented in Statistical Parametric Mapping toolbox (SPM8; www.fil.ion.ucl.ac.uk/spm) as previously published (Phillips, Bruno et al. 2011, Bruno, Majerus et al. 2012, Thibaut, Bruno et al. 2012). A full-factorial design was performed, three design matrices modelled the subject-effect (MCS patient 1, 2, 3), drug-effect (placebo versus zolpidem) and group effect (patients versus controls). After proportional scaling, we identified brain areas showing an impaired metabolism following both placebo and zolpidem intake as compared to healthy controls. We further investigated those brain regions showing a relative increased metabolism after zolpidem intake as compared to placebo. Results were considered significant at false discovery rate cluster level $p<0.001$ after a Bonferroni correction for multiple comparisons.

The results at group level analysis highlighted, after placebo, an impaired brain metabolism as compared with controls in the thalami and the left precuneus/posterior cingulate areas (see figure 9A). At a less conservative statistical threshold (i.e. $p<0.0001$ uncorrected for multiple comparison), an impaired metabolism was also observed in the bilateral superior and middle frontal gyri, the left precuneus/posterior cingulate, the bilateral precentral gyri, the left insula and right inferior parietal areas. After administration of zolpidem, brain metabolism impairment was confined to the thalami and the left precuneus/posterior cingulate areas, as compared with controls (figure 9B). At a less conservative threshold, metabolism impairment was also observed in the precentral gyri, the left superior frontal and temporal gyri, the left middle frontal gyrus, the precuneus and the right inferior parietal lobe. We identified increased brain metabolism in the bilateral superior frontal gyri and the right medial frontal cortex following zolpidem intake

as compared to placebo (see figure 9C). At a less conservative statistical threshold uncorrected for multiple comparisons, the increased metabolism involved also the left insula, the middle frontal gyri and the left inferior frontal and parietal areas. When we looked at the glucose uptake value in the highest peak voxel in the prefrontal cortex for all three subjects, we identified an increased metabolism following administration of zolpidem as compared to placebo (see figure 10).

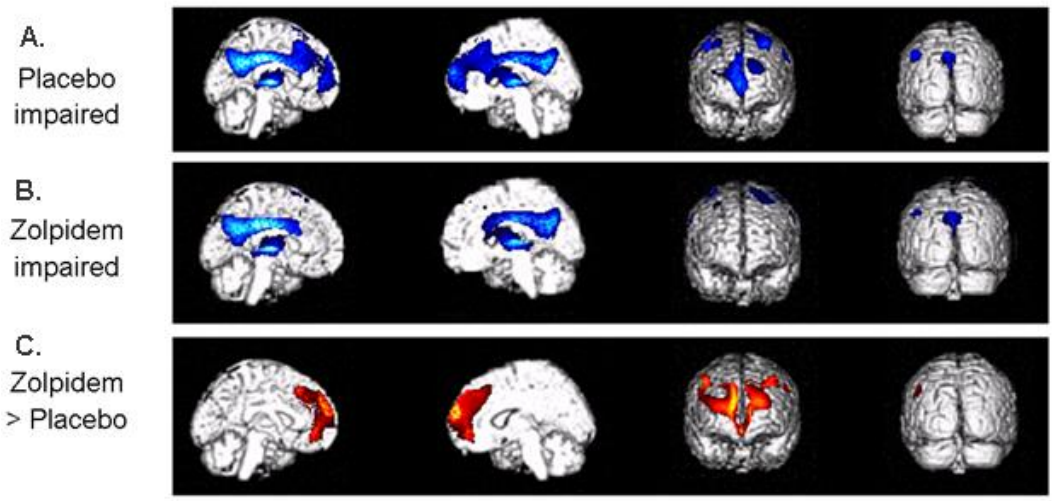


Figure 9. Impaired brain metabolism after placebo (A) and zolpidem (B) intake and areas showing relative recovery after zolpidem (C). Brain areas showing impaired metabolism (in blue) following placebo and zolpidem administration and regions which were impaired following placebo but showed relative recovery of activity after zolpidem intake (in red). For display purposes results are shown thresholded at uncorrected $p<0.001$. From left to right, medial right and left view, frontal and posterior view of a 3D rendered brain MRI. From (Chatelle, Thibaut et al. 2014).

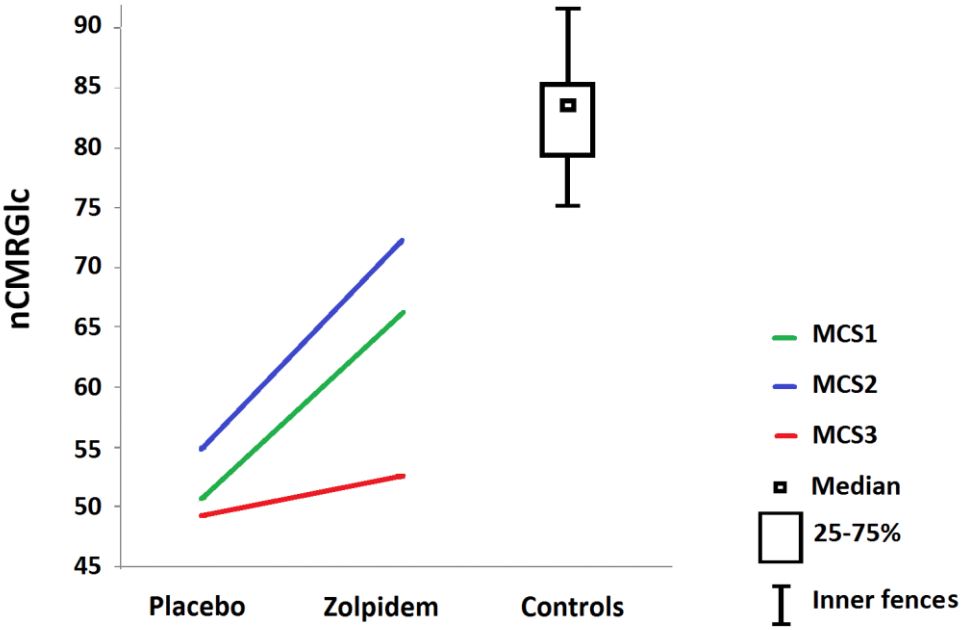


Figure 10: Normalized cerebral metabolic rate for glucose (nCMRGlc) values in prefrontal cortices after placebo. Values reported for the cluster using MNI peak coordinates x, y, z = -22, 56, 24 after placebo and zolpidem intake in patients, as compared to healthy controls (boxplot showing median, 25-75% quartiles and inner fences). From (Chatelle, Thibaut et al. 2014).

Summary and discussion

In this double-blind placebo-controlled study on three chronic post-anoxic patients in MCS who showed clinically significant paradoxical behavioural improvements (i.e. emergence from MCS) after zolpidem intake, we observed increased metabolism in the prefrontal cortices (following zolpidem intake), in line with the previously proposed mesocircuit model for recovery of consciousness in DOC (Schiff 2010).

Our findings support previous case-studies reporting a change in prefrontal cortex activity after zolpidem intake using single-photon emission computed tomography (measuring blood flow; Clauss, Guldenpfennig et al. 2000), FDG-PET (Brefel-Courbon, Payoux et al. 2007, Williams, Conte et al. 2013) and EEG (showing a decrease of low frequency activity; Williams, Conte et al. 2013).

After zolpidem intake we identified increased metabolism in prefrontal areas known to be involved in the "limbic loop" regulation of motivation and are a key centre of the mesocircuit model for

consciousness (Schiff 2010, Laureys and Schiff 2012). The mesocircuit hypothesis explaining the effect of zolpidem (Schiff 2010) supports the idea that, in normal cognitive processing, the striatum disinhibits the central thalamus via the GPi while the central thalamus promotes activity of prefrontal cortices. Therefore, if the activity in the striatum is reduced due to a severe brain injury, central thalamic and prefrontal activity is also decreased, possibly explaining the observed hypometabolism of the prefrontal regions in the hereby reported patients at baseline condition (i.e. after placebo administration). The mesocircuit model assumes that zolpidem directly inhibits the GPi, taking over the inhibition that is normally exerted by the striatum (one of the most sensitive areas to cerebral hypoxia; Calabresi, Centonze et al. 2000). This would hence increase the thalamic excitatory influence on the prefrontal cortices. The fact that we did not find significant structural lesions in the brain areas showing increased metabolism following zolpidem administration corroborates previous studies (Clauss, Guldenpfennig et al. 2000, Brefel-Courbon, Payoux et al. 2007). It further suggests that neurological deficits observed in those patients who respond to zolpidem might be mainly caused by inhibitory functional effects - phenomenon known as cerebral *diaschisis* (i.e. the loss of function in a portion of the brain as a result of its connection to another injured area; Glassman 1971, Feeney and Baron 1986, Tecco, Wuilmart et al. 1998, Witte, Bidmon et al. 2000), rather than by severely structurally damaged or dead brain tissue (Shames and Ring 2008). Here, we hypothesize that the observed reduced metabolism in the prefrontal cortices might be related to lesions in the striatum and/or thalami.

In contrast with two previous case-studies (Brefel-Courbon, Payoux et al. 2007, Williams, Conte et al. 2013), we did not observe a zolpidem-related increased metabolism in thalamic and striatal regions. This could be explained either by the fact that the technique used is not sensitive enough to highlight small functional activity changes in these areas, or because zolpidem induced changes might modify effective connectivity between areas which cannot be investigated using FDG-PET. Future neuroimaging studies looking at the effective connectivity in DOC zolpidem-responders would allow to better document the role of thalamo-cortical excitatory pathways underlying the observed increased activity in prefrontal areas. In addition, further studies on regional glucose metabolism changes at the individual level would help us better understand the mechanisms of recovery following zolpidem intake (Fridman, Beattie et al. 2014).

In parallel to the mesocircuit hypothesis, the “GABA impairment hypothesis” was recently proposed by Pistoia et al (Pistoia, Sara et al. 2014). This theory suggests that zolpidem (as well as baclofen, a GABA-B

agonist) may act on consciousness recovery through the restoration of a normal ratio between synaptic excitation and inhibition by reversing the impairment of GABA in patients with DOC. This hypothesis has the advantage to explain the potential mode of action of both zolpidem and baclofen on patient's recovery. In some patients with impaired balance of cortical subcortical-cortical connections, the use of GABA agonists can decrease excessive information and thus reorganize a balanced dialogue among different brain nuclei, allowing proper information processing. Nevertheless, the time course of the observed effect of these two drugs is different. Regarding zolpidem, the short term effects that disappear when the plasma drug concentration falls suggest rapid neurotransmitter changes. On the other hand the slow-onset effects of baclofen suggest a phenomenon of gradual neuroplasticity. Unfortunately, it was not possible to verify this hypothesis through the methods used in our study. However, both mesocircuit and GABA impairment hypotheses do not explain why less than 10% of patients react positively to those GABA agonist drugs.

Our results underline a key role of the prefrontal cortices in the recovery of functional communication and object use in hypoxic patients with chronic DOC. It is well known that the prefrontal cortex has a major role in executive function and working memory, a recovery of its functionality is therefore likely to influence the recovery of object use and functional communication. Our findings partly corroborate previous case (Nakayama, Okumura et al. 2006, Thibaut, Bruno et al. 2012) reporting a decreased metabolism in medial prefrontal and fronto-basal regions, cingulate gyrus and thalamus in severely brain-injured patients with DOC.

Taken together, the data suggest that the infrequent but existing paradoxical effect of zolpidem could be characteristic of patients who have suffered subcortical thalamic (as in all 3 cases here reported) and/or striatal functional lesions preventing prefrontal cortices to exert their normal function.

3.3 Non-invasive brain stimulations

3.3.1 Principles

tDCS is one of the easiest way to stimulate the brain focally. tDCS was first investigated in the 1960s, when researchers showed that transcranial stimulation could affect brain functioning by modifying cortical excitability (Creutzfeldt, Fromm et al. 1962, Bindmann, Lippold et al. 1964, Purpura and McMurtry 1965). However, only in the beginning of the 21th century tDCS widely attracted the attention of the scientific community. The interest in tDCS has been mainly focussed in understanding its underlying way of actions and its potential therapeutic applications (Paulus 2003, Fregni, Boggio et al. 2005, Boggio, Ferrucci et al. 2006, Ferrucci, Mameli et al. 2008, Kang, Kim et al. 2012, Nelson, McKinley et al. 2012), which remained yet to be entirely determined (Nitsche, Cohen et al. 2008, George, Padberg et al. 2009).

tDCS is a safe method to modulate cortical excitability through the emission of a weak (usually ≤ 2mA) direct current through the brain between two electrodes, from the anode to the cathode. It is a safe, cheap and easy to use device that could be easily integrated in a rehabilitation program. Anodal stimulation can enhance the stimulated area's functions whereas cathodal stimulation reduces them. Currently, a lot of clinical trials have been conducted to study the effect of tDCS on post-stroke motor and language deficits, in psychiatric disorders, chronic pain, memory impairment and tinnitus in order to reduce symptoms (Hummel, Voller et al. 2006, Antal, Terney et al. 2010, Baker, Rorden et al. 2010, Zaehle, Sandmann et al. 2011, Frank, Schecklmann et al. 2012, Loo, Alonzo et al. 2012).

Physiologically, anodal tDCS enhances excitability, whereas cathodal tDCS reduces it by decreasing or increasing the action potential threshold (Nitsche, Seeber et al. 2005). The establishment of the long-lasting after-effects depends on membrane potential changes as well as modulations of N-methyl-D-aspartate (NMDA) receptor efficacy (Liebetanz, Nitsche et al. 2002). In another word, tDCS does not induce the firing of otherwise resting neurons, but it modulates the spontaneous firing rate of neurons by acting on the membrane potential. This characteristic distinguishes tDCS from other stimulation techniques, such as TMS, which excites neurons directly.

As said above, tDCS can modulate neuronal activity through the induction of a relatively small electric current flowing constantly through the cerebral cortex via two electrodes, the anode and the cathode, placed on the scalp. Depending on the polarity of the stimulation, this technique can increase or decrease the cortical excitability and spontaneous activity of the neurons (Jacobson, Koslowsky et al. 2012). The neuronal hyperexcitability of the stimulated region is produced by the anode which lowers the depolarization threshold of the neuronal membrane (Purpura and McMurtry 1965, Jefferys 1995). Conversely, the cathode induces an increase in the depolarization threshold and, consequently, a decrease in neuronal activity. In general, the anode improves the function of the stimulated area whereas the cathode can either reduce the performance or have no effect (Jacobson, Koslowsky et al. 2012). In most protocols, the currents used are 1 or 2 mA applied for 5 to 30 minutes on a specific cortical area (Been, Ngo et al. 2007). Apart from the influx, which is lost at the scalp and skull level (40 to 60%), the remaining current reaches the cortex and neurons (Miranda , Lomarev et al. 2006). The short-term effects are a direct consequence of the neuronal excitability induced by the anode or cathode. The long-term effects, which last for about an hour (Nitsche and Paulus 2001), are thought to be related to the NMDA receptors which are activated by glutamate and are involved in cellular memory (Nitsche and Paulus 2001). It has been shown that tDCS showed no effect when NMDA receptors were blocked by antagonists (Liebetanz David 2002, Nitsche, Fricke et al. 2003). In contrast, the stimulation effects were prolonged with the NMDA receptor agonist, D-cycloserine (Nitsche, Jaussi et al. 2004). These results suggest that the anodal tDCS improves the efficiency of NMDA receptors on neuronal depolarization via two independent but synergic mechanisms (Nitsche and Paulus 2000). Firstly, high frequency (30Hz) pre-synaptic activity is induced by the anode. This lowers the pre- and postsynaptic depolarization threshold and improves the efficiency of the voltage-dependent NMDA receptors. Secondly, the changes in the intracellular calcium rate induced by the prolonged tDCS enhance changes to the NMDA receptors, the efficacy of which depends on a high intracellular level of calcium (Bennet 2000). Indeed, it has be shown that when a calcium inhibitor is injected, the long-term effects of anodal tDCS are abolished (Nitsche, Fricke et al. 2003).

However, despite all the above-mentioned studies, the tDCS mechanisms of action have not yet been fully understood. At present, it is commonly accepted that the short-term tDCS effects are the result of potential membrane modifications (induced by the anode or cathode) which are known to act on neuronal

excitability (Nitsche, Fricke et al. 2003). Long-term effects, however, are related to the modification of NMDA receptors excitability and certain ion channels such as calcium channels (Liebetanz David 2002). We can therefore conclude that the effects of tDCS depend on a combination of axonal and synaptic tDCS induced alterations.

Several studies showed that a single anodal stimulation of a damaged cortical area of post stroke or TBI patients could improve the function of the stimulated area. An anodal session of tDCS over C3 or C4, according to the 10-20 international system (Herwig, Satrapi et al. 2003), could enhance motor function (Boggio, Castro et al. 2006, Antal, Terney et al. 2010). Likewise the stimulation of the prefrontal cortex (F3 or F4) showed positive effects on memory (Jo, Kim et al. 2009, Kang, Kim et al. 2012) or attention (Kang, Baek et al. 2009). Nevertheless, the effects decreased between one and two hours after the stimulation (Nitsche and Paulus 2001). To overcome these limits, researchers tried chronic daily stimulation for one (Antal, Terney et al. 2010), two (Boggio, Rigonatti et al. 2008) or three weeks (Polanowska, Lesniak et al. 2013). Effects upon daily chronic tDCS were shown to last up to 4 weeks after the end of the stimulations.

In comparison to other non-invasive brain stimulation technique such as TMS, the major limitation of tDCS is the lack of a focal stimulation locus, making it difficult to precisely map cortical functions. Furthermore, contrary to the TMS, tDCS cannot produce temporally focused cortical effects. A study by Miranda et al. modelling the current distribution during tDCS over the left prefrontal dorsolateral cortex (Miranda, Lomarev et al. 2006) showed that only a fraction of the current applied to the anode (about 50%) could penetrate into the brain. Comparing to TMS, which allows a 1.5 to 3 cm penetration of the pulse in the brain (Zangen, Roth et al. 2005), tDCS seems unable to reach deeper brain structures such as the precuneus, a critical hub for consciousness recovery (Laureys, Faymonville et al. 2000). On the other hand, the use of tDCS is simple, handy and involves no dangerous risk. Moreover, tDCS is easier than TMS for efficiently blinding subjects and investigators in double-blind and sham-controlled trials (Gandiga, Hummel et al. 2006).

Given that tDCS showed encouraging results on patient's motor and cognitive functions, we decided to test its efficacy on behavioural recovery in patients with DOC.

3.3.2 tDCS as a tool to improve patients' signs of consciousness

In a first pilot study, we wanted to test the effect of prefrontal tDCS on patients with DOC, both UWS/VS and MCS, acute-subacute (< 3months) and chronic, and with traumatic and non-traumatic aetiologies. We aimed to assess the effect of a single session of anodal tDCS of the left DLPF cortex on consciousness, as evaluated by means of the CRS-R. Our primary question was whether anodal tDCS, as compared to sham stimulation, would improve consciousness in a convenience sample of UWS/VS and MCS patients. Our second question was whether the tDCS had an impact on CRS-R subscales. Finally, follow-up outcome data were acquired 12 months after inclusion using the Glasgow Outcome Scale-Extended to assess the long-term effect of tDCS.

For this study, we recruited 55 patients to receive both anodal and sham tDCS in a crossover study design. We included 25 UWS/VS (age: 42±17 years; 9 women; interval since insult: 24 ± 48 months; 6 post-traumatic) and 30 MCS (age: 43±19 years; 7 women; interval since insult: 43±63 months; 19 post-traumatic). Each patient received both anodal and sham tDCS stimulations in randomized order. A computer-generated randomization sequence was used to assign in a 1:1 ratio the first session as anodal tDCS or sham tDCS. Direct current was applied by a battery-driven constant current stimulator using saline-soaked surface sponge electrodes (7x5cm) with the anode positioned over the left DLPF and the cathode placed over the right supraorbital region, as previously described (Keeser, Meindl et al. 2011). During tDCS, the current was increased to 2 mA from the onset of stimulation and applied for 20 min. For the sham condition, the same electrode placement was used as in the stimulation condition, but the current was applied for 5 s, and was then ramped down to mimic the somatosensory artefact of real tDCS. Impedances were kept <10 kΩ and voltage <26 V. tDCS and sham were tested in random order in two separate sessions separated by 48 h (see figure 11). tDCS treatment effect was assessed by means of standardized CRS-R (Giacino 2004). Patients' outcome was assessed 12 months after the trial using the Extended Glasgow Outcome Scale to assess the long-term effects of tDCS on clinical evolution of patients (Jennett, Snoek et al. 1981). A good outcome was defined by a score >4 (i.e. return to independent living).

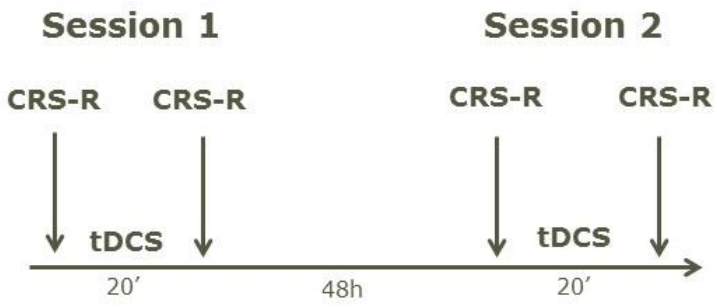


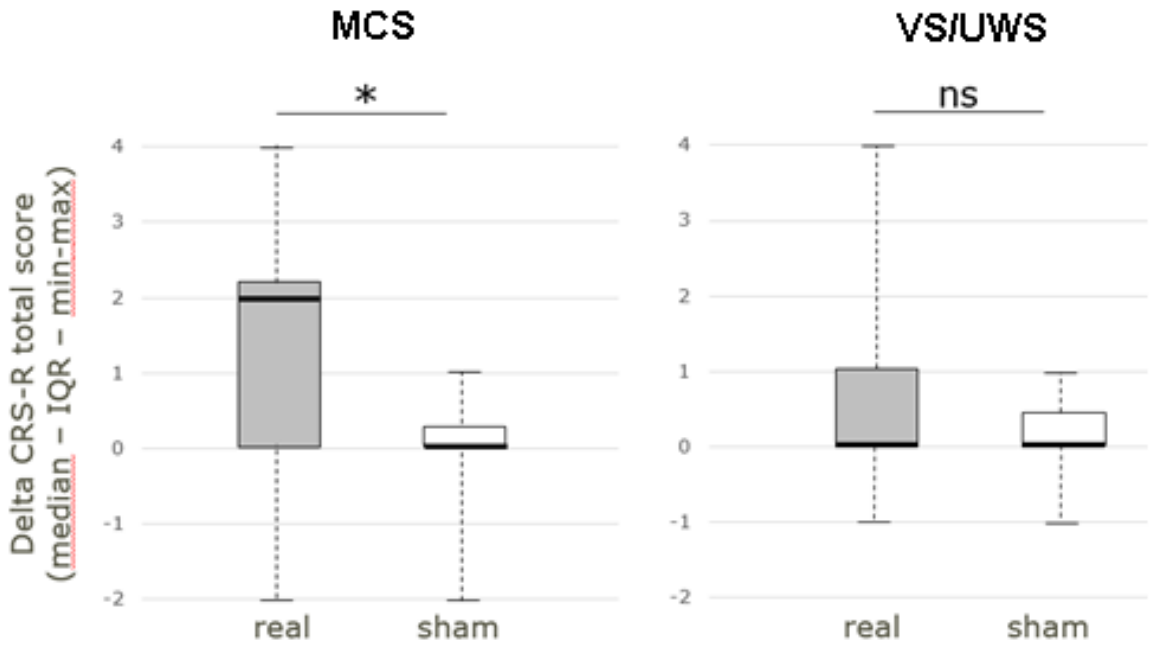
Figure 11: Protocol of the randomized double blind sham controlled study. Transcranial direct current stimulation (tDCS) was either real or sham. A Coma Recovery Scale Revised (CRS-R) was performed before and after each tDCS session.

Statistical analysis was performed using Stata (*Stata Statistical Software 11*.2 StataCorp, College Station, TX). The CRS-R responses were summarized using median (P25-P75). Statistical analysis of the cross-over data was based on the method proposed by Altman (Practical statistics for medical research, Chapman and Hall, London, pp. 467-471, 1991) and summarized here bellow. Evolution of CRS-R were considered as the difference between CRS-R total score after and before the stimulation (tDCS or sham). We looked for a period, interaction and treatment effect. The period effect compared the tDCS-sham response differences between the two periods (tDCS-sham and sham-tDCS). If there was no general tendency for subjects to do better in one of the two periods, we would expect the mean differences between the periods in the two treatment orders to be of the same size but having opposite sign (tDCS minus sham vs sham minus tDCS). The interaction effect referred to the calculation of the mean response after tDCS and sham together. In the absence of an interaction between treatment and period, a subject's average response to the two treatments would be the same regardless of the order in which they were received. Both, period and interaction effects, were tested using a Mann-Whitney U test. If no period and interaction effect was observed, then the treatment effect (tDCS versus sham) was assessed using a Wilcoxon signed-rank test. Results were considered significant at $p<0.05$. Multiple comparisons using Bonferroni correction (6 comparisons) had to be performed for the secondary end-point assessment (i.e. assessment of CRS-R *subscale* change according to tDCS vs sham) and results were considered significant at $p<0.0083$ (i.e. 0.05/6).

At the individual level, tDCS responders were defined as those patients who presented a sign of consciousness (i.e. command following; visual pursuit; recognition, manipulation, localization or functional use of objects; orientation to pain; intentional or functional communication; Giacino 2004) after tDCS that was not present before anodal nor before or after sham tDCS sessions.

32 patients (14 in UWS/VS; mean age of 46±17 years; 9 women; interval since insult: 44±72 months; 14 post-traumatic) first received anodal tDCS and 23 (11 in UWS/VS; mean age of 40±19 years; 7 women; interval since insult: 24±34 months; 11 post-traumatic) first received sham stimulation. No significant clinical or demographic differences were observed between the two groups.

At group level, there was a treatment effect for the MCS (p=0.003) but not for the UWS/VS (p=0.952) patients group (figure 12). No period or interaction effects were observed. No effect of tDCS on any of the CRS-R *subscales* was observed in any group (UWS/VS or MCS).



Median (black line) of Coma Recovery Scale-Revised (CRS-R) total scores delta (i.e., CRS-R post tDCS minus CRS-R pre tDCS) and interquartile range for patients (IQR, boxes) in minimally conscious state (MCS) and unresponsive wakefulness syndrome/vegetative state (UWS/VS), with minimal and maximal values. In grey, the results for the real tDCS and in with the sham tDCS. An asterisk (*) denotes statistical significance at $p < 0.05$ and NS stands for Non-Significant. From (Thibaut, Bruno et al. 2014).

At individual level, 13/30 (43%) patients in MCS showed a tDCS-related improvement (i.e. showed a clinical sign of consciousness never observed before). 2 acute (<3 months) patients in UWS/VS out of 25 (8%) showed a tDCS response (i.e. showed command following and visual pursuit present after the anodal stimulation not present at baseline or pre- or post- sham-tDCS). No tDCS related side effects were observed. No correlation between tDCS response and patient outcome was observed at 12 months follow-up.

Summary and discussion

In this work, we demonstrated that a single session of left DLPF tDCS may transiently improve CRS-R scores of patients in MCS without side effects. Our study illustrates the residual capacity for neural plasticity and temporary recovery of (minimal) signs of consciousness in some patients in MCS, but does not permit to make any claim regarding possible long-term tDCS effects in this setting. This is even more important since, at present, there are limited evidence-based pharmacological or non-pharmacological treatment options for severely brain-damaged patients with DOC, especially in the chronic setting (Bernat 2006, Giacino, Fins et al. 2014). Out of the 13 patients in MCS who showed a tDCS response, 5 were included >12 months after injury. This show that chronic patients, even years after the brain lesion, can still improve and recover some new signs of consciousness (even if no emergence from the MCS was observed in chronic patients). These clinical improvements in longstanding MCS corroborate previous evidence for late recovery and neural plasticity in MCS (Voss, Uluc et al. 2006, Luaute, Maucort-Boulch et al. 2010).

On the other hand, we observed no tDCS-related increase in CRS-R total scores in patients in UWS/VS, in line with previous studies showing more capacity for neural plasticity in patients in MCS (Monti 2012). It should be noted that, two acute UWS/VS patients showed improvement after tDCS. These two patients naturally recovered a communication few days following their inclusion in the study.

It could be that the observed tDCS-related transient improvements in consciousness as assessed by changes in CRS-R total score are related to the improvement in attention and working memory (D'Esposito, Detre et al. 1995), known to involve prefrontal cortical functioning (D'Esposito, Aguirre et al. 1998). The DLPF is thought to play a central integrative function on motor control and behaviour and to be a critical component of the decision-making network (Heekeren, Marrett et al. 2006).

Previous studies have shown that anodal tDCS over the left DLPF cortex has beneficial effects on working memory in neurological patients with Alzheimer's (Ferrucci, Mameli et al. 2008) and Parkinson's disease (Boggio, Ferrucci et al. 2006). Similarly, there is some evidence that tDCS of the left DLPF could improve attention in stroke (Kang, Beak et al. 2009) and mild traumatic brain injury (Kang, Kim et al. 2012) patients with attention deficits. A recent fMRI study showed that tDCS of the left DLPF cortex increased functional connectivity in the "default mode" (i.e. intrinsic cortical network) and bilateral frontal-parietal associative cortical networks (i.e. extrinsic networks; Keeser, Meindl et al. 2011) considered to be involved in internal and external awareness, respectively (Vanhaudenhuyse, Demertzi et al. 2011). As it is mentioned in the introduction, both networks are known to be dysfunctional in patients with DOC, as shown by previous PET (Thibaut, Bruno et al. 2012) and fMRI (Vanhaudenhuyse, Noirhomme et al. 2010) studies.

We here showed that tDCS is safe and, thus, could be tested as an alternative neuromodulatory tool to improve consciousness and cognitive function in severely brain-injured patients. A methodological limitation of tDCS is the absence of MRI-based mapping of the stimulated area. This limit is particularly constraining in patients with DOC, given the presence of focal brain damage, atrophy and injury-induced heterogeneity of their brain topography. Future studies could employ patient-tailored *structural* MRI-guided tDCS to overcome this limitation. Additional studies with *functional* MRI are warranted to document tDCS-specific changes in cerebral functional connectivity in DOC in order to better comprehend the mechanisms of action of tDCS, which remain only partially understood.

Moreover, the long-term effects of tDCS on clinical improvement in this patient population remains yet to be shown. Future controlled clinical trials should now employ long duration tDCS and its possible long-term effects, as it has been previously performed for other indications such as pain (Fregni, Boggio et al. 2006) and depression (Fregni, Liebetanz et al. 2007).

3.3.3 Clinical response to tDCS: understand its way of action

The mechanisms of action of tDCS remain only partly understood (for a complete review see Stagg and Nitsche 2011) and several clinical trials have shown that the proportion of tDCS responders may vary from 40 to 80% (Song, Vanneste et al. 2012, Goncalves, Borges et al. 2013, Ferrucci, Vergari et al. 2014). In our previous study, we reported that left DLPF tDCS could improve signs of consciousness in 43% of patients in MCS (Thibaut, Bruno et al. 2014). If these findings suggest the potential interest of tDCS as a treatment for DOC, they also highlight the lack of a clinical improvement following tDCS in more than half of the patient population. The natural step is to define the structural and functional brain features of those patients that are likely to respond to tDCS (Whyte 2014).

The aim of this retrospective study was to characterize the previously described (Thibaut, Bruno et al. 2014) subgroup of tDCS responders by means of multi-modal neuroimaging analyses, including FDG-PET, MRI and EEG. For this study, out of the 30 MCS patients included in our previous study, 24 patients underwent a brain FDG-PET acquisition, MRI acquisition and EEG registration on clinical demand. The FDG-PET and MRI scans of three patients were excluded from the statistical analysis due to suboptimal normalization. A group of age-matched healthy controls (n=17; mean age 47±13 years; 9 men) underwent both MRI and FDG-PET scans acquisition within one week of distance. As a reminder, tDCS responders were defined as patients who presented at least one additional sign of consciousness, as measure by the CRS-R, after tDCS that was never present before real tDCS, nor before or after the sham tDCS session (i.e. command following; visual pursuit; recognition, manipulation, localization or functional use of objects; orientation to pain; intentional or functional communication; Giacino, Kalmar et al. 2004).

EEG was recorded with a 16 channels cap (using the 10-20 positioning system Fp1, Fp2, Fz, F3, F4, Cz, C3, C4, T7, T8, Pz, P3, P4, Oz, O1, O2, referenced to the mastoid for 10 minutes). Basic rhythms were visually inspected by an EEG expert in order to discard artefacts and retained epochs. The remaining epochs were filtered between 0.75 and 40 Hz. For each subject and electrode the normalized power in each frequency bands was estimated (delta:1-4 Hz; theta: 4-7 Hz; alpha: 8-12 Hz; and beta 12-25Hz, as used in (Lehembre, Bruno et al. 2012). Then, we calculated the mean of all the electrodes rhythms together during the recording time. Student unpaired t tests were performed to compare the mean power averaged of each rhythms of

interest (i.e. bands) between responders and non-responders. Results were corrected for multiple comparison.

Structural MRI T1 data (T1-weighted 3D gradient echo images using 120 slices, repetition time = 2300 ms, echo time = 2.47 ms, voxel size = 1 x 1 x 1.2 mm^3, flip angle = 9 degrees, field of view = 256 x 256 mm^2) were acquired on a 3T scanner (Siemens Trio Tim, Munich, Germany). A T1-based voxel-based morphometry (VBM) analysis of brain structure (http://dbm.neuro.uni-jena.de/vbm/) was applied to search for potential morphological differences in grey matter volume between the two patient groups. For this analysis, we used DARTEL-based spatial normalization (Ashburner 2007) to allow the high-dimensional spatial normalization in order to increase the chance of correct normalization of the severely damaged brain of patients with disorders of consciousness (Takahashi, Ishii et al. 2010). A study template made from the average of T1 images from our patients and control subjects was used to facilitate the normalization procedure (Ashburner 2007, Peelle, Cusack et al. 2012, Di Perri, Bastianello et al. 2013). The design matrix separately modelled patients' (responders and non-responders) and healthy controls' grey matter density. Results were considered significant at family-wise whole-brain volume-corrected for multiple comparisons (FWE) $p<0.05$.

Brain metabolism was measured during rest using PET-CT (Gemini Big Bore TF, Philips Medical Systems) after intravenous injection of 300 MBq FDG (as previously reported; Laureys, Faymonville et al. 2000). In order to reduce the influence of the surrounding structures on the radiotracer concentration, phenomenon known as partial volume effect (PVE) - particularly critical when the relative proportion of brain tissue components is altered- a partial volume effect correction (PVEc) was applied to the PET images (Quarantelli, Berkouk et al. 2004). PET data were then pre-processed as previously published (Phillips, Bruno et al. 2011, Bruno, Majerus et al. 2012, Thibaut, Bruno et al. 2012), including spatial normalization, smoothing (using a Gaussian kernel of 14 mm full width at a half maximum) and proportional scaling, implemented in Statistical Parametric Mapping toolbox (SPM8; www.fil.ion.ucl.ac.uk/spm). The design matrix separately modelled patients' (responders and non-responders) and healthy controls' PET scans. Results were considered significant at family-wise whole-brain volume-corrected for multiple comparisons (FWE) $p<0.05$.

Statistical comparisons of clinical data between the two groups (patients' age, time since onset, aetiology – traumatic versus non-traumatic – CRS-R total score changes) were performed using student t tests implemented in Stata (*Stata Statistical Software 11*.2 StataCorp, College Station, TX) and considered significant at $p<0.05$ corrected for multiple comparisons.

Out of the 21 patients in MCS that were included in the analyses, 8 were tDCS responder (4 post-traumatic, 4 non-traumatic, 4 men) and 13 were non-responder (8 post-traumatic, 5 non-traumatic, 10 men). The responders and non-responders did not show a significant difference in age (mean ± SD; 38±19 vs 36±14y respectively; p=0.84), time since onset (6±8 vs 4±3y; p=0.45), or baseline CRS-R total score (median (IQR); 9(3) vs 9(7); p=0.29). At the group level, CRS-R total scores improvement (obtained before and after active tDCS) were higher in responders as compared to non-responders (see figure 15A).

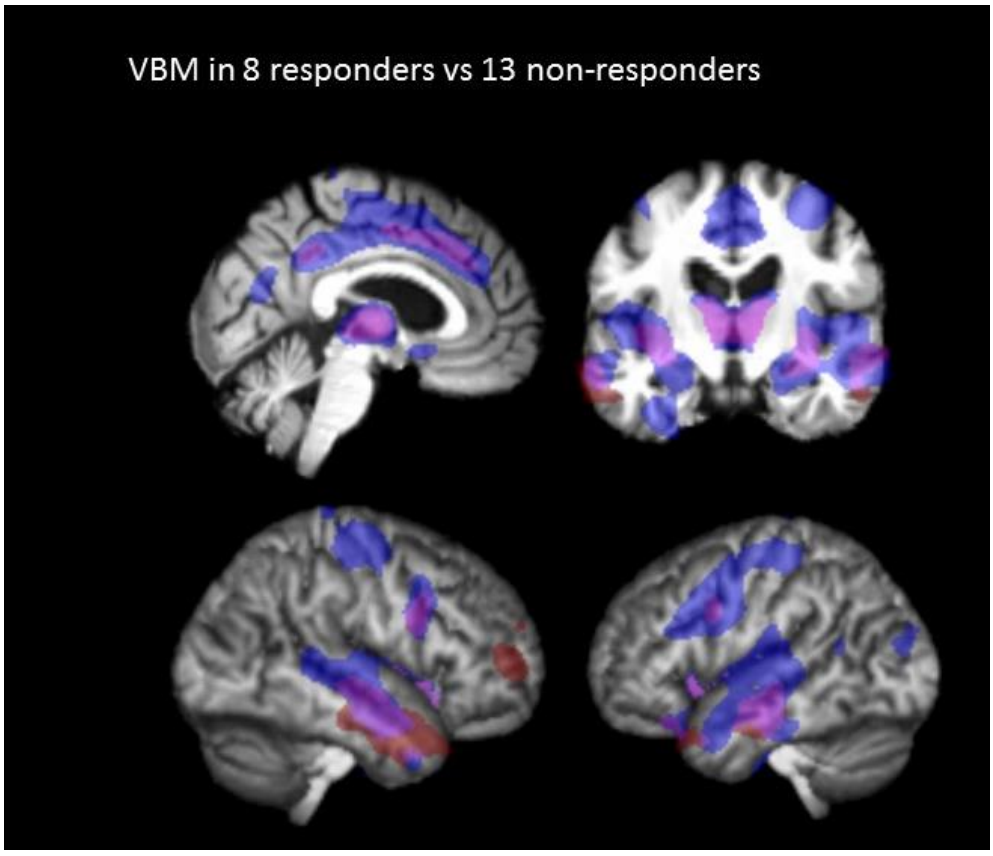


Figure 13: Voxel based morphometry (VBM): in red are represented the areas of grey matter atrophy in responders compared to healthy controls. In blue the areas of grey matter atrophy in non-responders compared to healthy controls. In pink the overlapping between responders and non-responders. Results are shown at FWE $p<0.05$ and are superimposed in sagittal slices in MNI space and projected in the lateral surfaces of the rendered MNI single subject brain. From (Thibaut, Di Perri et al. submitted).

VBM: Statistical analyses identified: A) reduced grey matter areas (as compared with healthy controls) in the subgroup of tDCS non-responders, and B) reduced grey matter areas in the subgroup of tDCS responders (as compared with healthy controls). Based on the VBM, responders showed decreased grey matter volume in the lateral temporal cortex, the thalamus, the lenticular nuclei, the left caudatum, the right amygdala and parahippocampal gyrus and to a certain extent the right DLPF cortex and the cingulate cortex. Non-responders showed decreased grey matter volume in the same regions observed in the tDCS responders (except for part of the temporal poles) but more extensively in the precuneus/cuneus and the cingulate cortex and additionally in the left frontal medium/inferior gyrus, the superior temporal gyri, the hippocampi, the left amygdala and to some extent the rolandic areas (see figure 13).

FDG-PET: Statistical analyses identified: A) brain areas showing hypometabolism (as compared with healthy control) in the subgroup of tDCS responders, B) brain areas showing hypometabolism in the subgroup of tDCS non-responders, and C) brain areas showing a relatively preserved metabolism in tDCS responders as compared to tDCS non-responders. Findings from FGD-PET in tDCS responders showed regional hypometabolism (as compared with healthy control) in the medial prefrontal cortex/anterior cingulate cortex, the medial thalamus bilaterally and the caudate. tDCS non-responders showed impaired metabolism in similar areas (albeit more extended in the medial prefrontal cortex, the caudate and left thalamus) and additionally in the precuneus and the left DLPF cortex. Areas showing preserved metabolism in responders as compared with non-responders were in the left DLPF cortex, the medial-prefrontal cortex, the precuneus, the caudate and the left thalamus (figure 14). Inversely, no brain areas appeared metabolically preserved in non-responders as compared with responders.

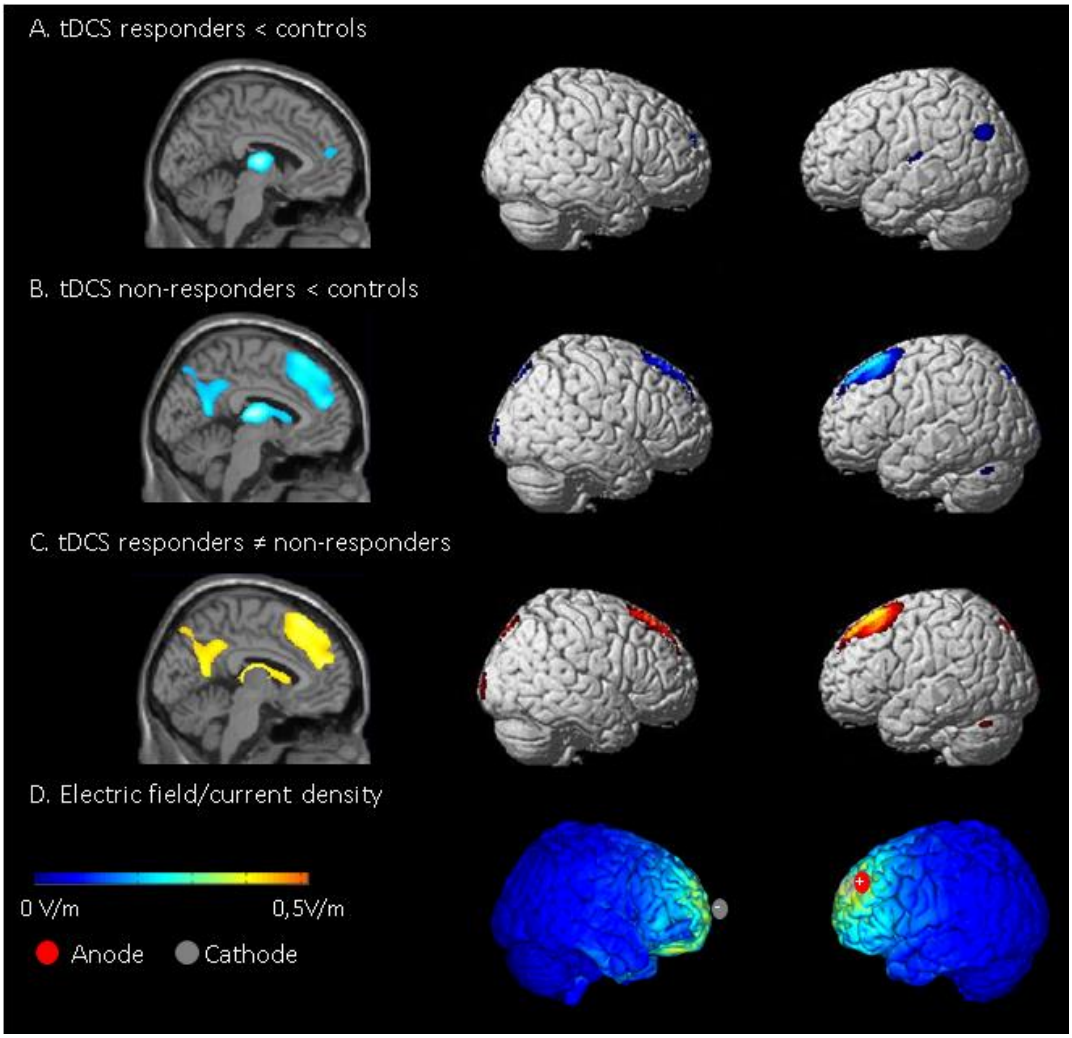


Figure 14: Positron emission tomography (PET): Brain areas showing hypometabolism (in blue), as compared to controls, in patients in a minimally conscious state (FEW corrected): (A) 8 tDCS-responders and (B) 13 non-responders. (C) Regions with less hypometabolism in responders as compared to non-responders (in red). (D) Theoretical (Ruffini, Fox et al. 2014) tDCS induced electric fields. Note that behavioural responsiveness to short duration left dorsolateral prefrontal cortex (DLPFC) tDCS correlates with less impaired metabolism in the areas presumed to be stimulated by tDCS (left DLPFC and mesiofrontal cortices) but also of distant cortical (precuneus) and subcortical (thalamus) regions. From (Thibaut, Di Perri et al. submitted).

When focusing on the mean glucose uptake in three critical regions for consciousness recovery (left DLPF cortex, precuneus and thalamus) a significant decreased metabolism was observed in non-responders as compared with responders (figure 15B).

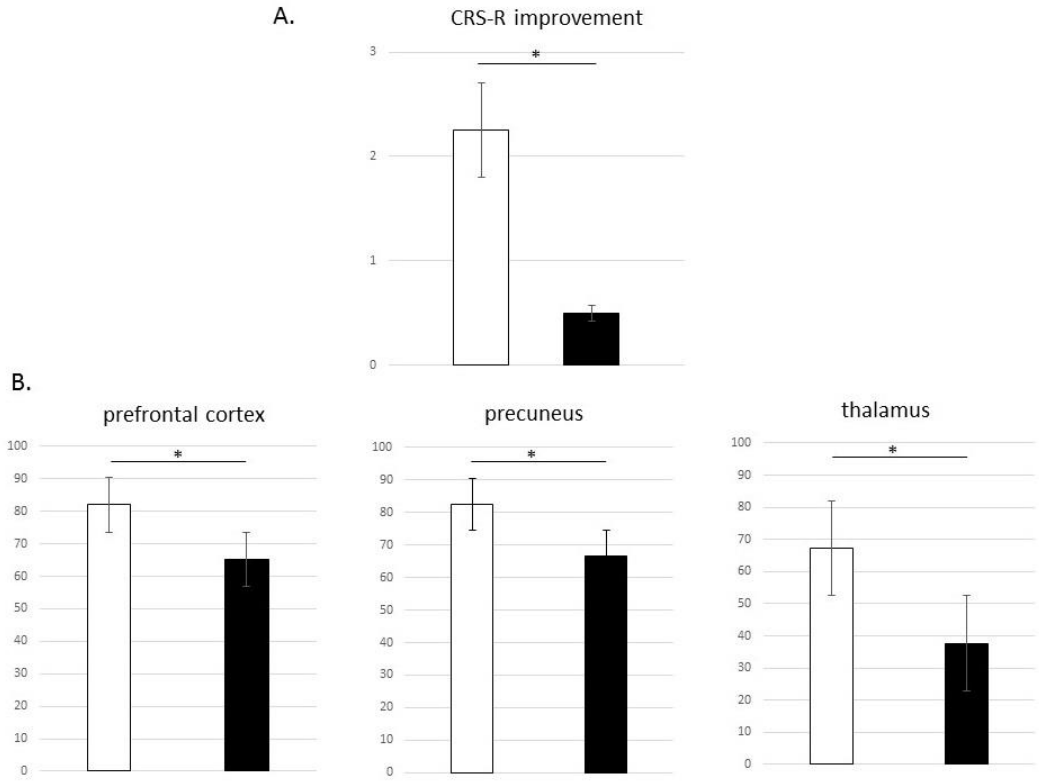


Figure 15: A. Mean and standard deviation of Coma Recovery Scale Revised (CRS-R) total score changes in tDCS responders and non-responders. B. Mean and standard deviation of normalized glucose metabolism (% of normal) in left dorsolateral prefrontal (DLPF) cortex, precuneus and left thalamus in tDCS-responders (white) and non-responders (black). An asterisk (*) denotes statistical significance at $p < 0.05$. From (Thibaut, Di Perri et al. submitted).

EEG: We failed to detect significant differences in the mean frequency bands (i.e. delta, theta, alpha, and beta) between the two groups.

Summary and discussion

In this retrospective study, we observed a common pattern of metabolic preservation (as detected by FDG-PET) and grey matter preservation (as detected by MRI), in tDCS responders as compared with non-

responders, whilst no specific behavioural patterns of improvement among the patients who showed clinical improvement following left DLPF cortex tDCS could be observed. The areas of preserved grey matter observed in responders as compared with non-responders involved the left frontal medium/inferior gyrus, the precuneus, the cingulate cortex, the superior temporal gyri, the hippocampi, the left amygdala and to some extent the rolandic areas. The areas metabolically preserved in responders as compared with non-responders included the left DLPFC, the medial-prefrontal cortex, the precuneus, and the thalamus.

The residual brain metabolism in the left DLPF cortex where the anode of the tDCS was positioned suggests that, independently from the variability of the cortical damage, a residual brain activity in the stimulated area is necessary for an effective stimulation. These results are in agreement with a previous study on patients with stroke showing that tDCS effects upon stimulation on motor area are limited when the pyramidal tract is damaged (as detected by diffusion tensor imaging; Schlaug, Renga et al. 2008). Moreover, a study using TMS coupled with EEG on patients with DOC further showed that cortical responses can be detectable only upon stimulation of a preserved cortical tissue (Gosseries, Sarasso et al. 2015).

Our results regarding the residual brain metabolism and preserved grey matter in the medial-prefrontal cortex, posterior cingulate/precuneus and thalamus in responders rather than non-responders highlight the role played by these structures in the recovery of consciousness. PET studies on unresponsive patients versus control subjects have, in fact, previously identified metabolic impairment in regions involving the medial-prefrontal cortex and the posterior cingulate/precuneus, also known as default mode network, and the lateral fronto-parietal regions including the DLPF cortex, also known as executive control network, suggesting their crucial role in the emergence of consciousness (Laureys, Goldman et al. 1999, Laureys 2004, Thibaut, Bruno et al. 2012). The default mode network and the executive control network have further been functionally related respectively to internal awareness (i.e. awareness of self) and external awareness, (i.e. awareness of the environment). Moreover, as we have discussed in the introduction, their metabolism has shown to be gradually restored going from a lower to a higher degree of consciousness (Thibaut, Bruno et al. 2012). In particular, the residual metabolic and structural integrity of the medial-prefrontal cortex and the residual metabolism in the thalamus observed in responders rather than non-responders seem to support the key role of these structures in the disturbances of consciousness, in the setting of a widespread deafferentation and neuronal cell loss as observed after severe brain injuries

(Schiff 2010, Fridman, Beattie et al. 2014). The observed residual metabolic and structural integrity of the posterior cingulate/precuneus corroborates a large amount of literature indicating this structure as a key component of the internal awareness network, namely the default mode network, and a critical hub for consciousness recovery (Laureys, Goldman et al. 1999, Laureys, Boly et al. 2006, Vanhaudenhuyse, Noirhomme et al. 2010, Vanhaudenhuyse, Demertzi et al. 2011, Fernandez-Espejo, Soddu et al. 2012, Thibaut, Bruno et al. 2012).

The circumstance that the metabolic and structural integrity of structures belonging to the default mode network seems to be necessary for the clinical improvement of MCS patients upon tDCS stimulation is consistent with recent studies showing that tDCS enhances diffusively brain functional connectivity, especially targeting the default mode network and the executive control network (Lang, Siebner et al. 2005, Keeser, Meindl et al. 2011, Pena-Gomez, Sala-Lonch et al. 2012, Stagg, Bachtiar et al. 2012, Clemens, Jung et al. 2014). In fact, recent studies combining prefrontal tDCS and resting-state fMRI have shown that prefrontal tDCS modulates large-scale patterns of resting-state connectivity in the human brain by increasing coactivation patterns both in regions close to anode and cathode stimulation sites and in more widespread and distant brain regions. Additionally, these effects appeared to be more pronounced for the default mode network (Keeser, Meindl et al. 2011, Pena-Gomez, Sala-Lonch et al. 2012, Clemens, Jung et al. 2014). These studies further suggest the extensive and widespread action of the tDCS and therefore the importance of intra and inter-network connectivity for its efficacy.

With regards to EEG, it has been shown that left DLPF cortex tDCS on healthy controls and patients with moderate traumatic brain injury can either improve EEG high frequency activity or decrease low frequency activity, both at rest and during cognitive tasks (Keeser, Padberg et al. 2011, Song, Shin et al. 2014, Ulam, Shelton et al. 2014). The routine clinical EEG data hereby collected did not show any statistically significant difference between the two patient groups. However, this might be explained by the suboptimal quality (small number of electrodes, analysis on whole brain) and accuracy of EEG as acquired in clinical setting.

It is important to stress that our findings must however be read taking into account some caveats:

i) This study lacks of a direct comparison between the two patient groups (responders versus non-responders). This is related to the limited size of the population and the high degree of variability

within the groups involving both neuroradiological findings and aetiology.

ii) We cannot use the findings of the present study to predict the clinical improvement upon tDCS at single subject level. In fact, our results could effectively be applied only at a group level. Furthers studies might be warranted to detect specific features to predict the outcome at individual level.

iii) The stimulation area might be considered so far only theoretical since patients had widespread brain lesion. A functional reorganization and /or the development of atrophy and/or scars under the site of stimulation might have occurred. Therefore, a single subject head model of the current field is needed in order detect the trajectory of the current in in those severely injured brains.

iv) Other acquired neuroimaging data, such as functional MRI and diffusion tensor imaging, were not analysed. In fact, functional MRI and diffusion imaging tensor are extremely sensitive to motion and metal artefacts. This resulted in the availability of a too small sample of good quality data to be included in a statistical analysis. Furthermore, when patients were sedated the fMRI data were excluded from the analysis by default, as sedation might have affected the results.

v) Finally, this study does not aim to investigate the effect of tDCS on brain metabolism. Here, we rather try to understand what might be the distinctive features of the subgroup of patients who respond to tDCS.

In conclusion, the present study showed that the transient improvement of signs of consciousness following left DLPF tDCS in patients in sub-acute and chronic MCS seems to require grey matter integrity and/or residual metabolic activity in three brain regions: (i) the presumed stimulated area (i.e. left DLPF cortex), (ii) long distance cortical areas such as the precuneus, and (iii) subcortical brain areas known to be involved conscious processes (i.e. thalamus).

3.4 Conclusion and future directions

These studies aimed at better understand the mechanisms of action and the potential benefits of two treatment options in patients with DOC, namely zolpidem and tDCS.

Our first study showed that the behavioural recovery of three MCS patients who responded to zolpidem (i.e. emerged from MCS) paralleled a metabolic recovery in the prefrontal cortex (Thibaut, Chatelle et al. 2014). We sought to explain these findings in the setting of the mesocircuit hypothesis, suggesting that zolpidem (GABA-A agonist) reduces the inhibition of thalamus by acting on the GPi (Schiff 2010) and, subsequently, increases the connection between the thalamus and the prefrontal cortex. In fact, the functional impairment of the prefrontal cortex is related to a functional phenomenon known as diastasis, not structural damage. Another mechanism we proposed is the GABA impairment hypothesis (Pistoia, Sara et al. 2014). This theory suggests that zolpidem reverses the impairment of GABA therefore, restores a normal ratio between synaptic excitation and inhibition. This hypothesis explains the potential mode of action of both zolpidem and baclofen on patient recovery. Nevertheless, it does not explain why we observed an improvement in brain metabolism in the prefrontal cortex following zolpidem intake in the 3 patients studied. To test this hypothesis we would need to perform [18F] Fluoroethylflumazenil-PET (FEF-PET) in order to detect benzodiazepine receptors (Grunder, Siessmeier et al. 2001). Further studies involving a larger number of patients or comparing zolpidem responders and non-responders using multimodal imaging analyses (MRI, PET, EEG) could help us to better understand the mechanism of zolpidem and the reason why only a small number of patients can benefit from this drug.

In this chapter we also showed the potential therapeutic effects of tDCS (Thibaut, Bruno et al. 2014), the likes of which has never before been tested on patients with DOC. Considering that almost half of the patients in MCS showed behavioural improvement after a single stimulation, the results of our pilot study seem promising. Nevertheless, we still did not know why some MCS patients respond to tDCS and others do not. To answer this question we compared brain metabolism and morphology in tDCS responders and tDCS non-responders.

We showed that the transient increase of signs of consciousness in patients with DOC upon tDCS requires residual metabolic activity and grey matter preservation in cortical and subcortical brain areas

important for consciousness recovery (i.e. left DLPF cortex, precuneus and thalamus – Thibaut, Di Perri et al. submitted). These findings underline the critical role of long-range cortico-thalamic connections in consciousness recovery, and provide important information for guidelines on the use of tDCS in DOC. Studies in which neuroimaging (MRI, PET and HD-EEG) is done before and after a tDCS session should be carried out to investigate the effect of tDCS on the brain of each patient and the differences between responders and non-responders such as to better identify the patients who could benefit from left DLPF tDCS.

Regarding tDCS studies on healthy volunteers, a recent meta-analysis concluded that tDCS over the prefrontal cortex does not have a significant effect on working memory outcome or language production tasks (Horvath, Forte et al. 2015). One hypothesis they propose to explain this finding is state-dependency: this concept suggests that the effect an external stimulus has on the brain is highly influenced by the state of the brain at the time of stimulus onset (Silvanto, Muggleton et al. 2008), as shown for TMS (Silvanto and Pascual-Leone 2008). This hypothesis suggests that the different state-dependent effects of the various studies included in the analysis influenced the null results obtained. As information in literature confirming this hypothesis is scares, future studies should include such details (e.g. the time of day, day of week, and duration of stimulation sessions, energy-levels, amount of sleep of individual participants) in order to better understand how these factors influence tDCS results.

Previous studies on healthy subjects showed that tDCS over the primary motor cortex could enhance motor performance of the non-dominant hand but not of the dominant one (e.i. Boggio, Castro et al. 2006). This suggests that tDCS can improve skills that are not yet at maximum potential, but has no effect on already well trained skills. That is, tDCS has limited effects on a healthy subject without cognitive or motor impairment, but could help patients with deficits to recover a dysfunctional ability.

Furthermore, several sessions of tDCS may be required in order to achieve the desired effect. A study of repeated tDCS over the primary motor cortex in healthy volunteers highlighted a consolidation mechanism which lasted up to 3 months after 5 tDCS sessions (Reis, Schambra et al. 2009). Unfortunately, not enough comparable multiple-day stimulation studies have been carried out to assess whether repeated tDCS sessions could be efficient at improving motor or cognitive skills in healthy volunteers. Nevertheless, in neurological patients with motor or cognitive deficits, tDCS has shown to be effective and its effects seem

to last several weeks or even months when the stimulation is repeated for 5 or 10 consecutive days (for a review of reaped tDCS on stroke patients see table 9). Based on the above-said, we believe the efficacy of tDCS to be relevant and proven in cognitive or motor deficit rather than in healthy controls and that repeated stimulation might be required to induce reliable improvements that could warrant its implementation in clinical daily practice.

Authors (year)	Study	Stimulation parameters	Patients	Assessment	Results
Stroke; motor					
Boggio et al. (2007)	Randomised, double blind, sham controlled, cross-over study	M1: anode or sham: ipsilesional hemisphere; cathode: contralesional, 1 mA, 35 cm2, 20 minutes; 1 weekly session for 1 month or 1 daily session during 10 consecutive days	9 patients, subcortical stroke; > 12 months post-insult	Hand function (Jebsen Taylor Test)	Transitory motor improve-ment after anodal and cathodal stimulation. Cumulative and lasting effect with daily stimulation
Linden-berg et al. (2010)	Randomised sham controlled	M1: anode or sham: ipsilesional hemisphere; cathode: contralesional hemisphere, 1.5 mA, 30 minutes, 5 consecutive days, associated with PT	20 patients Sylvian ischemic stroke. Sham group: 10 patients; > 5 months post insult	Stroke impairment (Upper-Extremity Fugl-Meyer Assessment) and Wolf Motor Function Test	Significant progress in the treated group, lasting one week after the intervention
Kim et al. (2010)	Randomised, double-blind, sham controlled	M1: anode or sham: ipsilesional hemisphere; cathode: contralesional, 2 mA, 25 cm2, – 10 sessions associated with OT	18 patients; > 2 months post insult	Fugl Meyer (upper limb) and Barthel Index	Functional progress in the three groups. Better for cathodal stimulation at 6 months
Nair et al. (2011)	Randomised, double-	M1: cathode: contralesional	14 chronic (> 1 year)	Range of motion,	tDCS + OT: motor

	blind, sham controlled	hemisphere; anode: opposite supraorbicular area; 1 mA; 30 min for 5 days. Combined with OT. 1 week follow-up	stroke patients	Upper-Extremity Fugl-Meyer Assessment, fMRI motor task	progress. Effects lasted one week
Khedr et al. (2013)	Randomised, double-blind, sham controlled	Anodal: anode or sham: ipsilesional hemisphere; cathode: supraorbicular area; Cathodal: cathode or sham: contralesional hemisphere; anode supraorbicular area; 2mA, 35 cm^2; 25 min during six consecutive days. 3 months follow-up	40 acute (< 28days) patients with ischemic stroke	NIHSS, Barthel index, Medical Research Council muscle strength scale, MEP	Better improvement for both cathodal and anodal tDCS compare to sham of the severity of stroke at 3 months follow-up
Ochi et al. (2013)	Randomised, double-blind, sham controlled, cross-over study	Anodal: anode or sham: ipsilesional hemisphere; cathode: supraorbicular area; Cathodal: cathode: contralesional hemisphere; anode: supraorbicular area; 2mA, 35 cm^2; 10 min during five days	18 chronic stroke patients	Fugl-Meyer assessment for the upper limb, Modified Ashworth Scale, Motor Activity Living	Anodal and cathodal tDCS showed improvement of motor function and spasticity. Progresses lasted one week
Stroke; aphasia					
Baker et al. (2010)	Randomised, double blind, sham controlled, cross-over study	Area predetermined by fMRI naming task; anodal or sham, 1 mA, 25cm^2, 20 min; cathode over the right shoulder. 5 consecutive days of stimulation. 7 days of washout	10 patients, > 10 months post-insult	Computeri-zed naming test; 2 additional untargeted word lists (1 for each stimulation type)	Improvement of naming accuracy of treated items. Effect persisted at least 1 week
Fiori et al. (2011)	Randomised, double blind, sham controlled,	Anode: Wernicke (CP5); cathode: contralateral supraorbicular area, 1 mA, 20 minutes, 5	3 patients, > 3 months post stroke	Naming task and reaction time	More correct answers and better reaction

	cross-over study	consecutive days. 3 weeks follow-up			time. Effect lasted 3 weeks
Marango-lo et al. (2013)	Randomised, double blind, sham controlled, cross-over study	Anode: Broca or Wernicke or sham; cathode: right supraorbicular area; 1 mA, 35 cm^2,20 min; 10 consecutive days. 1month follow-up. Coupled with speech therapy	12 chronic (> 6months) patients with left hemisphere stroke	Battery for the analysis of aphasic disorders, Token test, selective, divided and sustained attention tests, visual memory test	Improvement of aphasia after anodal tDCS over Broca's area. Improvement lasted 1 month
Polanows-ka et al., (2013)	Randomised, double blind, sham controlled study	Broca area: anode or sham; cathode: right supraorbicular area; 1 mA; 10 min. 15 sessions (5days/w, during 3w). Combine with speech therapy. 3 months follow-up	24 stroke patients with left MCA stroke (2 to 24 weeks post onset)	Naming accuracy and naming time	tDCS: higher effect sizes in naming time, until 3-month follow-up
Vestito et al. (2014)	Pilot, single-blind, sham-controlled, cross-over study	Anode: Broca's area; cathode: right supraorbicular area; 1.5 mA; 25cm^2; 20 min for 10 days. 21 weeks follow-up	Chronic stroke patients (> 1 year post insult)	Aachener Aphasie Test, Boston Naming Test	Improvement of naming with positive effect until the 16th week
Stroke, other					
Olma et al. (2013)	Randomised, double blind, sham controlled cross-over study	Anode, or sham: ipsilesional calcarine sulcus (MRI navigation system); cathode: Cz; 1.5 mA; 25cm^2; 20 min for 5 consecutive days. 4 weeks follow-up	12 chronic stroke patients and homonymous visual field defects	Motion detection task	Improvement in motion perception. Still present at 14- and 28-day follow-up

Table 9: Studies on the effects of repeated transcranial direct current stimulations on motor and cognitive deficit in stroke patients. All studies reported prolonged effects after 5 to 15 days of stimulations that lasted up to 4 months. Abbreviations: NIHSS: National Institutes of Health Stroke Scale; M1: primary motor area; fMRI: functional magnetic resonance imaging; MEP: motor evoked potential; PT: physical therapy; OT: occupational therapy; MCA: middle cerebral artery.

Clinical recommendations

Our next step will be to determine whether or not the short-term effects of tDCS can be improved sufficiently in terms of efficacy and duration to make it suitable for use in clinical practice. We have started a study to test the effects of repeated stimulation sessions carried out 5 days consecutively and to evaluate the benefits, in terms of CRS-R, a week from the end of the stimulations. The results will tell us if tDCS could be used as a therapeutic tool on a daily basis in clinical practice, in rehabilitation centres, nursing homes or even at the patient's home. Moreover, we will test if an increased number of stimulations could also enhance the beneficial effect (as measured by effect size) and increase the number of patients who respond to the treatment.

4. Conclusion and perspectives

“There is nothing permanent except change.”

Heraclitus

Managing pain and promoting recovery in patients with DOC is a major challenge to overcome. In this work, we first tried to improve our understanding of pain assessment tools (NCS-R) and of spasticity in non-communicative severely brain-injured patients. Next, we studied potential pharmacological (zolpidem) and non-pharmacological (tDCS) treatments for promoting recovery in these patients.

Neuroimaging studies have suggested that patients in MCS and some patients in UWS/VS retain sufficient cortical activity to process pain in a similar way to healthy subjects (Boly, Faymonville et al. 2008, Markl, Yu et al. 2013). These findings highlight the importance of managing potential pain in patients with DOC independent of their diagnosis at the bedside, which might be incorrect (Schnakers, Chatelle et al. 2012, Thibaut, Chatelle et al. 2014). The NCS-R was developed to behaviourally assess pain in non-communicative severely brain-injured patients (Chatelle, Majerus et al. 2012). In this thesis, we have shown that the NCS-R constitutes a sensitive and appropriate behavioural tool to assess pain in patients with DOC by showing that the NCS-R total score is related to cortical processing of pain (Chatelle, Thibaut et al. 2014). In the future, we should investigate the sensitivity of the scale to analgesic treatment. This would allow clinicians to evaluate the efficacy of a treatment and adapt it rapidly in order to manage patients' comfort and promote recovery. Additionally, further studies will need to investigate the clinical usability of the scale to handle chronic pain in patients with DOC. Above and beyond this, we need to develop clear guidelines regarding the assessment and the management of pain in this population to help clinicians in the daily management of patients.

A potential source of discomfort in chronic patients is spasticity. Spasticity could also be a main confounder in behavioural assessment of consciousness. We showed here that almost 90% of patients in UWS/VS and MCS suffer from spasticity, while 62% of patients have severe invalidating spasticity (Thibaut, Deltombe et al. in press). We also identified a positive correlation between pain (assessed by the NCS-R) and the severity of this motor disorder. This result suggests a strong potential impact of spasticity on patients' quality of life and demonstrates the importance of understanding and treating spasticity as well as the other motor and muscle dysfunctions (e.g. tendon retraction, joint fixation, muscular connective tissue and fat content, reorganization of CNS motor areas and their activation) commonly associated with it. In patients with spasticity the hyperexcitability of the stretch reflex is neurophysiologically characterized by an increase of the "H max/M max" ratio. This could be due to an exaggerated facilitation of the H-reflex

to voluntary muscle contraction and/or to the lack of inhibition associated with muscle relaxation (Schieppati 1987, Nielsen, Petersen et al. 1993). A first step could be to use the H max/M max ratio and compare it between the population of stroke patients and patients with DOC. If the observed spasticity in this population of patients is more related to hypertonia, dyskinesia or ankyloses than spasticity itself, we should observe a similar or smaller H max/M max ratio in DOC as compared to stroke patients. Additionally, the H max/M max ratio would not correlate with the MAS scores. This would suggest than scales adapted to non-communicative patients with tendon retraction, joint fixation and ankyloses, should be developed to evaluate all motor disorders and not only spasticity. Looking at the patients' structural brain lesions could also help us improve our understanding of the mechanisms underlying spasticity in this population.

Regarding the treatment, we have shown a positive effect of soft splinting on spasticity of the intrinsic hand muscles. This technique seems to be a worthy alternative to conventional rigid splints for chronic patients with DOC. To evaluate the potential improvement of the effects of soft splinting and to attest its utility in clinical daily practice, further studies assessing the long-term effects of daily application are needed. We should also study other treatments that can be used on both upper and lower limbs, such as botulinum toxin A. Indeed, this drug has been shown to efficiently reduce spasticity in stroke patients, improve arm posture, hand hygiene, facilitate dressing for caregivers, and decrease pain (Simpson, Alexander et al. 1996, Bhakta, Cozens et al. 2000, Brashear, Gordon et al. 2002). It would be worth studying the effect of botulinum toxin A on patients with DOC and investigating the effects on patient comfort (e.g. using the NCS-R during mobilization and/or care), as well as the impact on caregivers and bedside assessment of consciousness.

The onset of spasticity could be explained by an anarchic neuronal reorganization after brain lesion (Sheean 2002). If we could stimulate the motor regions to promote a healthy cortical reorganization after the brain damaged area, it may result in a reduction in motor impairment. We know that non-invasive techniques such as anodal tDCS can increase the neuronal excitability under the stimulated area (Nitsche and Paulus 2000). It could be hypothesized that stimulating the damaged motor area after a brain lesion could stimulate an appropriate and healthy reorganization of the cortex and may potentially reduce the occurrence of spasticity in brain-injured patients.

Regarding the curative treatments, we have demonstrated that tDCS on the left prefrontal dorsolateral cortex may transiently improve signs of consciousness in almost 50% of patients in MCS both in acute and chronic stages (> 3 months post insult) (Thibaut, Bruno et al. 2014). Additionally, we have found that clinical effects after tDCS seem to be associated with some residual brain metabolism and grey matter preservation in the stimulated area (i.e. left prefrontal cortex), long-distance cortical areas (i.e. precuneus) and subcortical areas (i.e. thalamus) (Thibaut, Di Perri et al. submitted). If these findings suggest that not all patients could respond to this treatment, it highlights the need for improved patient characterization in order to guide treatments in this population.

In our work, we focused on the effect of a single stimulation on recovery of signs of consciousness, although it may take more than one stimulation to some patients to respond (Vestito, Rosellini et al. 2014). Therefore, it could be interesting to look at the effects of repeated tDCS sessions to know if it may lengthen the duration of the benefits as well as increase the proportion of responders. Another potential option for increasing the number of responders would be to use a multi-focal tDCS to stimulate the entire external awareness network (Vanhaudenhuyse, Demertzi et al. 2011, Thibaut, Bruno et al. 2012). This would increase the probability to stimulate relatively preserved brain areas and therefore target higher cognitive function in DOC. Mechanistically, this multifocal stimulation of the external consciousness network could increase and strengthen long distance functional connectivity inside this network which is thought to correlate with the recovery of consciousness in DOC (Vanhaudenhuyse, Noirhomme et al. 2010). Previous fMRI (Keeser, Meindl et al. 2011, Pena-Gomez, Sala-Lonch et al. 2012) and EEG (Keeser, Padberg et al. 2011) studies in normal volunteers have indeed shown some preliminary evidence that tDCS might increase cerebral functional connectivity. It would be interesting to record an EEG at rest before and after the stimulation in order to objectively measure any effect on cortical connectivity. This could be used to try to predict clinical efficacy based on objective EEG markers (e.g. entropy, time-frequency and connectivity measures; Lehembre, Bruno et al. 2012).

Treatment combination is another possibility to increase the effect on recovery. We have shown that zolpidem induces a metabolic increase in the prefrontal cortex (Thibaut, Chatelle et al. 2014). On the other side, we have highlighted the critical role of the prefrontal cortex and the thalamus in tDCS responders. These results highlight the key role played by thalamo-cortical connectivity, and especially the connectivity

between the prefrontal cortex and the thalamus in recovery of signs of consciousness, which has previously been suggested by the literature (Laureys, Faymonville et al. 2000, Laureys, Antoine et al. 2002, Crone, Soddu et al. 2014, Monti, Rosenberg et al. 2015). Further treatments to improve the revalidation of patients with DOC should focus on these brain areas. The combination of zolpidem and tDCS, both acting on the prefrontal cortex, could increase the effects of tDCS and/or increase the number of patients who could respond to zolpidem. In addition, other drugs could be tested to prolong and enlarge the effects of the stimulation on cognitive functions. For example, combining tDCS with an NMDA receptor agonist, such as D-Cycloserine, that has already shown to potentiate the duration of cortical excitability enhancements induced by tDCS (Nitsche, Jaussi et al. 2004), may strengthen the effects of tDCS in patients.

To provide the most efficient treatment to patients with DOC, we could also investigate other non-invasive brain stimulation techniques such as transcranial pulsed current stimulation (tPCS). This technique, tested in healthy volunteers, is thought to stimulate deeper brain structures than tDCS including the subcortical arousal network (Jaberzadeh, Bastani et al. 2014). Moreover, it has been hypothesized that tDCS and tPCS are two complementary techniques (Castillo Saavedra, Morales-Quezada et al. 2014). tDCS is thought to improve cognitive abilities (especially if impaired) by increasing the neuronal firing under the stimulated area, whereas tPCS may facilitate behavioural performance of tasks that have been previously learned through a more widespread increase of cortical and subcortical connectivity (e.g. Castillo Saavedra, Morales-Quezada et al. 2014). It would be interesting to investigate the effects of tDCS and tPCS, individually as well as combined, on cognitive function and to assess stimulation-induced cortical changes using EEG and neuroimaging techniques.

In conclusion, more work has to be done to strengthen our understanding of the mechanisms of pain, motor impairments (such as spasticity), and potential treatments to promote the recovery of consciousness in patients with DOC. This will help improve daily care, comfort, and rehabilitation in this population in acute as well as in chronic stages. We think that tDCS, which is a safe, easy to use, and inexpensive technique, has great potential for promoting rehabilitation during both acute and chronic stages. Continuing this research on both symptomatic and curative treatments will help us develop clear guidelines to help clinicians improve the quality of life of patients with DOC.

5. References

(1981). "Guidelines for the determination of death. Report of the medical consultants on the diagnosis of death to the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research." *JAMA* 246(19): 2184-2186.
Ada, L., N. O'Dwyer and E. O'Neill (2006). "Relation between spasticity, weakness and contracture of the elbow flexors and upper limb activity after stroke: an observational study." *Disabil Rehabil* 28(13-14): 891-897.
Al-Zamil, Z. M., N. Hassan and W. Hassan (1995). "Reduction of elbow flexor and extensor spasticity following muscle stretch." *Neurorehabil Neural Repair* 9(3): 161-165.
American Congress of Rehabilitation Medecine (1995). "Recommendations for use of uniform nomenclature pertinent to patients with severe alterations of consciousness." *Arch Phys Med Rehabil* 76: 205-209.
American Geriatrics Society (2002). "The management of persistent pain in older persons." *Clinical Practice guideline* 50: S205-S224.
Ansari, N. N. and S. Naghdi (2007). "The effect of Bobath approach on the excitability of the spinal alpha motor neurones in stroke patients with muscle spasticity." *Electromyogr Clin Neurophysiol* 47(1): 29-36.
Antal, A., D. Terney, S. Kuhnl and W. Paulus (2010). "Anodal transcranial direct current stimulation of the motor cortex ameliorates chronic pain and reduces short intracortical inhibition." *J Pain Symptom Manage* 39(5): 890-903.
Ashburner, J. (2007). "A fast diffeomorphic image registration algorithm." *NeuroImage* 38(1): 95-113.
Attia, J. and D. J. Cook (1998). "Prognosis in anoxic and traumatic coma." *Crit Care Clin* 14(3): 497-511.
Baars, B. J., T. Z. Ramsoy and S. Laureys (2003). "Brain, conscious experience and the observing self." *Trends in Neuroscience* 26: 671-675.
Bagnato, S., C. Boccagni and G. Galardi (2013). "Structural epilepsy occurrence in vegetative and minimally conscious states." *Epilepsy Res* 103(1): 106-109.
Baker, J. M., C. Rorden and J. Fridriksson (2010). "Using transcranial direct-current stimulation to treat stroke patients with aphasia." *Stroke* 41(6): 1229-1236.
Basaran, A., U. Emre, K. I. Karadavut, O. Balbaloglu and N. Bulmus (2012). "Hand splinting for poststroke spasticity: a randomized controlled trial." *Top Stroke Rehabil* 19(4): 329-337.
Been, G., T. Ngo, S. Miller and P. Fitzgerald (2007). "The use of tDCS and CVS as methods of noninvasive brain stimulation." *Brain Res Rev* 56: 346-361.
Bekinschtein, T. A., M. R. Coleman, J. Niklison, 3rd, J. D. Pickard and F. F. Manes (2008). "Can electromyography objectively detect voluntary movement in disorders of consciousness?" *J Neurol Neurosurg Psychiatry* 79(7): 826-828.
Bekinschtein, T. A., F. F. Manes, M. Villarreal, A. M. Owen and V. Della-Maggiore (2011). "Functional imaging reveals movement preparatory activity in the vegetative state." *Front Hum Neurosci* 5: 5.
Bennet, M. (2000). "The concept of long term potentiation of transmission at synapses." *Progr Neurobiol* 60: 109-137.
Bernat, J. L. (1998). "A defense of the whole-brain concept of death." *Hastings Cent Rep* 28(2): 14-23.
Bernat, J. L. (2006). "Chronic disorders of consciousness." *Lancet* 367(9517): 1181-1192.

Bhakta, B. B., J. A. Cozens, M. A. Chamberlain and J. M. Bamford (2000). "Impact of botulinum toxin type A on disability and carer burden due to arm spasticity after stroke: a randomised double blind placebo controlled trial." *J Neurol Neurosurg Psychiatry* 69(2): 217-221.
Bindmann, L., O. Lippold and J. Redfearn (1964). "The action of brief polarizing currents on the cerebral cortex of the rat during current flow and in the production of long-lasting after-effects." *J Physiol* 172: 369-382.
Bingel, U., M. Quante, R. Knab, B. Bromm, C. Weiller and C. Buchel (2002). "Subcortical structures involved in pain processing: evidence from single-trial fMRI." *Pain* 99(1-2): 313-321.
Biswal, B. B., J. Van Kylen and J. S. Hyde (1997). "Simultaneous assessment of flow and BOLD signals in resting-state functional connectivity maps." *NMR Biomed* 10(4-5): 165-170.
Bobath, B. (1979). "The application of physiological principles to stroke rehabilitation." *Practitioner* 223(1338): 793-794.
Bodart, O., S. Laureys and O. Gosseries (2013). "Coma and disorders of consciousness: scientific advances and practical considerations for clinicians." *Semin Neurol* 33(2): 83-90.
Boggio, P., R. Ferrucci, S. Rigonatti, P. Covre, M. Nitsche, A. Pascual-Leone and F. Fregni (2006). "Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease." *J Neurol Sci* 249(1): 31-38.
Boggio, P. S., L. O. Castro, E. A. Savagim, R. Braite, V. C. Cruz, R. R. Rocha, S. P. Rigonatti, M. T. Silva and F. Fregni (2006). "Enhancement of non-dominant hand motor function by anodal transcranial direct current stimulation." *Neurosci Lett* 404(1-2): 232-236.
Boggio, P. S., A. Nunes, S. P. Rigonatti, M. A. Nitsche, A. Pascual-Leone and F. Fregni (2007). "Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients." *Restor Neurol Neurosci* 25(2): 123-129.
Boggio, P. S., S. P. Rigonatti, R. B. Ribeiro, M. L. Myczkowski, M. A. Nitsche, A. Pascual-Leone and F. Fregni (2008). "A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression." *Int J Neuropsychopharmacol* 11(2): 249-254.
Bohannon, R. and M. Smith (1987). "Inter rater reliability of a modified Ashworth Scale of muscle spasticity." *Phys Ther* 67: 206-207.
Boly, M., E. Balteau, C. Schnakers, C. Degueldre, G. Moonen, A. Luxen, C. Phillips, P. Peigneux, P. Maquet and S. Laureys (2007). "Baseline brain activity fluctuations predict somatosensory perception in humans." *Proc Natl Acad Sci* 104(29): 12187-12192.
Boly, M., Balteau, E., Schnakers, C., Degueldre, C., Moonen, G., Luxen, A., Phillips, C., Peigneux, P., Maquet, P., Laureys, S. (2007). "Baseline brain activity fluctuations predict somatosensory perception in humans." *Proc Natl Acad Sci* 104: 12187-12192.
Boly, M., M. E. Faymonville, C. Schnakers, P. Peigneux, B. Lambermont, C. Phillips, P. Lancellotti, A. Luxen, M. Lamy, G. Moonen, P. Maquet and S. Laureys (2008). "Perception of pain in the minimally conscious state with PET activation: an observational study." *The Lancet Neurology* 7(11): 1013-1020.
Boly, M., M. E. Faymonville, C. Schnakers, P. Peigneux, B. Lambermont, C. Phillips, P. Lancellotti, A. Luxen, M. Lamy, G. Moonen, P. Maquet and S. Laureys (2008). "Perception of pain in the minimally conscious state with PET activation: an observational study." *Lancet Neurol* 7(11): 1013-1020.

Bornhovd, K., M. Quante, V. Glauche, B. Bromm, C. Weiller and C. Buchel (2002). "Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: A single trial fMRI study." *Brain* 125: 1326-1336.
Bovend'Eerdt, T. J., M. Newman, K. Barker, H. Dawes, C. Minelli and D. T. Wade (2008). "The effects of stretching in spasticity: a systematic review." *Arch Phys Med Rehabil* 89(7): 1395-1406.
Boyd, R. and H. K. Graham (1999). "Objective measurement of clinical findings in the use of botulinum toxin type A in the management of spasticity in children with cerebral palsy." *Eur J Neurol* 6: 23-36.
Brainin, M. (2013). "Poststroke spasticity: Treating to the disability." *Neurology* 80(3 Suppl 2): S1-4.
Brashear, A., M. F. Gordon, E. Elovic, V. D. Kassicieh, C. Marciniak, M. Do, C. H. Lee, S. Jenkins and C. Turkel (2002). "Intramuscular injection of botulinum toxin for the treatment of wrist and finger spasticity after a stroke." *N Engl J Med* 347(6): 395-400.
Brashear, A., R. Zafonte, M. Corcoran, N. Galvez-Jimenez, J. M. Gracies, M. F. Gordon, A. McAfee, K. Ruffing, B. Thompson, M. Williams, C. H. Lee and C. Turkel (2002). "Inter- and intrarater reliability of the Ashworth Scale and the Disability Assessment Scale in patients with upper-limb poststroke spasticity." *Arch Phys Med Rehabil* 83(10): 1349-1354.
Brefel-Courbon, C., P. Payoux, F. Ory, A. Sommet, T. Slaoui, G. Raboyeau, B. Lemesle, M. Puel, J. L. Montastruc, J. F. Demonet and D. Cardebat (2007). "Clinical and imaging evidence of zolpidem effect in hypoxic encephalopathy." *Ann Neurol* 62(1): 102-105.
Brown, G. W. and G. F. Hayden (1985). "Nonparametric methods. Clinical applications." *Clin Pediatr (Phila)* 24(9): 490-498.
Bruno, M., O. Gosseries, D. Ledoux, R. Hustinx and S. Laureys (2011). "Assessment of consciousness with electrophysiological and neurological and neurological imaging techniques." *Curr Opin Crit Care* 17(2): 146-151.
Bruno, M., D. Ledoux, A. Vanhaudenhuyse, O. Gosseries, A. Thibaut and S. Laureys (2012). Prognosis of patients with alered states of consciousness. *Coma and Consciousness*. S. C. a. L. S. London, Springer-Verlag.
Bruno, M. A., J. L. Bernheim, D. Ledoux, F. Pellas, A. Demertzi and S. Laureys (2011). "A survey on self-assessed well-being in a cohort of chronic locked-in syndrome patients: happy majority, miserable minority." *BMJ Open* 1(1): e000039.
Bruno, M. A., O. Gosseries, D. Ledoux, R. Hustinx and S. Laureys (2011). "Assessment of consciousness with electrophysiological and neurological imaging techniques." *Curr Opin Crit Care* 17(2): 146-151.
Bruno, M. A., D. Ledoux, B. Lambermont, F. Damas, C. Schnakers, A. Vanhaudenhuyse, O. Gosseries and S. Laureys (2011). "Comparison of the Full Outline of UnResponsiveness and Glasgow Liege Scale/Glasgow Coma Scale in an intensive care unit population." *Neurocrit Care* 15(3): 447-453.
Bruno, M. A., D. Ledoux, A. Vanhaudenhuyse, O. Gosseries, A. Thibaut and S. Laureys (2012). Prognosis of patients with alered states of consciousness. *Coma and Consciousness*. Springer. London, Springer-Verlage.
Bruno, M. A., S. Majerus, M. Boly, A. Vanhaudenhuyse, C. Schnakers, O. Gosseries, P. Boveroux, M. Kirsch, A. Demertzi, C. Bernard, R. Hustinx, G. Moonen and S. Laureys (2012). "Functional neuroanatomy underlying the clinical subcategorization of minimally conscious state patients." *J Neurol* 259(6): 1087-1098.

Bruno, M. A., A. Vanhaudenhuyse, A. Thibaut, G. Moonen and S. Laureys (2011). "From unresponsive wakefulness to minimally conscious PLUS and functional locked-in syndromes: recent advances in our understanding of disorders of consciousness." *J Neurol* 258(7): 1373-1384.
Calabresi, P., D. Centonze and G. Bernardi (2000). "Cellular factors controlling neuronal vulnerability in the brain: a lesson from the striatum." *Neurology* 55(9): 1249-1255.
Calvet, J. and J. Coll (1959). "[Meningitis of sinusoid origin with the form of coma vigil]." *Rev Otoneuroophtalmol* 31: 443-445.
Castillo Saavedra, L., L. Morales-Quezada, D. Doruk, J. Rozinsky, L. Coutinho, P. Faria, I. Perissinotti, Q. M. Wang and F. Fregni (2014). "QEEG indexed frontal connectivity effects of transcranial pulsed current stimulation (tPCS): A sham-controlled mechanistic trial." *Neurosci Lett* 577: 61-65.
Chatelle, C. and S. Laureys (2011). Understanding disorders of consciousness. *The Oxford Handbook of Neuroethics*. J. Illes and B. Sahakian. New York, Oxford University Press: 119-133.
Chatelle, C., S. Majerus, J. Whyte, S. Laureys and C. Schnakers (2012). "A sensitive scale to assess nociceptive pain in patients with disorders of consciousness." *J Neurol Neurosurg Psychiatry* 83(12): 1233-1237.
Chatelle, C., A. Thibaut, M. A. Bruno, M. Boly, C. Bernard, R. Hustinx, C. Schnakers and S. Laureys (2014). "Nociception coma scale-revised scores correlate with metabolism in the anterior cingulate cortex." *Neurorehabil Neural Repair* 28(2): 149-152.
Chatelle, C., A. Thibaut, O. Gosseries, M. A. Bruno, A. Demertzi, C. Bernard, R. Hustinx, L. Tshibanda, M. A. Bahri and S. Laureys (2014). "Changes in cerebral metabolism in patients with a minimally conscious state responding to zolpidem." *Front Hum Neurosci* 8: 917.
Chatelle, C., A. Thibaut, J. Whyte, M. D. De Val, S. Laureys and C. Schnakers (2014). "Pain issues in disorders of consciousness." *Brain Injury* in press.
Clauss, R. and W. Nel (2006). "Drug induced arousal from the permanent vegetative state." *NeuroRehabilitation* 21(1): 23-28.
Clauss, R. P., W. M. Guldenpfennig, H. W. Nel, M. M. Sathekge and R. R. Venkannagari (2000). "Extraordinary arousal from semi-comatose state on zolpidem. A case report." *S Afr Med J* 90(1): 68-72.
Clemens, B., S. Jung, G. Mingoia, D. Weyer, F. Domahs and K. Willmes (2014). "Influence of anodal transcranial direct current stimulation (tDCS) over the right angular gyrus on brain activity during rest." *PLoS One* 9(4): e95984.
Cohen, L., B. Chaaban and M. O. Habert (2004). "Transient improvement of aphasia with zolpidem." *N Engl J Med* 350(9): 949-950.
Coleman, M. R., T. Bekinschtein, M. M. Monti, A. M. Owen and J. D. Pickard (2009). "A multimodal approach to the assessment of patients with disorders of consciousness." *Prog Brain Res* 177: 231-248.
Cologan, V., X. Drouot, S. Parapatics, A. Delorme, G. Gruber, G. Moonen and S. Laureys (2013). "Sleep in the unresponsive wakefulness syndrome and minimally conscious state." *J Neurotrauma* 30(5): 339-346.
Coupar, F., A. Pollock, P. Rowe, C. Weir and P. Langhorne (2012). "Predictors of upper limb recovery after stroke: a systematic review and meta-analysis." *Clin Rehabil* 26(4): 291-313.
Creutzfeldt, O., G. Fromm and H. Kapp (1962). "Influence of transcortical d-c currents on cortical neuronal activity. ." *Exp Neurol* 5: 436-452.

Crone, J. S., A. Soddu, Y. Holler, A. Vanhaudenhuyse, M. Schurz, J. Bergmann, E. Schmid, E. Trinka, S. Laureys and M. Kronbichler (2014). "Altered network properties of the fronto-parietal network and the thalamus in impaired consciousness." *Neuroimage Clin* 4: 240-248.
Cruse, D., S. Chennu, C. Chatelle, T. Bekinschtein, D. Fernández-Espejo, C. Junqué, J. Pickard, S. Laureys and A. and Owen (2011). "Bedside detection of awareness in the vegetative state." *The Lancet* 378(9809): 2088-2094.
Cruse, D., S. Chennu, C. Chatelle, T. A. Bekinschtein, D. Fernandez-Espejo, J. D. Pickard, S. Laureys and A. M. Owen (2011). "Bedside detection of awareness in the vegetative state: a cohort study." *Lancet* 378(9809): 2088-2094.
Cruse, D., S. Chennu, C. Chatelle, D. Fernandez-Espejo, T. A. Bekinschtein, J. D. Pickard, S. Laureys and A. M. Owen (2012). "Relationship between etiology and covert cognition in the minimally conscious state." *Neurology* 78(11): 816-822.
D'Esposito, M., G. K. Aguirre, E. Zarahn, D. Ballard, R. K. Shin and J. Lease (1998). "Functional MRI studies of spatial and nonspatial working memory." *Brain Res Cogn Brain Res* 7(1): 1-13.
D'Esposito, M., J. A. Detre, D. C. Alsop, R. K. Shin, S. Atlas and M. Grossman (1995). "The neural basis of the central executive system of working memory." *Nature* 378(6554): 279-281.
Dean, C. M., F. H. Mackey and P. Katrak (2000). "Examination of shoulder positioning after stroke: A randomised controlled pilot trial." *Aust J Physiother* 46(1): 35-40.
Demertzi, A., F. Gomez, J. S. Crone, A. Vanhaudenhuyse, L. Tshibanda, Q. Noirhomme, M. Thonnard, V. Charland-Verville, M. Kirsch, S. Laureys and A. Soddu (2014). "Multiple fMRI system-level baseline connectivity is disrupted in patients with consciousness alterations." *Cortex* 52: 35-46.
Demertzi, A., E. Racine, M.-A. Bruno, D. Ledoux, O. Gosseries, A. Vanhaudenhuyse, M. Thonnard, A. Soddu, G. Moonen and S. Laureys (2013). "Pain Perception in Disorders of Consciousness: Neuroscience, Clinical Care, and Ethics in Dialogue." *Neuroethics* 6(1): 37-50.
Demertzi, A., A. Soddu and S. Laureys (2013). "Consciousness supporting networks." *Curr Opin Neurobiol* 23(2): 239-244.
Demertzi, A., A. Vanhaudenhuyse, S. Bredart, L. Heine, C. di Perri and S. Laureys (2013). "Looking for the Self in Pathological Unconsciousness." *Front Hum Neurosci* 7: 538.
Di, H., M. Boly, X. Weng, D. Ledoux and S. Laureys (2008). "Neuroimaging activation studies in the vegetative state: predictors of recovery?" *Clin Med* 8(5): 502-507.
Di, H. B., S. M. Yu, X. C. Weng, S. Laureys, D. Yu, J. Q. Li, P. M. Qin, Y. H. Zhu, S. Z. Zhang and Y. Z. Chen (2007). "Cerebral response to patient's own name in the vegetative and minimally conscious states." *Neurology* 68(12): 895-899.
Di Perri, C., S. Bastianello, A. J. Bartsch, C. Pistarini, G. Maggioni, L. Magrassi, R. Imberti, A. Pichiecchio, P. Vitali, S. Laureys and F. Di Salle (2013). "Limbic hyperconnectivity in the vegetative state." *Neurology* 81(16): 1417-1424.
Di Perri, C., A. Thibaut, L. Heine, A. Soddu, A. Demertzi and S. Laureys (2014). "Measuring consciousness in coma and related states." *World J Radiol* 6(8): 589-597.
Dietz, V. and T. Sinkjaer (2007). "Spastic movement disorder: impaired reflex function and altered muscle mechanics." *Lancet Neurol* 6(8): 725-733.

Doan, Q. V., A. Brashear, P. J. Gillard, S. F. Varon, A. M. Vandenburgh, C. C. Turkel and E. P. Elovic (2012). "Relationship between disability and health-related quality of life and caregiver burden in patients with upper limb poststroke spasticity." *PM R* 4(1): 4-10.
Dockery, C. A., R. Hueckel-Weng, N. Birbaumer and C. Plewnia (2009). "Enhancement of planning ability by transcranial direct current stimulation." *J Neurosci* 29(22): 7271-7277.
Elovic, E. P., L. K. Simone and R. Zafonte (2004). "Outcome assessment for spasticity management in the patient with traumatic brain injury: the state of the art." *J Head Trauma Rehabil* 19(2): 155-177.
Estraneo, A., P. Moretta, V. Loreto, B. Lanzillo, L. Santoro and L. Trojano (2010). "Late recovery after traumatic, anoxic, or hemorrhagic long-lasting vegetative state." *Neurology* 75(3): 239-245.
Feeney, D. M. and J. C. Baron (1986). "Diaschisis." *Stroke* 17(5): 817-830.
Feldman, P. A. (1990). Upper extremity casting and splinting. *The practical management of spasticity in children and adults*. M. B. G. J. Whyte. Philadephia, Lea & Febiger.
Fernandez-Espejo, D., A. Soddu, D. Cruse, E. M. Palacios, C. Junque, A. Vanhaudenhuyse, E. Rivas, V. Newcombe, D. K. Menon, J. D. Pickard, S. Laureys and A. M. Owen (2012). "A role for the default mode network in the bases of disorders of consciousness." *Ann Neurol* 72(3): 335-343.
Ferrucci, R., F. Mameli, I. Guidi, S. Mrakic-Sposta, M. Vergari, S. Marceglia, F. Cogiamanian, S. Barbieri, E. Scarpini and A. Priori (2008). "Transcranial direct current stimulation improves recognition memory in Alzheimer disease." *Neurology* 71(7): 493-498.
Ferrucci, R., M. Vergari, F. Cogiamanian, T. Bocci, M. Ciocca, E. Tomasini, M. De Riz, E. Scarpini and A. Priori (2014). "Transcranial direct current stimulation (tDCS) for fatigue in multiple sclerosis." *NeuroRehabilitation* 34(1): 121-127.
Fiori, V., M. Coccia, C. V. Marinelli, V. Vecchi, S. Bonifazi, M. G. Ceravolo, L. Provinciali, F. Tomaiuolo and P. Marangolo (2011). "Transcranial direct current stimulation improves word retrieval in healthy and nonfluent aphasic subjects." *J Cogn Neurosci* 23(9): 2309-2323.
Flinn, S. R. and K. Craven (2014). "Upper limb casting in stroke rehabilitation: rationale, options, and techniques." *Top Stroke Rehabil* 21(4): 296-302.
Foltz, E. L. and L. E. White, Jr. (1962). "Pain "relief" by frontal cingulumotomy." *J Neurosurg* 19: 89-100.
Frank, E., M. Schecklmann, M. Landgrebe, J. Burger, P. Kreuzer, T. B. Poeppl, T. Kleinjung, G. Hajak and B. Langguth (2012). "Treatment of chronic tinnitus with repeated sessions of prefrontal transcranial direct current stimulation: outcomes from an open-label pilot study." *J Neurol* 259(2): 327-333.
Fregni, F., P. S. Boggio, M. C. Lima, M. J. Ferreira, T. Wagner, S. P. Rigonatti, A. W. Castro, D. R. Souza, M. Riberto, S. D. Freedman, M. A. Nitsche and A. Pascual-Leone (2006). "A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury." *Pain* 122(1-2): 197-209.
Fregni, F., P. S. Boggio, M. Nitsche, F. Bermpohl, A. Antal, E. Feredoes, M. A. Marcolin, S. P. Rigonatti, M. T. Silva, W. Paulus and A. Pascual-Leone (2005). "Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory." *Exp Brain Res* 166(1): 23-30.
Fregni, F., D. Liebetanz, K. K. Monte-Silva, M. B. Oliveira, A. A. Santos, M. A. Nitsche, A. Pascual-Leone and R. C. Guedes (2007). "Effects of transcranial direct current stimulation coupled with repetitive electrical stimulation on cortical spreading depression." *Exp Neurol* 204(1): 462-466.

Fridman, E. A., B. J. Beattie, A. Broft, S. Laureys and N. D. Schiff (2014). "Regional cerebral metabolic patterns demonstrate the role of anterior forebrain mesocircuit dysfunction in the severely injured brain." *Proc Natl Acad Sci U S A*.
Fridman, E. A., B. J. Beattie, A. Broft, S. Laureys and N. D. Schiff (2014). "Regional cerebral metabolic patterns demonstrate the role of anterior forebrain mesocircuit dysfunction in the severely injured brain." *Proc Natl Acad Sci U S A* 111(17): 6473-6478.
Fridman, E. A., J. Calvar, M. Bonetto, E. Gamzu, B. Z. Krimchansky, F. Meli, R. C. Leiguarda and R. Zafonte (2009). "Fast awakening from minimally conscious state with apomorphine." *Brain Inj* 23(2): 172-177.
Fridman, E. A., B. Z. Krimchansky, M. Bonetto, T. Galperin, E. R. Gamzu, R. C. Leiguarda and R. Zafonte (2010). "Continuous subcutaneous apomorphine for severe disorders of consciousness after traumatic brain injury." *Brain Inj* 24(4): 636-641.
Fuhrmann, A., G. Hein, N. Tsai, M. Naumer and R. Knight (2008). "Temporal characteristic of audiovisual information processing." *J Neurosci* 28: 5344-5349.
Gandiga, P. C., F. C. Hummel and L. G. Cohen (2006). "Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation." *Clin Neurophysiol* 117(4): 845-850.
George, M. S., F. Padberg, T. E. Schlaepfer, J. P. O'Reardon, P. B. Fitzgerald, Z. H. Nahas and M. A. Marcolin (2009). "Controversy: Repetitive transcranial magnetic stimulation or transcranial direct current stimulation shows efficacy in treating psychiatric diseases (depression, mania, schizophrenia, obsessive-complusive disorder, panic, posttraumatic stress disorder)." *Brain Stimul* 2(1): 14-21.
Ghotbi, N., N. Nakhostin Ansari, S. Naghdi and S. Hasson (2011). "Measurement of lower-limb muscle spasticity: intrarater reliability of Modified Modified Ashworth Scale." *J Rehabil Res Dev* 48(1): 83-88.
Giacino, J., K. Kalmar and J. Whyte (2004). "The JFK Coma Recovery Scale-Revised: measurement characteristics and diagnostic utility." *Arch Phys Med Rehabil* 85(12): 2020 - 2029.
Giacino, J. T. (2004). "The vegetative and minimally conscious states: consensus-based criteria for establishing diagnosis and prognosis." *NeuroRehabilitation* 19(4): 293-298.
Giacino, J. T., S. Ashwal, N. Childs, R. Cranford, B. Jennett, D. I. Katz, J. P. Kelly, J. H. Rosenberg, J. Whyte, R. D. Zafonte and N. D. Zasler (2002). "The minimally conscious state: definition and diagnostic criteria." *Neurology* 58(3): 349-353.
Giacino, J. T., J. J. Fins, S. Laureys and N. D. Schiff (2014). "Disorders of consciousness after acquired brain injury: the state of the science." *Nat Rev Neurol* 10(2): 99-114.
Giacino, J. T., J. J. Fins, S. Laureys and N. D. Schiff (2014). "Disorders of consciousness after acquired brain injury: the state of the science." *Nat Rev Neurol*.
Giacino, J. T., K. Kalmar and J. Whyte (2004). "The JFK Coma Recovery Scale-Revised: measurement characteristics and diagnostic utility." *Arch Phys Med Rehabil* 85(12): 2020-2029.
Giacino, J. T., M. A. Kezmarsky, J. DeLuca and K. D. Cicerone (1991). "Monitoring rate of recovery to predict outcome in minimally responsive patients." *Arch Phys Med Rehabil* 72(11): 897-901.
Giacino, J. T., J. Whyte, E. Bagiella, K. Kalmar, N. Childs, A. Khademi, B. Eifert, D. Long, D. I. Katz, S. Cho, S. A. Yablon, M. Luther, F. M. Hammond, A. Nordenbo, P. Novak, W. Mercer, P. Maurer-Karattup and M. Sherer (2012). "Placebo-controlled trial of amantadine for severe traumatic brain injury." *N Engl J Med* 366(9): 819-826.
Glassman, R. B. (1971). "Recovery following sensorimotor cortical damage: evoked potentials, brain stimulation and motor control." *Exp Neurol* 33(1): 16-29.

Goncalves, G. S., I. C. Borges, B. T. Goes, M. E. de Mendonca, R. G. Goncalves, L. B. Garcia, K. N. Sa, M. R. Coutinho, B. G. Castro Filho, F. Fregni and A. F. Baptista (2013). "Effects of tDCS Induced Motor Cortex Modulation on Pain in HTLV-1: A Blind Randomized Clinical Trial." *Clin J Pain*.
Gosseries, O., M.-A. l. Bruno, A. Vanhaudenhuyse, S. Laureys and C. Schnakers (2009). Chapter 15 - Consciousness in the Locked-in Syndrome. *The Neurology of Consciousness*. L. Steven and T. Giulio. San Diego, Academic Press: 191-203.
Gosseries, O., M. A. Bruno, C. Chatelle, A. Vanhaudenhuyse, C. Schnakers, A. Soddu and S. Laureys (2011). "Disorders of consciousness: what's in a name?" *NeuroRehabilitation* 28(1): 3-14.
Gosseries, O., S. Sarasso, S. Casarotto, M. Boly, C. Schnakers, M. Napolitani, M. Bruno, D. Ledoux, J. Tshibanda, M. Massimini, S. Laureys and M. Rosanova (2015). "On the Cerebral Origin of EEG Responses to TMS: Insights From Severe Cortical Lesions." *Brain Stimul* 8(1): 142-149.
Gracies, J. M. (2001). "Physical modalities other than stretch in spastic hypertonia." *Phys Med Rehabil Clin N Am* 12(4): 769-792, vi.
Gracies, J. M. (2005). "Pathophysiology of spastic paresis. I: Paresis and soft tissue changes." *Muscle Nerve* 31(5): 535-551.
Gracies, J. M., N. Bayle, M. Vinti, S. Alkandari, P. Vu, C. M. Loche and C. Colas (2010). "Five-step clinical assessment in spastic paresis." *Eur J Phys Rehabil Med* 46(3): 411-421.
Gracies, J. M., K. Burke, N. J. Clegg, R. Browne, C. Rushing, D. Fehlings, D. Matthews, A. Tilton and M. R. Delgado (2010). "Reliability of the Tardieu Scale for assessing spasticity in children with cerebral palsy." *Arch Phys Med Rehabil* 91(3): 421-428.
Gracies, J. M., R. Fitzpatrick, L. Wilson, D. Burke and S. C. Gandevia (1997). "Lycra garments designed for patients with upper limb spasticity: mechanical effects in normal subjects." *Arch Phys Med Rehabil* 78(10): 1066-1071.
Gracies, J. M., J. E. Marosszeky, R. Renton, J. Sandanam, S. C. Gandevia and D. Burke (2000). "Short-term effects of dynamic lycra splints on upper limb in hemiplegic patients." *Arch Phys Med Rehabil* 81(12): 1547-1555.
Grunder, G., T. Siessmeier, C. Lange-Asschenfeldt, I. Vernaleken, H. G. Buchholz, P. Stoeter, A. Drzezga, H. Luddens, F. Rosch and P. Bartenstein (2001). "[18F]Fluoroethylflumazenil: a novel tracer for PET imaging of human benzodiazepine receptors." *Eur J Nucl Med* 28(10): 1463-1470.
Habbal, D., O. Gosseries, Q. Noirhomme, J. Renaux, D. Lesenfants, T. Bekinschtein, S. Majerus, S. Laureys and C. Schnakers (2014). "Volitional electromyographic responses in disorders of consciousness." *Brain Injury* in press.
Hale, L. A., V. U. Fritz, C. L. Richards and F. Dumas (1990). "Effects of prolonged muscle stretch on reflex and voluntary muscle activations in children with spastic cerebral palsy." *Scand J Rehabil Med* 22(4): 171-180.
Harlaar, J., J. G. Becher, C. J. Snijders and G. J. Lankhorst (2000). "Passive stiffness characteristics of ankle plantar flexors in hemiplegia." *Clin Biomech (Bristol, Avon)* 15(4): 261-270.
Hart, A. (2001). "Mann-Whitney test is not just a test of medians: differences in spread can be important." *BMJ* 323(7309): 391-393.
Harvey, L., R. Herbert and J. Crosbie (2002). "Does stretching induce lasting increases in joint ROM? A systematic review." *Physiother Res Int* 7(1): 1-13.

Haupt, W. F. and J. Rudolf (1999). "European brain death codes: a comparison of national guidelines." *J Neurol* 246(6): 432-437.
Heekeren, H. R., S. Marrett, D. A. Ruff, P. A. Bandettini and L. G. Ungerleider (2006). "Involvement of human left dorsolateral prefrontal cortex in perceptual decision making is independent of response modality." *Proc Natl Acad Sci U S A* 103(26): 10023-10028.
Hefter, H., W. H. Jost, A. Reissig, B. Zakine, A. M. Bakheit and J. Wissel (2012). "Classification of posture in poststroke upper limb spasticity: a potential decision tool for botulinum toxin A treatment?" *Int J Rehabil Res* 35(3): 227-233.
Held, J. and E. Pierrot-Deseilligny (1969). "Reeducation Motrice des Affections Neurologiques." *Paris*.
Herwig, U., P. Satrapi and C. Schonfeldt-Lecuona (2003). "Using the international 10-20 EEG system for positioning of transcranial magnetic stimulation." *Brain Topogr* 16(2): 95-99.
Hesse, S., H. Mach, S. Frohlich, S. Behrend, C. Werner and I. Melzer (2012). "An early botulinum toxin A treatment in subacute stroke patients may prevent a disabling finger flexor stiffness six months later: a randomized controlled trial." *Clin Rehabil* 26(3): 237-245.
Horvath, J. C., J. D. Forte and O. Carter (2015). "Quantitative review finds no evidence of cognitive effects in healthy populations from single-session transcranial direct current stimulation (tDCS)." *Brain Stimul* in press.
Hummel, F. C., B. Voller, P. Celnik, A. Floel, P. Giraux, C. Gerloff and L. G. Cohen (2006). "Effects of brain polarization on reaction times and pinch force in chronic stroke." *BMC Neurosci* 7: 73.
Hummel, P., van Dijk, M. (2006). "Pain assessment: Current status and challenges." *Seminars in Fetal & Neonatal Medicine* 11: 237-245.
Huskisson, E. C. (1982). "Measurement of pain." *J Rheumatol* 9(5): 768-769.
Iannetti, G. D., N. P. Hughes, M. C. Lee and A. Mouraux (2008). "Determinants of laser-evoked EEG responses: pain perception or stimulus saliency?" *J Neurophysiol* 100(2): 815-828.
Ingvar, M. (1999). "Pain and functional imaging." *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences* 354(1387): 1347-1358.
International Association for the Study of Pain, I. (1979). "Pain terms: a list definitions and notes on usage." *Pain* 6: 249-252.
Jaberzadeh, S., A. Bastani and M. Zoghi (2014). "Anodal transcranial pulsed current stimulation: A novel technique to enhance corticospinal excitability." *Clin Neurophysiol* 125(2): 344-351.
Jacobson, L., M. Koslowsky and M. Lavidor (2012). "tDCS polarity effects in motor and cognitive domains: a meta-analytical review." *Exp Brain Res* 216(1): 1-10.
Jagger, J., J. A. Jane and R. Rimel (1983). "The Glasgow coma scale: to sum or not to sum?" *Lancet* 2(8341): 97.
Jefferys, J. (1995). "Nonsynaptic modulation of neuronal activity in th brain: electric currents and extracellular ions." *Physiol Rev* 75: 689-723.
Jennett, B. and F. Plum (1972). "Persistent vegetative state after brain damage. A syndrome in search of a name." *Lancet* 1(7753): 734-737.
Jennett, B., J. Snoek, M. R. Bond and N. Brooks (1981). "Disability after severe head injury: observations on the use of the Glasgow Outcome Scale." *J Neurol Neurosurg Psychiatry* 44(4): 285-293.
Jo, J. M., Y. H. Kim, M. H. Ko, S. H. Ohn, B. Joen and K. H. Lee (2009). "Enhancing the working memory of stroke patients using tDCS." *Am J Phys Med Rehabil* 88(5): 404-409.

Juengling, F., J. Kassubek, H. Huppertz, T. Krause and T. Els (2005). "Separating functional and structural damade in persistent vegetative state using combined voxel-based analysis of 3-D MRI and FDG-PET." *J Neurol Sci* 228(2): 179-184.
Kampfl, A., E. Schmutzhard, G. Franz, B. Pfausler, H. P. Haring, H. Ulmer, S. Felber, S. Golaszewski and F. Aichner (1998). "Prediction of recovery from post-traumatic vegetative state with cerebral magnetic-resonance imaging." *Lancet* 351(9118): 1763-1767.
Kaneko, F., T. Murakami, K. Onari, H. Kurumadani and K. Kawaguchi (2003). "Decreased cortical excitability during motor imagery after disuse of an upper limb in humans." *Clin Neurophysiol* 114(12): 2397-2403.
Kang, E. K., M. J. Baek, S. Kim and N. J. Paik (2009). "Non-invasive cortical stimulation improves post-stroke attention decline." *Restor Neurol Neurosci* 27(6): 645-650.
Kang, E. K., M. J. Beak, S. Kim and N. J. Paik (2009). "Non-invasive cortical stimulation improves post-stroke attention decline." *Restor Neurol Neurosci* 27(6): 654-650.
Kang, E. K., D. Y. Kim and N. J. Paik (2012). "Transcranial direct current stimulation of the left prefrontal cortex improves attention in patients with traumatic brain injury: A pilot study." *J Rehabil Med* 44(4): 346-350.
Kassubek, J., F. D. Juengling, T. Els, J. Spreer, M. Herpers, T. Krause, E. Moser and C. H. Lucking (2003). "Activation of a residual cortical network during painful stimulation in long-term postanoxic vegetative state: a 15O-H2O PET study." *J Neurol Sci* 212(1-2): 85-91.
Katalinic, O. M., L. A. Harvey, R. D. Herbert, A. M. Moseley, N. A. Lannin and K. Schurr (2010). "Stretch for the treatment and prevention of contractures." *Cochrane Database Syst Rev*(9): CD007455.
Katz, R. T., G. P. Rovai, C. Brait and W. Z. Rymer (1992). "Objective quantification of spastic hypertonia: correlation with clinical findings." *Arch Phys Med Rehabil* 73(4): 339-347.
Keeser, D., T. Meindl, J. Bor, U. Palm, O. Pogarell, C. Mulert, J. Brunelin, H. J. Moller, M. Reiser and F. Padberg (2011). "Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI." *J Neurosci* 31(43): 15284-15293.
Keeser, D., F. Padberg, E. Reisinger, O. Pogarell, V. Kirsch, U. Palm, S. Karch, H. J. Moller, M. A. Nitsche and C. Mulert (2011). "Prefrontal direct current stimulation modulates resting EEG and event-related potentials in healthy subjects: a standardized low resolution tomography (sLORETA) study." *Neuroimage* 55(2): 644-657.
Kendall (1938). "A new measure of rank correlation." *Biometrika* 30: 81-89.
Kheder, A. and K. P. Nair (2012). "Spasticity: pathophysiology, evaluation and management." *Pract Neurol* 12(5): 289-298.
Khedr, E. M., O. A. Shawky, D. H. El-Hammady, J. C. Rothwell, E. S. Darwish, O. M. Mostafa and A. M. Tohamy (2013). "Effect of anodal versus cathodal transcranial direct current stimulation on stroke rehabilitation: a pilot randomized controlled trial." *Neurorehabil Neural Repair* 27(7): 592-601.
Kim, D. Y., J. Y. Lim, E. K. Kang, D. S. You, M. K. Oh, B. M. Oh and N. J. Paik (2010). "Effect of transcranial direct current stimulation on motor recovery in patients with subacute stroke." *Am J Phys Med Rehabil* 89(11): 879-886.
Kouloulas, E. J., A. G. Papadeas, X. Michail, D. E. Sakas and E. J. Boviatsis (2013). "Prognostic value of time-related Glasgow coma scale components in severe traumatic brain injury: a prospective evaluation with respect to 1-year survival and functional outcome." *Int J Rehabil Res* 36(3): 260-267.

Kretschmer, E. (1940). "Das apallische syndrom." *Z ges Neurol Psychiat* 169: 576-579.
Lance, J. (1980). *Spasticity: Disorders Motor Control*. Miami, FL: Year Book Medical Publishers, In: Feldman RG, Young RP, Koella WP eds.
Landsness, E., M. A. Bruno, Q. Noirhomme, B. Riedner, O. Gosseries, C. Schnakers, M. Massimini, S. Laureys, G. Tononi and M. Boly (2011). "Electrophysiological correlates of behavioural changes in vigilance in vegetative state and minimally conscious state." *Brain* 134(Pt 8): 2222-2232.
Lang, N., H. R. Siebner, N. S. Ward, L. Lee, M. A. Nitsche, W. Paulus, J. C. Rothwell, R. N. Lemon and R. S. Frackowiak (2005). "How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain?" *Eur J Neurosci* 22(2): 495-504.
Langtry, H. D. and P. Benfield (1990). "Zolpidem. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential." *Drugs* 40(2): 291-313.
Lannin, N. A., A. Cusick, A. McCluskey and R. D. Herbert (2007). "Effects of splinting on wrist contracture after stroke: a randomized controlled trial." *Stroke* 38(1): 111-116.
Lannin, N. A., I. Novak and A. Cusick (2007). "A systematic review of upper extremity casting for children and adults with central nervous system motor disorders." *Clin Rehabil* 21(11): 963-976.
Lapeyre, E., J. B. Kuks and W. J. Meijler (2010). "Spasticity: revisiting the role and the individual value of several pharmacological treatments." *NeuroRehabilitation* 27(2): 193-200.
Lapitskaya, N., O. Gosseries, V. De Pasqua, A. R. Pedersen, J. F. Nielsen, A. M. de Noordhout and S. Laureys (2013). "Abnormal corticospinal excitability in patients with disorders of consciousness." *Brain Stimul* 6(4): 590-597.
Lapitskaya, N., S. K. Moerk, O. Gosseries, J. F. Nielsen and A. M. de Noordhout (2013). "Corticospinal excitability in patients with anoxic, traumatic, and non-traumatic diffuse brain injury." *Brain Stimul* 6(2): 130-137.
Laureys, S. (2004). "Functional neuroimaging in the vegetative state." *NeuroRehabilitation* 19(4): 335-341.
Laureys, S. (2005). "The neural correlate of (un)awareness: lessons from the vegetative state." *Trends Cogn Sci* 9(12): 556-559.
Laureys, S. (2005). "Science and society: death, unconsciousness and the brain." *Nat Rev Neurosci* 6(11): 899-909.
Laureys, S. (2007). "Eyes open, brain shut." *Scientific American* 296(5): 84-89.
Laureys, S., S. Antoine, M. Boly, S. Elincx, M. E. Faymonville, J. Berre, B. Sadzot, M. Ferring, X. De Tiege, P. van Bogaert, I. Hansen, P. Damas, N. Mavroudakis, B. Lambermont, G. Del Fiore, J. Aerts, C. Degueldre, C. Phillips, G. Franck, J. L. Vincent, M. Lamy, A. Luxen, G. Moonen, S. Goldman and P. Maquet (2002). "Brain function in the vegetative state." *Acta Neurol Belg* 102(4): 177-185.
Laureys, S., M. Boly and P. Maquet (2006). "Tracking the recovery of consciousness from coma." *J Clin Invest* 116(7): 1823-1825.
Laureys, S., G. G. Celesia, F. Cohadon, J. Lavrijsen, J. Leon-Carrion, W. G. Sannita, L. Sazbon, E. Schmutzhard, K. R. von Wild, A. Zeman and G. Dolce (2010). "Unresponsive wakefulness syndrome: a new name for the vegetative state or apallic syndrome." *BMC Med* 8: 68.
Laureys, S., M. Faymonville and P. Maquet (2002). "Quelle conscience durant le coma?" *Pour la science* 302: 122-128.

Laureys, S., M. Faymonville, P. Peigneux, P. Damas, B. Lambermont, G. Del Fiore, C. Degueldre, J. Aerts, A. Luxen and G. Franck (2002). "Cortical processing of noxious somatosensory stimuli in the persistent vegetative state." *Neuroimage* 17(2): 732 - 741.
Laureys, S., M. E. Faymonville, A. Luxen, M. Lamy, G. Franck and P. Maquet (2000). "Restoration of thalamocortical connectivity after recovery from persistent vegetative state." *Lancet* 355(9217): 1790-1791.
Laureys, S., M. E. Faymonville, A. Luxen, M. Lamy, G. Franck and P. Maquet (2000). "Restoration of thalamocortical connectivity after recovery from persistent vegetative state." *Lancet* 355(9217): 1790-1791.
Laureys, S., M. E. Faymonville, G. Moonen, A. Luxen and P. Maquet (2000). "PET scanning and neuronal loss in acute vegetative state." *Lancet* 355(9217): 1825-1826; author reply 1827.
Laureys, S., M. E. Faymonville, P. Peigneux, P. Damas, B. Lambermont, G. Del Fiore and e. al (2002). "Cortical processing of noxious somatosensory stimuli in the persistent vegetative state." *Neuroimage* 17(2): 732-741.
Laureys, S., M. E. Faymonville, P. Peigneux, P. Damas, B. Lambermont, G. Del Fiore, C. Degueldre, J. Aerts, A. Luxen, G. Franck, M. Lamy, G. Moonen and P. Maquet (2002). "Cortical processing of noxious somatosensory stimuli in the persistent vegetative state." *Neuroimage* 17(2): 732-741.
Laureys, S., S. Goldman, C. Phillips, P. Van Bogaert, J. Aert, A. Luxen, G. Franck and P. Maquet (1999). "Impaired effective cortical connectivity in vegetative state: Preliminary investigation using PET." *Neuroimage* 9: 377-382.
Laureys, S., S. Goldman, C. Phillips, P. Van Bogaert, J. Aerts, A. Luxen, G. Franck and P. Maquet (1999). "Impaired effective cortical connectivity in vegetative state: preliminary investigation using PET." *Neuroimage* 9(4): 377-382.
Laureys, S., C. Lemaire, P. Maquet, C. Phillips and G. Franck (1999). "Cerebral metabolism during vegetative state and after recovery to consciousness." *J Neurol Neurosurg Psychiatry* 67(1): 121.
Laureys, S., A. M. Owen and N. D. Schiff (2004). "Brain function in coma, vegetative state, and related disorders." *Lancet Neurol* 3(9): 537-546.
Laureys, S. and N. D. Schiff (2012). "Coma and consciousness: Paradigms (re)framed by neuroimaging." *Neuroimage* 61(2): 478-491.
Ledoux, D., M. Bruno, C. Schnakers, G. J, M. Ventura, L. Vanopdenbosch, E. Peeters, E. Lannoo, T. Willemart and S. Laureys (2008). Outcome of vegetative and minimally conscious states: results from the Belgian Federal expertise network. *Eighteeth Meeting of the European Neurological Society*. eds. Nice, France. 255: 24.
Lee, K. C., L. Carson, E. Kinnin and V. Patterson (1989). "The Ashworth Scale: A reliable and reproducible method of measuring spasticity." *J Neurol Rehab* 3: 205-209.
Lehembre, R., M. A. Bruno, A. Vanhaudenhuyse, C. Chatelle, V. Cologan, Y. Leclercq, A. Soddu, B. Macq, S. Laureys and Q. Noirhomme (2012). "Resting-state EEG study of comatose patients: a connectivity and frequency analysis to find differences between vegetative and minimally conscious states." *Funct Neurol* 27(1): 41-47.
Leong, B. (2002). "Critical review of passive muscle stretch: implications for the treatment of children in vegetative and minimally conscious states." *Brain Inj* 16(2): 169-183.

Leong, B. (2002). "The vegetative and minimally conscious states in children: spasticity, muscle contracture and issues for physiotherapy treatment." *Brain Injury* 16(3): 217-230.
Liebetanz, D., M. A. Nitsche, F. Tergau and W. Paulus (2002). "Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability." *Brain* 125(Pt 10): 2238-2247.
Liebetanz David , N. M. A., Tergau Frithjof and Paulus Walter (2002). "Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability." *Brain Research Reviews* 125: 2238-2247.
Lindenberg, R., V. Renga, L. L. Zhu, D. Nair and G. Schlaug (2010). "Bihemispheric brain stimulation facilitates motor recovery in chronic stroke patients." *Neurology* 75(24): 2176-2184.
Lockwood, P. L., G. D. Iannetti and P. Haggard (2012). "Transcranial magnetic stimulation over human secondary somatosensory cortex disrupts perception of pain intensity." *Cortex*.
Loeser, J. D. and R. D. Treede (2008). "The Kyoto protocol of IASP Basic Pain Terminology." *Pain* 137(3): 473-477.
Loo, C. K., A. Alonzo, D. Martin, P. B. Mitchell, V. Galvez and P. Sachdev (2012). "Transcranial direct current stimulation for depression: 3-week, randomised, sham-controlled trial." *Br J Psychiatry* 200(1): 52-59.
Luaute, J., D. Maucort-Boulch, L. Tell, F. Quelard, T. Sarraf, J. Iwaz, D. Boisson and C. Fischer (2010). "Long-term outcomes of chronic minimally conscious and vegetative states." *Neurology* 75(3): 246-252.
Lui, F., D. Duzzi, M. Corradini, M. Serafini, P. Baraldi and C. A. Porro (2008). "Touch or pain? Spatio-temporal patterns of cortical fMRI activity following brief mechanical stimuli." *Pain* 138(2): 362-374.
Lule, D., Q. Noirhomme, S. C. Kleih, C. Chatelle, S. Halder, A. Demertzi, M. A. Bruno, O. Gosseries, A. Vanhaudenhuyse, C. Schnakers, M. Thonnard, A. Soddu, A. Kubler and S. Laureys (2013). "Probing command following in patients with disorders of consciousness using a brain-computer interface." *Clin Neurophysiol* 124(1): 101-106.
Majerus, S., M. A. Bruno, C. Schnakers, J. T. Giacino and S. Laureys (2009). "The problem of aphasia in the assessment of consciousness in brain-damaged patients." *Prog Brain Res* 177: 49-61.
Majerus, S., H. Gill-Thwaites, K. Andrews and S. Laureys (2005). "Behavioral evaluation of consciousness in severe brain damage." *Prog Brain Res* 150: 397-413.
Malhotra, S., A. D. Pandyan, S. Rosewilliam, C. Roffe and H. Hermens (2011). "Spasticity and contractures at the wrist after stroke: time course of development and their association with functional recovery of the upper limb." *Clin Rehabil* 25(2): 184-191.
Marangolo, P., V. Fiori, M. A. Calpagnano, S. Campana, C. Razzano, C. Caltagirone and A. Marini (2013). "tDCS over the left inferior frontal cortex improves speech production in aphasia." *Front Hum Neurosci* 7: 539.
Marciniak, C. (2011). "Poststroke hypertonicity: upper limb assessment and treatment." *Top Stroke Rehabil* 18(3): 179-194.
Markl, A., T. Yu, D. Vogel, F. Muller, B. Kotchoubey and S. Lang (2013). "Brain processing of pain in patients with unresponsive wakefulness syndrome." *Brain Behav* 3(2): 95-103.
Massimini, M., M. Boly, A. Casali, M. Rosanova and G. Tononi (2009). "A perturbational approach for evaluating the brain's capacity for consciousness." *Prog Brain Res* 177: 201-214.

Mayer, N. H. and A. Esquenazi (2003). "Muscle overactivity and movement dysfunction in the upper motoneuron syndrome." *Phys Med Rehabil Clin N Am* 14(4): 855-883.
McComas, A. J. (1994). "Human neuromuscular adaptations that accompany changes in activity." *Med Sci Sports Exerc* 26(12): 1498-1509.
Mehrholz, J., Y. Major, D. Meissner, S. Sandi-Gahun, R. Koch and M. Pohl (2005). "The influence of contractures and variation in measurement stretching velocity on the reliability of the Modified Ashworth Scale in patients with severe brain injury." *Clin Rehabil* 19(1): 63-72.
Mehrholz, J., K. Wagner, D. Meissner, K. Grundmann, C. Zange, R. Koch and M. Pohl (2005). "Reliability of the Modified Tardieu Scale and the Modified Ashworth Scale in adult patients with severe brain injury: a comparison study." *Clin Rehabil* 19(7): 751-759.
Menon, V., J. M. Ford, K. O. Lim, G. H. Glover and A. Pfefferbaum (1997). "Combined event-related fMRI and EEG evidence for temporal-parietal cortex activation during target detection." *Neuroreport* 8(14): 3029-3037.
Miranda , P., M. Lomarev and M. Hallett (2006). "Modeling the current distribution during transcranial direct current stimulation." *Clin Neurophysiol* 117: 1623-1629.
Miranda, P. C., M. Lomarev and M. Hallett (2006). "Modeling the current distribution during transcranial direct current stimulation." *Clin Neurophysiol* 117(7): 1623-1629.
Monti, M. M. (2012). "Cognition in the vegetative state." *Annu Rev Clin Psychol* 8: 431-454.
Monti, M. M., M. R. Coleman and A. M. Owen (2009). "Executive functions in the absence of behavior: functional imaging of the minimally conscious state." *Prog Brain Res* 177: 249-260.
Monti, M. M., M. Rosenberg, P. Finoia, E. Kamau, J. D. Pickard and A. M. Owen (2015). "Thalamo-frontal connectivity mediates top-down cognitive functions in disorders of consciousness." *Neurology* 84(2): 167-173.
Monti, M. M., A. Vanhaudenhuyse, M. R. Coleman, M. Boly, J. D. Pickard, L. Tshibanda, A. M. Owen and S. Laureys (2010). "Willful modulation of brain activity in disorders of consciousness." *N Engl J Med* 362(7): 579-589.
Monti, M. M., A. Vanhaudenhuyse, M. R. Coleman, M. Boly, J. D. Pickard, L. Tshibanda, A. M. Owen and S. Laureys (2010). "Willful Modulation of Brain Activity in Disorders of Consciousness." *N Engl J Med*.
Morris, S. L., K. J. Dodd and M. E. Morris (2004). "Outcomes of progressive resistance strength training following stroke: a systematic review." *Clin Rehabil* 18(1): 27-39.
Moskopp, D., C. Stahle and H. Wassmann (1995). "Problems of the Glasgow Coma Scale with early intubated patients." *Neurosurg Rev* 18(4): 253-257.
Moulton, E. A., G. Pendse, L. R. Becerra and D. Borsook (2012). "BOLD responses in somatosensory cortices better reflect heat sensation than pain." *J Neurosci* 32(17): 6024-6031.
Mouraux, A., A. Diukova, M. C. Lee, R. G. Wise and G. D. Iannetti (2011). "A multisensory investigation of the functional significance of the "pain matrix"." *Neuroimage* 54(3): 2237-2249.
Mouraux, A. and G. D. Iannetti (2009). "Nociceptive laser-evoked brain potentials do not reflect nociceptive-specific neural activity." *J Neurophysiol* 101(6): 3258-3269.
Nair, D. G., V. Renga, R. Lindenberg, L. Zhu and G. Schlaug (2011). "Optimizing recovery potential through simultaneous occupational therapy and non-invasive brain-stimulation using tDCS." *Restor Neurol Neurosci* 29(6): 411-420.

Nakayama, N., A. Okumura, J. Shinoda, T. Nakashima and T. Iwama (2006). "Relationship between regional cerebral metabolism and consciousness disturbance in traumatic diffuse brain injury without large focal lesions: an FDG-PET study with statistical parametric mapping analysis." *JNNP* 77: 856-862.
Nakayama, N., A. Okumura, J. Shinoda, T. Nakashima and T. Iwama (2006). "Relationship between regional cerebral metabolism and consciousness disturbance in traumatic diffuse brain injury without large focal lesions: an FDG-PET study with statistical parametric mapping analysis." *J Neurol Neurosurg Psychiatry* 77(7): 856-862.
Nelson, J. T., R. A. McKinley, E. J. Golob, J. S. Warm and R. Parasuraman (2012). "Enhancing vigilance in operators with prefrontal cortex transcranial direct current stimulation (tDCS)." *Neuroimage*.
Neuhaus, B. E., E. R. Ascher, B. A. Coullon, M. V. Donohue, A. Einbond, J. M. Glover, S. R. Goldberg and V. L. Takai (1981). "A survey of rationales for and against hand splinting in hemiplegia." *Am J Occup Ther* 35(2): 83-90.
Nielsen, J., N. Petersen, M. Ballegaard, F. Biering-Sorensen and O. Kiehn (1993). "H-reflexes are less depressed following muscle stretch in spastic spinal cord injured patients than in healthy subjects." *Exp Brain Res* 97(1): 173-176.
Nitsche, M., K. Fricke, U. Henschke, A. Schlitterlau, D. Liebetanz, N. Lang, S. Henning, F. Tergau and W. Paulus (2003). "Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans." *J Physiol* 553: 293-301.
Nitsche, M., W. Jaussi, D. Liebetanz, N. Lang, F. Tergau and W. Paulus (2004). "Consolidation of externally induced human motor cortical neuroplasticity by D-cycloserine." *Neuropsychoparmacology* 29: 1573-1578.
Nitsche, M. and W. Paulus (2000). "Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation." *J Physiol* 527(3): 633-639.
Nitsche, M. and W. Paulus (2001). "Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans." *Neurology* 57: 1899-1901.
Nitsche, M. A., L. G. Cohen, E. M. Wassermann, A. Priori, N. Lang, A. Antal, W. Paulus, F. Hummel, P. S. Boggio, F. Fregni and A. Pascual-Leone (2008). "Transcranial direct current stimulation: State of the art 2008." *Brain Stimul* 1(3): 206-223.
Nitsche, M. A., W. Jaussi, D. Liebetanz, N. Lang, F. Tergau and W. Paulus (2004). "Consolidation of human motor cortical neuroplasticity by D-cycloserine." *Neuropsychopharmacology* 29(8): 1573-1578.
Nitsche, M. A. and W. Paulus (2000). "Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation." *J Physiol* 527 Pt 3: 633-639.
Nitsche, M. A. and W. Paulus (2001). "Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans." *Neurology* 57: 1899-1901.
Nitsche, M. A., A. Seeber, K. Frommann, C. C. Klein, C. Rochford, M. S. Nitsche, K. Fricke, D. Liebetanz, N. Lang, A. Antal, W. Paulus and F. Tergau (2005). "Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex." *J Physiol* 568(Pt 1): 291-303.
Ochi, M., S. Saeki, T. Oda, Y. Matsushima and K. Hachisuka (2013). "Effects of anodal and cathodal transcranial direct current stimulation combined with robotic therapy on severely affected arms in chronic stroke patients." *J Rehabil Med* 45(2): 137-140.

Olma, M. C., R. A. Dargie, J. R. Behrens, A. Kraft, K. Irlbacher, M. Fahle and S. A. Brandt (2013). "Long-Term Effects of Serial Anodal tDCS on Motion Perception in Subjects with Occipital Stroke Measured in the Unaffected Visual Hemifield." *Front Hum Neurosci* 7: 314.
Owen, A. M., M. R. Coleman, D. K. Menon, I. S. Johnsrude, J. M. Rodd, M. H. Davis, K. Taylor and J. D. Pickard (2005). "Residual auditory function in persistent vegetative state: a combined PET and fMRI study." *Neuropsychol Rehabil* 15(3-4): 290-306.
Owen, A. M., N. D. Schiff and S. Laureys (2009). "A new era of coma and consciousness science." *Prog Brain Res* 177: 399-411.
Pandyan, A., G. Johnson, C. Price, R. Curless, M. Barnes and H. Rodgers (1999). "A review of the properties and limitations of the Ashworth and modified Ashworth Scales as measures of spasticity." *Clinical Rehab* 13: 373-383.
Paulus, W. (2003). "Transcranial direct current stimulation (tDCS)." *Suppl Clin Neurophysiol* 56: 249-254.
Peelle, J. E., R. Cusack and R. N. Henson (2012). "Adjusting for global effects in voxel-based morphometry: gray matter decline in normal aging." *NeuroImage* 60(2): 1503-1516.
Pena-Gomez, C., R. Sala-Lonch, C. Junque, I. C. Clemente, D. Vidal, N. Bargallo, C. Falcon, J. Valls-Sole, A. Pascual-Leone and D. Bartres-Faz (2012). "Modulation of large-scale brain networks by transcranial direct current stimulation evidenced by resting-state functional MRI." *Brain Stimul* 5(3): 252-263.
Peyron, R., L. Garcia-Larrea, M. C. Gregoire, P. Convers, A. Richard, F. Lavenne, F. G. Barral, F. Mauguiere, D. Michel and B. Laurent (2000). "Parietal and cingulate processes in central pain. A combined positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) study of an unusual case." *Pain* 84(1): 77-87.
Peyron, R., García-Larrea, L., Grégoire M.C., Costes, N., Convers, P., Lavenne, F., Mauguière, F., Michel, D., Laurent, B. (1999). "Haemodynamic brain responses to acute pain in humans : sensory and attentional networks." *Brain* 122: 1765-1779.
Peyron, R., Laurent, B., Garcia-Larrea, L. (2000). "Functional imaging of brain responses to pain. A review and meta-analysis." *Neurophysiologie Clinique* 30(5): 263-288.
Phillips, C. L., M. A. Bruno, P. Maquet, M. Boly, Q. Noirhomme, C. Schnakers, A. Vanhaudenhuyse, M. Bonjean, R. Hustinx, G. Moonen, A. Luxen and S. Laureys (2011). ""Relevance vector machine" consciousness classifier applied to cerebral metabolism of vegetative and locked-in patients." *Neuroimage* 56(2): 797-808.
Pilon, M. and S. J. Sullivan (1996). "Motor profile of patients in minimally responsive and persistent vegetative states." *Brain Injury* 10(6): 421-437.
Pistoia, F., M. Sara, S. Sacco, M. Franceschini and A. Carolei (2014). "Silencing the brain may be better than stimulating it. The GABA effect." *Curr Pharm Des* 20(26): 4154-4166.
Ploner, M., J. Gross, L. Timmermann and A. Schnitzler (2002). "Cortical representation of first and second pain sensation in humans." *Proc Natl Acad Sci U S A* 99(19): 12444-12448.
Ploner, M., F. Schmitz, H. J. Freund and A. Schnitzler (1999). "Parallel activation of primary and secondary somatosensory cortices in human pain processing." *J Neurophysiol* 81(6): 3100-3104.
Plum, F. and J. B. Posner (1972). "The diagnosis of stupor and coma." *Contemp Neurol Ser* 10: 1-286.
Polanowska, K. E., M. M. Lesniak, J. B. Seniow, W. Czepiel and A. Czlonkowska (2013). "Anodal transcranial direct current stimulation in early rehabilitation of patients with post-stroke non-fluent

aphasia: A randomized, double-blind, sham-controlled pilot study." *Restor Neurol Neurosci* 31(6): 761-771.
Posner, J., C. Saper, N. Schiff and F. Plum (2007). *Plum and Posner's diagnosis of stupor and coma*. New York, Oxford University Press.
Purpura, D. and J. McMurtry (1965). "Intracellular activities and evoked potential changes during polarization of motor cortex." *J Neurophysiol* 28: 166-185.
PVS, T. M.-S. T. F. o. (1994). "Medical aspects of the persistent vegetative state (2). The Multi-Society Task Force on PVS." *The New England journal of medicine* 330(22): 1572-1579.
Quarantelli, M., K. Berkouk, A. Prinster, B. Landeau, C. Svarer, L. Balkay, B. Alfano, A. Brunetti, J. C. Baron and M. Salvatore (2004). "Integrated software for the analysis of brain PET/SPECT studies with partial-volume-effect correction." *J Nucl Med* 45(2): 192-201.
Rainville, P., Duncan, G.H., Price, D.D., Carrier, B., Bushnell, M.C. (1997). "Pain affect encoded in human anterior cingulate but not somatosensory cortex." *Science* 277(5328): 968-971.
Rees, G. (2007). "Neural correlates of the contents of visual awareness in humans." *Philos Trans R Soc Lond Biol Sci* 362(1481): 877-886.
Reis, J., H. M. Schambra, L. G. Cohen, E. R. Buch, B. Fritsch, E. Zarahn, P. A. Celnik and J. W. Krakauer (2009). "Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation." *Proc Natl Acad Sci U S A* 106(5): 1590-1595.
Ronga, I., E. Valentini, A. Mouraux and G. D. Iannetti (2012). "Novelty is not enough: laser-evoked potentials are determined by stimulus saliency, not absolute novelty." *J Neurophysiol*.
Ronga, I., E. Valentini, A. Mouraux and G. D. Iannetti (2013). "Novelty is not enough: laser-evoked potentials are determined by stimulus saliency, not absolute novelty." *J Neurophysiol* 109(3): 692-701.
Rowley, G. and K. Fielding (1991). "Reliability and accuracy of the Glasgow Coma Scale with experienced and inexperienced users." *Lancet* 337(8740): 535-538.
Ruffini, G., M. D. Fox, O. Ripolles, P. C. Miranda and A. Pascual-Leone (2014). "Optimization of multifocal transcranial current stimulation for weighted cortical pattern targeting from realistic modeling of electric fields." *Neuroimage* 89: 216-225.
Sanger, D. J. (2004). "The pharmacology and mechanisms of action of new generation, non-benzodiazepine hypnotic agents." *CNS Drugs* 18 Suppl 1: 9-15; discussion 41, 43-15.
Sara, M., F. Pistoia, E. Mura, P. Onorati and S. Govoni (2009). "Intrathecal baclofen in patients with persistent vegetative state: 2 hypotheses." *Arch Phys Med Rehabil* 90(7): 1245-1249.
Sara, M., S. Sacco, F. Cipolla, P. Onorati, C. Scoppetta, G. Albertini and A. Carolei (2007). "An unexpected recovery from permanent vegetative state." *Brain Inj* 21(1): 101-103.
Satkunam, L. E. (2003). "Rehabilitation medicine: 3. Management of adult spasticity." *CMAJ* 169(11): 1173-1179.
Schieppati, M. (1987). "The Hoffmann reflex: a means of assessing spinal reflex excitability and its descending control in man." *Prog Neurobiol* 28(4): 345-376.
Schiff, N. D. (2010). "Recovery of consciousness after brain injury: a mesocircuit hypothesis." *Trends Neurosci* 33(1): 1-9.
Schiff, N. D., J. T. Giacino, K. Kalmar, J. D. Victor, K. Baker, M. Gerber, B. Fritz, B. Eisenberg, T. Biondi, J. O'Connor, E. J. Kobylarz, S. Farris, A. Machado, C. McCagg, F. Plum, J. J. Fins and A. R. Rezai (2007).

"Behavioural improvements with thalamic stimulation after severe traumatic brain injury." *Nature* 448(7153): 600-603.
Schlaug, G., V. Renga and D. Nair (2008). "Transcranial direct current stimulation in stroke recovery." *Arch Neurol* 65(12): 1571-1576.
Schnakers, C., C. Chatelle, A. Demertzi, S. Majerus and S. Laureys (2012). "What about pain in disorders of consciousness?" *AAPS J* 14(3): 437-444.
Schnakers, C., C. Chatelle, A. Demertzi, S. Majerus and S. Laureys (2012). "What about Pain in Disorders of Consciousness?" *AAPS J*.
Schnakers, C., C. Chatelle, A. Vanhaudenhuyse, S. Majerus, D. Ledoux, M. Boly, M. A. Bruno, P. Boveroux, A. Demertzi, G. Moonen and S. Laureys (2010). "The Nociception Coma Scale: a new tool to assess nociception in disorders of consciousness." *Pain* 148(2): 215-219.
Schnakers, C., J. Giacino, K. Kalmar, S. Piret, E. Lopez, M. Boly, R. Malone and S. Laureys (2006). "Does the FOUR score correctly diagnose the vegetative and minimally conscious states?" *Ann Neurol* 60(6): 744-745; author reply 745.
Schnakers, C., R. Hustinx, G. Vandewalle, S. Majerus, G. Moonen, M. Boly, A. Vanhaudenhuyse and S. Laureys (2008). "Measuring the effect of amantadine in chronic anoxic minimally conscious state." *J Neurol Neurosurg Psychiatry* 79(2): 225-227.
Schnakers, C., S. Majerus, J. Giacino, A. Vanhaudenhuyse, M. Bruno, M. Boly, G. Moonen, P. Damas, B. Lambermont and M. Lamy (2008). "A French validation study of the Coma Recovery Scale-Revised (CRS-R)." *Brain Injury* 22(10): 786 - 792.
Schnakers, C., S. Majerus, J. Giacino, A. Vanhaudenhuyse, M. A. Bruno, M. Boly, G. Moonen, P. Damas, B. Lambermont, M. Lamy, F. Damas, M. Ventura and S. Laureys (2008). "A French validation study of the Coma Recovery Scale-Revised (CRS-R)." *Brain Injury* 22(10): 786-792.
Schnakers, C., S. Majerus, S. Goldman, M. Boly, P. Van Eeckhout, S. Gay, F. Pellas, V. Bartsch, P. Peigneux, G. Moonen and S. Laureys (2008). "Cognitive function in the locked-in syndrome." *J Neurol* 255(3): 323-330.
Schnakers, C., F. Perrin, M. Schabus, R. Hustinx, S. Majerus, G. Moonen, M. Boly, A. Vanhaudenhuyse, M. A. Bruno and S. Laureys (2009). "Detecting consciousness in a total locked-in syndrome: an active event-related paradigm." *Neurocase* 15(4): 271-277.
Schnakers, C., A. Vanhaudenhuyse, J. Giacino, M. Ventura, M. Boly, S. Majerus, G. Moonen and S. Laureys (2009). "Diagnostic accuracy of the vegetative and minimally conscious state: clinical consensus versus standardized neurobehavioral assessment." *BMC Neurol* 9: 35.
Schnakers, C. and N. D. Zasler (2007). "Pain assessment and management in disorders of consciousness." *Curr Opin Neurol* 20(6): 620-626.
Sergent, C. and S. Dehaene (2004). "Neural process underlying conscious perception: experimental findings and global neuronal workspace framework." *J Physiol Paris* 98(4-6): 374-384.
Shackman, A. J., T. V. Salomons, H. A. Slagter, A. S. Fox, J. J. Winter and R. J. Davidson (2011). "The integration of negative affect, pain and cognitive control in the cingulate cortex." *Nat Rev Neurosci* 12(3): 154-167.
Shah, S. (2007). "Wrist splint for upper motor neuron paralysis." *Stroke* 38(8): e74; author reply e75.
Shames, J. L. and H. Ring (2008). "Transient reversal of anoxic brain injury-related minimally conscious state after zolpidem administration: a case report." *Arch Phys Med Rehabil* 89(2): 386-388.

Sheean, G. (2002). "The pathophysiology of spasticity." *Eur J Neurol* 9(1): 3-9.
Shiel, A., S. A. Horn, B. A. Wilson, M. J. Watson, M. J. Campbell and D. L. McLellan (2000). "The Wessex Head Injury Matrix (WHIM) main scale: a preliminary report on a scale to assess and monitor patient recovery after severe head injury." *Clin Rehabil* 14(4): 408-416.
Sikes, R. W. and B. A. Vogt (1992). "Nociceptive neurons in area 24 of rabbit cingulate cortex." *J Neurophysiol* 68(5): 1720-1732.
Silva, S., X. Alacoque, O. Fourcade, K. Samii, P. Marque, R. Woods, J. Mazziotta, F. Chollet and I. Loubinoux (2010). "Wakefulness and loss of awareness: brain and brainstem interaction in the vegetative state." *Neurology* 74(4): 313-320.
Silvanto, J., N. Muggleton and V. Walsh (2008). "State-dependency in brain stimulation studies of perception and cognition." *Trends Cogn Sci* 12(12): 447-454.
Silvanto, J. and A. Pascual-Leone (2008). "State-dependency of transcranial magnetic stimulation." *Brain Topogr* 21(1): 1-10.
Simpson, D. M., D. N. Alexander, C. F. O'Brien, M. Tagliati, A. S. Aswad, J. M. Leon, J. Gibson, J. M. Mordaunt and E. P. Monaghan (1996). "Botulinum toxin type A in the treatment of upper extremity spasticity: a randomized, double-blind, placebo-controlled trial." *Neurology* 46(5): 1306-1310.
Sommerfeld, D. K., E. U. Eek, A. K. Svensson, L. W. Holmqvist and M. H. von Arbin (2004). "Spasticity after stroke: its occurrence and association with motor impairments and activity limitations." *Stroke* 35(1): 134-139.
Song, J. J., S. Vanneste, P. Van de Heyning and D. De Ridder (2012). "Transcranial direct current stimulation in tinnitus patients: a systemic review and meta-analysis." *ScientificWorldJournal* 2012: 427941.
Song, M., Y. Shin and K. Yun (2014). "Beta-frequency EEG activity increased during transcranial direct current stimulation." *Neuroreport* 25(18): 1433-1436.
Stagg, C. J., V. Bachtiar, J. O'Shea, C. Allman, R. A. Bosnell, U. Kischka, P. M. Matthews and H. Johansen-Berg (2012). "Cortical activation changes underlying stimulation-induced behavioural gains in chronic stroke." *Brain* 135(Pt 1): 276-284.
Stagg, C. J. and M. A. Nitsche (2011). "Physiological basis of transcranial direct current stimulation." *Neuroscientist* 17(1): 37-53.
Stender, J., O. Gosseries, M. A. Bruno, V. Charland-Verville, A. vanhaudenhuyse, A. Demertzi, C. Chatelle, A. Thibaut, L. Heine, A. Soddu, M. Boly, C. Schnakers, A. Gjedde and S. Laureys (2014). "Diagnostic precision of multimodal neuroimaging methods in disorders of consciousness: a clinical validation study." *Lancet* in press.
Stender, J., O. Gosseries, M. A. Bruno, V. Charland-Verville, A. Vanhaudenhuyse, A. Demertzi, C. Chatelle, M. Thonnard, A. Thibaut, L. Heine, A. Soddu, M. Boly, C. Schnakers, A. Gjedde and S. Laureys (2014). "Diagnostic precision of PET imaging and functional MRI in disorders of consciousness: a clinical validation study." *Lancet*.
Stender, J., O. Gosseries, M. A. Bruno, V. Charland-Verville, A. Vanhaudenhuyse, A. Demertzi, C. Chatelle, M. Thonnard, A. Thibaut, L. Heine, A. Soddu, M. Boly, C. Schnakers, A. Gjedde and S. Laureys (2014). "Diagnostic precision of PET imaging and functional MRI in disorders of consciousness: a clinical validation study." *Lancet* 384(9942): 514-522.

Stoll, J., C. Chatelle, O. Carter, C. Koch, S. Laureys and W. Einhauser (2013). "Pupil responses allow communication in locked-in syndrome patients." *Curr Biol* 23(15): R647-648.
Sunnerhagen, K. S., J. Olver and G. E. Francisco (2013). "Assessing and treating functional impairment in poststroke spasticity." *Neurology* 80(3 Suppl 2): S35-44.
Svensson, J., S. Borg and P. Nilsson (2014). "Costs and quality of life in multiple sclerosis patients with spasticity." *Acta Neurol Scand* 129(1): 13-20.
Takahashi, R., K. Ishii, N. Miyamoto, T. Yoshikawa, K. Shimada, S. Ohkawa, T. Kakigi and K. Yokoyama (2010). "Measurement of gray and white matter atrophy in dementia with Lewy bodies using diffeomorphic anatomic registration through exponentiated lie algebra: A comparison with conventional voxel-based morphometry." *AJNR. American journal of neuroradiology* 31(10): 1873-1878.
Talmadge, R. J., R. R. Roy, S. C. Bodine-Fowler, D. J. Pierotti and V. R. Edgerton (1995). "Adaptations in myosin heavy chain profile in chronically unloaded muscles." *Basic Appl Myol* 5(2): 117-137.
Tardieu, G., S. Shentoub and R. Delarue (1954). "A la recherche d'une technique de mesure de la spasticité." *Rev Neurol* 91: 143-144.
Teasdale, G. and B. Jennett (1974). "Assessment of coma and impaired consciousness. A practical scale." *Lancet* 2(7872): 81-84.
Tecco, J. M., P. Wuilmart, L. Lasseaux, D. Wikler, Jr., P. Damhaut, S. Goldman and C. Gilles (1998). "Cerebello-thalamo-cerebral diaschisis: a case report." *J Neuroimaging* 8(2): 115-116.
Thakker, M. M. and P. A. Rubin (2004). "Pharmacology and clinical applications of botulinum toxins A and B." *Int Ophthalmol Clin* 44(3): 147-163.
The Multi-Society Task Force on PVS (1994). "Medical aspects of the persistent vegetative state (1). The Multi-Society Task Force on PVS." *N Engl J Med* 330(21): 1499-1508.
Thibaut, A., M. A. Bruno, C. Chatelle, O. Gosseries, A. Vanhaudenhuyse, A. Demertzi, C. Schnakers, M. Thonnard, V. Charland-Verville, C. Bernard, M. Bahri, C. Phillips, M. Boly, R. Hustinx and S. Laureys (2012). "Metabolic activity in external and internal awareness networks in severely brain-damaged patients." *J Rehabil Med* 44(6): 487-494.
Thibaut, A., M. A. Bruno, C. Chatelle, O. Gosseries, A. Vanhaudenhuyse, A. Demertzi, C. Schnakers, M. Thonnard, V. Charland-Verville, C. Bernard, M. A. Bahri, C. Phillips, M. Boly, R. Hustinx and S. Laureys (2012). "Metabolic activity in external and internal awareness networks in severely brain-damaged patients." *J Rehabil Med*.
Thibaut, A., M. A. Bruno, D. Ledoux, A. Demertzi and S. Laureys (2014). "tDCS in patients with disorders of consciousness: sham-controlled randomised double blind study." *Neurology* 82(13): 1112-1118.
Thibaut, A., C. Chatelle, O. Gosseries, M. A. Bruno, A. Demertzi, C. Bernard, R. Hustinx, L. Tshibanda, M. A. Bahri and S. Laureys (2014). "Changes in cerebral metabolism in patients with a minimally conscious state responding to zolpidem." *Front Hum Neurosci* 8: 917.
Thibaut, A., C. Chatelle, O. Gosseries, S. Laureys and M. A. Bruno (2013). "[Transcranial direct current stimulation: a new tool for neurostimulation]." *Rev Neurol (Paris)* 169(2): 108-120.
Thibaut, A., C. Chatelle, J. Stender, A. Demertzi, C. Bernard, R. Hustinx, S. Laureys and M. A. Bruno (2014). Positron emission tomography imaging in altered states of consciousness: Coma, sleep and hypnosis. *PET and SPECT in Neurology*. R. Dierckx, A. Otte, E. Vries, A. Waarde and K. Leenders. aris, Springer.

Thibaut, A., C. Chatelle, S. Wannez, T. Deltombe, J. Stender, C. Schnakers, S. Laureys and O. Gosseries (2014). "Spasticity in disorders of consciousness: A behavioral study." *Eur J Phys Rehabil Med* Nov 6 [Epub ahead of print].
Thibaut, A., C. Chatelle, J. Whyte, M. D. De Val, S. Laureys and C. Schnakers (2014). "Pain issues in disorders of consciousness." *Brain Inj* 28(9): 1202-1208.
Thibaut, A., C. Chatelle, E. Ziegler, M. A. Bruno, S. Laureys and O. Gosseries (2013). "Spasticity after stroke: Physiology, assessment and treatment." *Brain Inj*.
Thibaut, A., T. Deltombe, S. Wannez, O. Gosseries, E. Ziegler, C. Dieni, M. Deroy and S. Laureys (in press). "Impact of soft splints on upper limb spasticity in chronic patients with disorders of consciousness: a randomized, single-blind, controlled trial." *Brain Inj* in press.
Thibaut, A., C. Di Perri, C. Chatelle, M. A. Bruno, S. Wannez, C. Bernard, R. Hustinx and S. Laureys (submitted). "Clinical response to tDCS depends on residual brain metabolism and grey matter integrity in patients with minimally conscious state " *Brain Stimulation*.
Thonnard, M., O. Gosseries, A. Demertzi, Z. Lugo, A. Vanhaudenhuyse, B. Marie-Aurelie, C. Chatelle, A. Thibaut, V. Charland-Verville, D. Habbal, C. Schnakers and S. Laureys (2014). "Effect of zolpidem in chronic disorders of consciousness: a prospective open-label study." *Funct Neurol*: 1-6.
Tremblay, F., F. Malouin, C. L. Richards and F. Dumas (1990). "Effects of prolonged muscle stretch on reflex and voluntary muscle activations in children with spastic cerebral palsy." *Scand J Rehabil Med* 22(4): 171-180.
Ulam, F., C. Shelton, L. Richards, L. Davis, B. Hunter, F. Fregni and K. Higgins (2014). "Cumulative effects of transcranial direct current stimulation on EEG oscillations and attention/working memory during subacute neurorehabilitation of traumatic brain injury." *Clin Neurophysiol*.
Urban, P., T. Wolf, M. Uebele, J. Marx, T. Vogt, P. Stoeter, T. Bauermann, C. Weibrich, G. Vucurevic, A. Schneider and J. Wissel (2010). "Occurence and clinical predictors of spasticity after ischemic stroke." *Stroke* 41(9): 2016-2020.
Vaccarino, A. L. and R. Melzack (1989). "Analgesia produced by injection of lidocaine into the anterior cingulum bundle of the rat." *Pain* 39(2): 213-219.
Vanhaudenhuyse, A., M. Boly, E. Balteau, C. Schnakers, G. Moonen, A. Luxen, M. Lamy, C. Degueldre, J. F. Brichant, P. Maquet, S. Laureys and M. E. Faymonville (2009). "Pain and non-pain processing during hypnosis: a thulium-YAG event-related fMRI study." *Neuroimage* 47(3): 1047-1054.
Vanhaudenhuyse, A., A. Demertzi, M. Schabus, Q. Noirhomme, S. Bredart, M. Boly, C. Phillips, A. Soddu, A. Luxen, G. Moonen and S. Laureys (2011). "Two distinct neuronal networks mediate the awareness of environment and of self." *J Cogn Neurosci* 23(3): 570-578.
Vanhaudenhuyse, A., J. Giacino, C. Schnakers, K. Kalmar, C. Smart, M. Bruno, O. Gosseries, G. Moonen and S. Laureys (2008). "Blink to visual threat does not herald consciousness in the vegetative state." *Neurology* 71(17): 1374-1375.
Vanhaudenhuyse, A., Q. Noirhomme, L. J. Tshibanda, M. A. Bruno, P. Boveroux, C. Schnakers, A. Soddu, V. Perlbarg, D. Ledoux, J. F. Brichant, G. Moonen, P. Maquet, M. D. Greicius, S. Laureys and M. Boly (2010). "Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients." *Brain* 133(Pt 1): 161-171.
Vestito, L., S. Rosellini, M. Mantero and F. Bandini (2014). "Long-term effects of transcranial direct-current stimulation in chronic post-stroke aphasia: a pilot study." *Front Hum Neurosci* 8: 785.

Vogt, B. A. (2005). "Pain and emotion interactions in subregions of the cingulate gyrus." *Nat Rev Neurosci* 6(7): 533-544.
Voss, H. U., A. M. Uluc, J. P. Dyke, R. Watts, E. J. Kobylarz, B. D. McCandliss, L. A. Heier, B. J. Beattie, K. A. Hamacher, S. Vallabhajosula, S. J. Goldsmith, D. Ballon, J. T. Giacino and N. D. Schiff (2006). "Possible axonal regrowth in late recovery from the minimally conscious state." *J Clin Invest* 116(7): 2005-2011.
Ward, A. B. (2012). "A literature review of the pathophysiology and onset of post-stroke spasticity." *Eur J Neurol* 19(1): 21-27.
Watanabe, T. (2004). "The role of therapy in spasticity management." *Am J Phys Med Rehabil* 83(10 Suppl): S45-49.
Watt, J., D. Sims, F. Harckham, L. Schmidt, A. McMillan and J. Hamilton (1986). "A prospective study of inhibitive casting as an adjunct to physiotherapy for cerebral-palsied children." *Dev Med Child Neurol* 28(4): 480-488.
Weir, C. J., A. P. Bradford and K. R. Lees (2003). "The prognostic value of the components of the Glasgow Coma Scale following acute stroke." *QJM* 96(1): 67-74.
Whyte, J. (2014). "Disorders of consciousness: the changing landscape of treatment." *Neurology* 82(13): 1106-1107.
Whyte, J. and R. Myers (2009). "Incidence of clinically significant responses to zolpidem among patients with disorders of consciousness: a preliminary placebo controlled trial." *Am J Phys Med Rehabil* 88(5): 410-418.
Whyte, J., R. Rajan, A. Rosenbaum, D. Katz, K. Kalmar, R. Seel, B. Greenwald, R. Zafonte, D. Demarest, R. Brunner and D. Kaelin (2014). "Zolpidem and restoration of consciousness." *Am J Phys Med Rehabil* 93(2): 101-113.
Wijdicks, E. F. (2006). "Clinical scales for comatose patients: the Glasgow Coma Scale in historical context and the new FOUR Score." *Rev Neurol Dis* 3(3): 109-117.
Wijdicks, E. F., W. R. Bamlet, B. V. Maramattom, E. M. Manno and R. L. McClelland (2005). "Validation of a new coma scale: The FOUR score." *Ann Neurol* 58(4): 585-593.
Wijdicks, E. F., E. Kokmen and P. C. O'Brien (1998). "Measurement of impaired consciousness in the neurological intensive care unit: a new test." *J Neurol Neurosurg Psychiatry* 64(1): 117-119.
Williams, S. T., M. M. Conte, A. M. Goldfine, Q. Noirhomme, O. Gosseries, M. Thonnard, B. Beattie, J. Hersh, D. I. Katz, J. D. Victor, S. Laureys and N. D. Schiff (2013). "Common resting brain dynamics indicate a possible mechanism underlying zolpidem response in severe brain injury." *Elife* 2(0).
Wilson, F. C., V. Elder, E. McCrudden and S. Caldwell (2009). "Analysis of Wessex Head Injury Matrix (WHIM) scores in consecutive vegetative and minimally conscious state patients." *Neuropsychol Rehabil* 19(5): 754-760.
Wissel, J., L. D. Schelosky, J. Scott, W. Christe, J. H. Faiss and J. Mueller (2010). "Early development of spasticity following stroke: a prospective, observational trial." *J Neurol* 257(7): 1067-1072.
Witte, O. W., H. J. Bidmon, K. Schiene, C. Redecker and G. Hagemann (2000). "Functional differentiation of multiple perilesional zones after focal cerebral ischemia." *J Cereb Blood Flow Metab* 20(8): 1149-1165.
Yelnik, A., O. Simon, D. Bensmail, E. Chaleat-Valayer, P. Decq, P. Dehail, V. Quentin, P. Marque, B. Parratte, F. Pellas, M. Rousseaux, J. Trocello, M. Uzzan and N. Dumarcet (2009). "Drug treatments for spasticity." *Ann Phys Rehabil Med* 52: 746-756.

Yelnik, A. P., O. Simon, B. Parratte and J. M. Gracies (2010). "How to clinically assess and treat muscle overactivity in spastic paresis." *J Rehabil Med* 42(9): 801-807.
Young, R. (1994). "Spasticity: a review." *Neurology* 44: S12-S20.
Yu, T., S. Lang, D. Vogel, A. Markl, F. Muller and B. Kotchoubey (2013). "Patients with unresponsive wakefulness syndrome respond to the pain cries of other people." *Neurology* 80(4): 345-352.
Zaehle, T., P. Sandmann, J. D. Thorne, L. Jancke and C. S. Herrmann (2011). "Transcranial direct current stimulation of the prefrontal cortex modulates working memory performance: combined behavioural and electrophysiological evidence." *BMC Neurosci* 12: 2.
Zangen, A., Y. Roth, B. Voller and M. Hallett (2005). "Transcranial magnetic stimulation of deep brain regions: evidence for efficacy of the H-coil." *Clin Neurophysiol* 116(4): 775-779.
Zeman, A. (2005). "What in the world is consciousness?" *Prog Brain Res* 150: 1-10.
Zorowitz, R. D., P. J. Gillard and M. Brainin (2013). "Poststroke spasticity: sequelae and burden on stroke survivors and caregivers." *Neurology* 80(3 Suppl 2): S45-52.

6. Appendix

Scientific publications