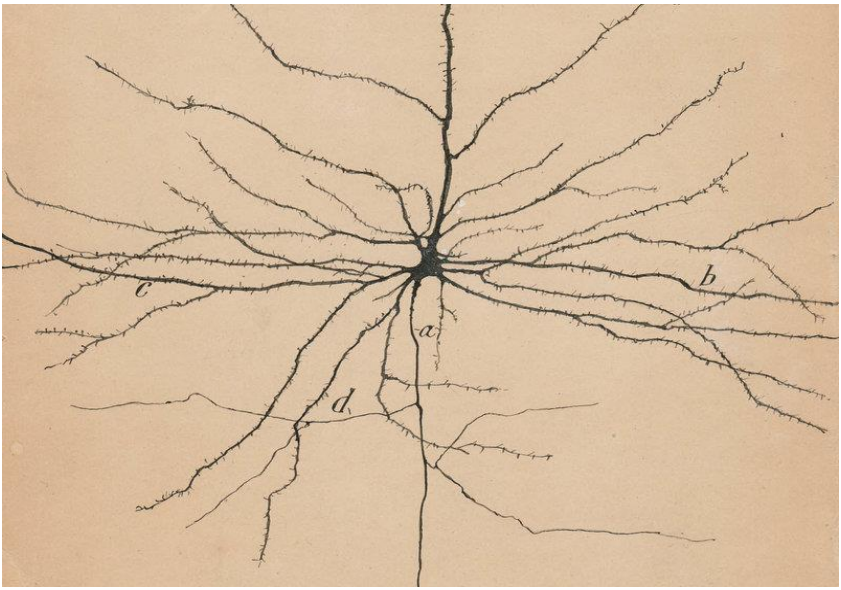
Under the supervision of
Prof Steven LAUREYS,
Coma Science Group,
GIGA-Consciousness

NON-INVASIVE BRAIN STIMULATION IN POST COMATOSE STATES

Géraldine Martens



THESE PRESENTEE EN VUE DE L'OBTENTION DU GRADE DE

Docteur en Sciences de la Motricité

Année académique 2019-2020

NON-INVASIVE BRAIN STIMULATION IN POST COMATOSE STATES

Géraldine Martens

Under the supervision of Prof. Steven Laureys and Dr. Aurore Thibaut

Thèse présentée en vue de l'obtention du grade de

Docteur en Docteur en Sciences de la Motricité

Promotor

Prof. Steven Laureys, MD, PhD, Université de Liège

Co-promotor

Dr Aurore Thibaut, PhD, Université de Liège

Assessment committee

Prof. Benjamin Rohaut, MD, PhD, Sorbonne Université & Columbia University NY

Prof. Yves Vandermeeren, MD, PhD, Université Catholique de Louvain

Prof. Jean-François Kaux, MD, PhD, Université de Liège

Prof. Bernard Rogister, MD, PhD, Université de Liège

Prof. Anne-Françoise Donneau, PhD, Université de Liège

Prof. Didier Ledoux, MD, PhD, Université de Liège

Cover: Santiago Ramón Y Cajal dradwing, © Instituto Cajal del Consjo Superior de Investigaciones Científicas, Madrid

---

The studies presented in this thesis have been financially supported by: LUMINOUS project (EU-H2020-fetopen-ga686764), US National Institute on Disability, Independent Living, and Rehabilitation Research (90DPTB0011), DOCMA project (EU-H2020-MSCA–RISE–778234), Cefaly Technology, Modus ULiège, the LEAR Foundation, Wallonie-Bruxelles International (WBI.World), Wallonia-Brussels Federation, James S. McDonnell Foundation, Tiny Blue Dot Foundation, Belgian National Funds for Scientific Research (FRS-FNRS), Bial Foundation and Human Brain Project (EU-H2020-fetflagship-hbp-sga1-ga720270). All authors report no conflict of interest.

Table of contents

Acknowledgments

First of all, I would like to thank my mentor and friend: Aurore, for being such a unique and brilliant person! You did not only initiate my own interest in research; you paved the way toward an incredible scientific journey for many of us. Since the very beginning, you have been a true pillar: advising me at every step, supporting me at every discouragement, and you carried on by being my beacon in the darkness during the whole ride – no matter where. Your kindness, your encouragements and your guidance were, by far, the most important contributions to the present work, and I couldn't be more grateful for it. The best is yet to come!

I owe a lot to my promotor Steven; thank you for providing us with this uniquely stimulating working environment, for your support and your humor, under all circumstances. Being part of the Coma Science Group, this family you founded, is the most rewarding experience I have got so far.

Over the years, colleagues have become best friends: I would like to address a special thought to the members of the "Club des cinq": Charlotte, Héléna, Sarah and Charlène. I am deeply thankful to my incredible crew at the Coma Science Group for making every day as enjoyable as possible: Manon, Alice, Estelle, Olivia, Léandro, Jitka, Bernard, Nicolas L, Sepehr, Raja, Audrey, Stephen, Fede, Marie-Michèle, Camille, Olivier, Georgios, Athéna, Cécile, Aminata, Floriane, Evelyne, Solène, Clément, Marta, Masachika; and to the former members of this dream-team: Lizette, Enrico, Alexandra, Caroline, Séverine, Vanessa and Carol.

I would like to thank the Boston team as well: Joe for giving me this unique opportunity to work with your lab and for being a flawless supervisor! You showed your exceptional support could cross any barrier and I'm grateful for it. Yelena, you are a true role model for all of us, I could only admire the combination of your scientific skills and professionalism with your kindness and empathy. Rebecca, I am so glad we could prove work friends can become real friends, thank you for lighting up my stay in Boston and I hope to welcome you and Julia again in Belgium. Amber, thank you for bringing the touch of fun everyone needs. Michael, thank you for helping me during my whole stay and beyond. Chris, thank you for everything you taught me and welcome to Europe! Many thanks as well to Andrea, Joe O. and the rest of the Spaulding team for their dedication and support. Many friends over there made my stay exceptional; special thanks to Diede, Jay, Charlotte, Matt, Catherine, Dana & Will and the MEME crew that continues spreading both knowledge and friendship all over the world.

I would like to thank the members of my doctoral thesis committee as well: Prof. Jean-François Kaux, Dr Anne-Françoise Donneau and Dr Didier Ledoux, for the time commitment, the support and the scientific insight provided during my whole PhD training. To the new members of the jury: Prof. Bernard Rogister, Dr Benjamin Rohaut and Prof. Yves Vandermeeren, thank you for taking the time to evaluate my work.

The present dissertation has been written in a wide variety of environments and I would like to address many thanks to the Therapiezentrum Burgau and especially Martin and Andreas for welcoming me during these intense times.

This would not have been possible without the close and essential support of my family: Maman, Papa & Christine, Delphine, Chloé & Raphaël, Flo, Ariane & Antoine, Didier & Graziella, Christian, Alice. Many thanks as well to my extended family, the 'in-laws' Goffart-Dubois team: Odile, Bernard, Cédric, Guillaume, Emma & Albert, Nicole, Patrice and Juliette.

I am blessed with friends that were always present to clear my head or patiently listen to my complaints: Pascaline & Vincent, Sergine & Pierre, Marine & Rodrigue, Julien R, Mélanie, Babu & Léa (and Iris!), Damien, the M&Ms, Julien B, Charlotte DW, Nicolas & Sharon, Jérémy & Hélène, Floriane W, Gauthier & Laura (and Iris! – not a typo) and Stephen B; thank you all! And to my dear friend 'Mama Chloé': you were taken from us way too early, and as painful as it can be, I know you would have been proud of me today.

Last but not least, to my sweetheart François: thank you for being my unconditional support during this whole journey, and you know better than anyone else it was a hell of a ride! For every new step I took, I knew I could rely on your comfort and advice; you always got my back. While perfectly balancing the amount of support you provided and the liberty you gave me to play with my own rules, you couldn't have been a greater aid. You truly are the best life partner I could wish for and I'm looking forward to the rest of our adventures.

Scientific Publications

The present thesis is based on the following publications:

Articles:

Martens, G., Ibanez-Soria, D., Soria-Frisch, A., Barra, A., Piarulli, A., Gosseries, O., Salvador, R., Rojas, A., Nitsche, M., Laureys, S., Ruffini, G., & Thibaut, A. A novel closed-loop EEG-tDCS approach to promote responsiveness of patients in minimally conscious state: a study protocol. *Submitted*

Martens, G., Kroupi, E., Bodien, Y., Cassol, H., Barra, A., Martial, C., Annen, J., Soria-Frisch, A., Gosseries, O., Ruffini, G., Laureys, S., & Thibaut, A. Behavioral and electrophysiological effects of network-based frontoparietal tDCS in patients with severe brain injury: a randomized controlled trial. *Submitted*

Martens, G., Bodien, Y. Thomas, A., & Giacino, J. Temporal profile of recovery of communication in patients with disorders of consciousness following severe brain injury. *Archives of Physical Medicine and Rehabilitation.* doi:10.1016/j.apmr.2020.01.015

Martens, G., Bodien, Y., Sheau, K., Christoforou, A., & Giacino, J. T. (2019). Which behaviours are first to emerge during recovery of consciousness after severe brain injury? *Annals of physical and rehabilitation medicine*. doi:10.1016/j.rehab.2019.10.004

Martens, G., Fregni, F., Carrière, M., Barra, A., Laureys, S., & Thibaut, A. (2019). Single tDCS session of motor cortex in patients with disorders of consciousness: a pilot study. *Brain Injury*, 1-5.

Martens G., Deltombe T., Foidart-Dessalle M., Laureys S., & Thibaut A. (2018). Clinical and electrophysiological investigation of spastic muscle overactivity in patients with disorders of consciousness following severe brain injury. *Clinical Neurophysiology*, 130(2), 207-213.

Martens, G., Lejeune, N., O'Brien, A. T., Fregni, F., Martial, C., Wannez, S., Laureys, S. & Thibaut, A. (2018). Randomized controlled trial of home-based 4-week tDCS in chronic minimally conscious state. *Brain stimulation*, 11(5), 982-990.

Martens, G., Laureys, S., & Thibaut, A. (2017). Spasticity management in disorders of consciousness. *Brain sciences*, 7(12), 162.

Book chapters:

Martens, G., Blandiaux, S., Wolff, A., Gosseries, O., Laureys, S., & Chatelle, C. (2018). Evaluation de la douleur chez le patient cérébrolésé en état de conscience altérée (Pain assessment in brain-injured patients with disorders of consciousness). In *Etats de conscience altérée* (pp.58-70). Sauramps Medical.

Martens, G., Foidart-Dessalle, M., Laureys, S., & Thibaut, A. (2018). How Does Spasticity Affect Patients with Disorders of Consciousness? In *Coma and Disorders of Consciousness* (pp. 119-135). Springer, Cham.

Other publications:

Article:

Martens, G., Deflandre, D., Schwartz, C., Dardenne, N., & Bury, T. (2018). Reproducibility of the Evolution of Stride Biomechanics During Exhaustive Runs. *Journal of human kinetics*, *64*(1), 57-69.

Articles (as co-author):

Aubinet, C., Cassol, H., Gosseries, O., Bahri, M. A., Larroque, S., Majerus, S., Martial, C., **Martens, G.**, Carrière, M., Chatelle, C., Laureys, S., & Thibaut, A. (2020). Brain metabolism but not grey matter volume underlies the presence of language function in the minimally conscious state. *Neurorehabilitation and Neural Repair*. 34(2), 172-184

Lejeune, N., Thibaut, A., **Martens, G.**, Martial, C., Wannez, S., Laureys, S., & Chatelle, C. (2019). Can the Nociception Coma Scale-Revised be used in patients with a tracheostomy? *Archives of physical medicine and rehabilitation*. doi:10.1016/j.apmr.2019.09.020

Bodien, Y., **Martens, G.**, Ostrow, J., Sheau, K., Giacino, J. (2020). Cognitive Impairment, Clinical Symptoms and Functional Disability in Patients Emerging from the Minimally Conscious State. *NeuroRehabilitation*. 46(1), 65-74.

Thibaut, A., Piarulli, A., **Martens, G.**, Chatelle, C., & Laureys, S. (2019). Effect of multichannel transcranial direct current stimulation to reduce hypertonia in individuals with prolonged disorders of consciousness: A randomized controlled pilot study. *Annals of Physical and Rehabilitation Medicine*. 62(6), 418-425.

Cassol, H., Martial, C., Annen, J., **Martens, G.**, Charland-Verville, V., Majerus, S., & Laureys, S. (2019). A systematic analysis of distressing near-death experience accounts. *Memory*, 1-8.

Gallucci, A., Lucena, P. H., **Martens, G.**, Thibaut, A., & Fregni, F. (2018). Transcranial direct current stimulation to prevent and

treat surgery-induced opioid dependence: a systematic review. *Pain management*, *9*(1), 93-106.

Thibaut, A., Chennu, S., Chatelle, C., **Martens, G.**, Annen, J., Cassol, H., & Laureys, S. (2018). Theta network centrality correlates with tDCS response in disorders of consciousness. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, *11*(6), 1407-1409.

Thibaut, A., Chatelle, C., Vanhaudenhyse, A., **Martens, G.**, Cassol, H., Barra, A., Martial, C., Carrière, M., & Laureys, S. (2018). Transcranial direct current stimulation unveils covert consciousness. *Brain Stimulation*, 11(3), 642-644

Thibaut, A., Wannez, S., Deltombe, T., **Martens, G.**, Laureys, S., & Chatelle, C. (2018). Physical therapy in patients with disorders of consciousness: Impact on spasticity and muscle contracture. *NeuroRehabilitation*, *42*(2), 199-205.

Cassol, H., Aubinet, C., Thibaut, A., Wannez, S., Martial, C., **Martens, G.** & Laureys, S. (2018). Diagnostic, pronostic et traitements des troubles de la conscience. *Neurologie - Psychiatrie – Gériatrie*, *8*, 47–49.

Chennu, S., Annen, J., Wannez, S., Thibaut, A., Chatelle, C., Cassol, H., **Martens, G.**, Schnakers, C., Gosseries, O., Menon, D., & Laureys, S. (2017). Brain networks predict metabolism, diagnosis and prognosis at the bedside in disorders of consciousness. *Brain*, *140*(8), 2120-2132.

Wannez, S., Heine, L., Thonnard, M., Gosseries, O., Laureys, S. et al. [Coma Science Group collaborators, including **Martens, G.**] (2017). The repetition of behavioral assessments in disorders of consciousness. *Annals of Neurology*, *81*(6), 883-889.

Wannez, S., Gosseries, O., Azzolini, D., Martial, C., Cassol, H., Aubinet, C., Annen, J., **Martens, G.**, Bodart, O., Heine, L., Charland-Verville, V., Thibaut, A., Chatelle, C., Vanhaudenhuyse, A., Demertzi, A., Schnakers, C., Donneau, A.-F. & Laureys, S. (2017). Prevalence of Coma-Recovery Scale-Revised signs of consciousness in patients in a minimally conscious state. *Neuropsychological Rehabilitation, 11*, 1–10.

Huang, W., Wannez, S., Fregni, F., Hu, X., Jing, S., **Martens, G.**, He, M., Di, H., Laureys, S., & Thibaut, A. (2017). Repeated stimulation of the posterior parietal cortex in patients in minimally conscious state: A sham-controlled randomized clinical trial. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, *10*(3), 718-720.

Book chapter (as co-author):

Bonin, E., **Martens, G.**, Cassol, H., Chatelle, C., Laureys, S., & Thibaut, A. PET Imaging in Altered States of Consciousness: Coma, Sleep, and Hypnosis. In *PET and SPECT in Neurology*. Springer, In press

Summary

After a severe brain injury leading to a period of coma, a possible scenario is that the patient remains with an altered state of consciousness for a prolonged period. These disorders of consciousness (DOC) encompass the unresponsive wakefulness syndrome (UWS); a state of awakening with only reflexive movements and the minimally conscious state (MCS); where fluctuating but reproducible signs of consciousness are observed. The ability to functionally use objects or communicate then marks the transition to the emergence of the MCS (EMCS). The management of patient with DOC represents a medical challenge from both diagnostic and treatment perspectives. Given the absence of subjective report, the brain injury-associated cognitive and motor deficits and the fluctuations in vigilance that characterize them, the misdiagnosis rates can go up to 40%, with dramatic impact on their care. Furthermore, therapeutic approaches to increase their level of consciousness and ameliorate their functional status are lacking and poorly investigated. The present thesis had therefore two aims: i) better characterizing the path to recovery from a behavioral perspective (Part One) and ii) investigate the use of non-invasive brain stimulation, more specifically transcranial direct current stimulation (tDCS), and its different application parameters, as a treatment option (Part Two).

In **Part One**, we present two retrospectives studies using data collected by therapists in a specialized rehabilitation setting. We used repeated administrations of the Coma Recovery Scale-Revised (CRS-

R), the current gold standard for behavioral assessment of DOC patients, to pin down the initial **transition from unconscious states** (i.e., coma or UWS) **to recovery of consciousness** (i.e., MCS or EMCS). Among the 13 CRS-R behaviors depicting consciousness, visual pursuit most often marked the transition while the time to recovery of consciousness was approximately six weeks after injury. We then focused on a specific and highly clinically relevant behavior that is the **recovery of communication**; anticipated by both relatives and therapists as it substantially ameliorates the interactions and the care. Within our 8-week observation period, the ability to answer some close-ended questions, despite of accuracy (i.e., intentional communication) was usually recovered within 40 days after injury while correctly answering six out of six close-ended questions (i.e., functional communication) reappeared about nine days later.

In **Part Two**, we develop four studies: a pilot trial, two randomized controlled trials and a study protocol, aiming at answering the following questions regarding the use of tDCS as a therapeutic option: In what kind of setting can we apply it? Where should we stimulate? When?

Which setting – In a feasibility and efficacy randomized controlled trial, we investigated the **home-based application** of tDCS, applied for a prolonged period of 20 days over the left prefrontal cortex of 27 chronic MCS patients following traumatic or non-traumatic insult. There was a significant behavioral treatment effect at the group level, as long as at least 80% of the planned sessions were applied. No severe adverse events were reported.

Where – *T*he first pilot study investigated the effects of a single session of tDCS applied over the **motor cortex** in ten UWS and MCS patients, with traumatic and non-traumatic etiologies. No

behavioral treatment effect was identified at the group level while at the individual level, two patients responded to tDCS by showing a new sign of consciousness for the first time after active and not sham stimulation. In a randomized controlled trial performed on 46 patients in UWS, MCS or EMCS with traumatic or non-traumatic etiologies, we used multifocal network-based tDCS to stimulate the **frontoparietal network**, also known as the external awareness network. Again, there was no group level behavioral treatment effect while at the individual level, seven patients responded positively to tDCS. Seven other patients negatively responded by losing a sign of consciousness after active stimulation that was present before. These patients presented an initial significantly higher complexity of the EEG signal in the theta band.

When – Finally, we developed an original study protocol based on **brain-state dependent application** of tDCS, in a closed-loop fashion. Based on electroencephalographic entropy patterns as markers of vigilance, we aim to compare the behavioral and electrophysiological effects of tDCS applied at high and low levels of vigilance and hypothesize this approach will significantly impact the individual response to tDCS.

Overall, the present findings show that patients with DOC have a strong potential for recovery in the subacute phase of their injury, and that false despair should be avoided in the early stages. These patients could benefit from tDCS, which has a proven efficacy when applied over the prefrontal cortex and when repeating the amount of sessions. Caregivers and relatives can be safely involved to apply this type of treatment and there is a potential in determining the timing of stimulations based on the brain's spontaneous activity.

Résumé

A la suite d'une lésion cérébrale sévère ayant mené à un coma, il est possible de présenter une altération prolongée de la conscience. Ces états de conscience altérée (ECA) comprennent le syndrome d'éveil non-répondant (ENR) ; un état d'éveil avec la présence de comportements réflexes uniquement, et l'état de conscience minimale (ECM) ; où l'on peut observer des signes fluctuants, mais reproductibles de conscience. La capacité d'utiliser des objets ou de communiquer de manière fonctionnelle marque alors l'émergence de l'ECM (EECM). La prise en charge des patients en ECA représente un défi médical d'un point de vue à la fois du diagnostic et des perspectives de traitement. Étant donné l'impossibilité de compte-rendu personnel, les déficits cognitifs et moteurs associés à l'atteinte cérébrale, ainsi que les fluctuations de vigilance qui caractérisent ces patients, le taux d'erreur diagnostique peut atteindre 40%, avec des conséquences dramatiques sur leur prise en charge. De plus, les options thérapeutiques visant à augmenter leur niveau de conscience et améliorer leur statut fonctionnel manquent et sont peu investiguées. Cette thèse avait dès lors deux objectifs : i) mieux caractériser la récupération comportementale (première partie) et ii) explorer l'usage de la stimulation cérébrale non-invasive, et plus particulièrement la stimulation transcrânienne à courant continu (ou tDCS) et ses différents modes d'application, comme option de traitement (seconde partie).

Dans la **première partie**, il est question de deux études rétrospectives qui ont été réalisées en centre de rééducation spécialisé. Nous avons utilisé des évaluations répétées avec l'échelle de récupération du coma (CRS-R) – la mesure étalon pour l'évaluation comportementale des patients en ECA à l'heure actuelle – afin de déterminer la première **transition depuis des états inconscients** (c-à-d. coma ou ENR) **vers la récupération de la conscience** (c-à-d. ECM ou EECM). Parmi les 13 signes comportementaux de conscience de la CRS-R, la poursuite visuelle annonçait le plus souvent cette transition. De plus, la durée de récupération de la conscience était d'environ six semaines après la lésion. Nous nous sommes ensuite concentrés sur un comportement spécifique et d'un grand intérêt clinique qu'est la **récupération de la communication** ; très attendue par les proches et les soignants, car elle améliore de manière substantielle les interactions et les soins. Sur la période des huit semaines d'observation, la capacité de répondre à certaines questions fermées (c-à-d. la communication intentionnelle) était récupérée endéans 40 jours après la lésion tandis que la capacité de répondre correctement à au moins six questions fermées (c-à-d. la communication fonctionnelle) était récupérée environ neuf jours plus tard.

Dans la **seconde partie**, quatre études sont développées : une étude pilote, deux essais randomisés contrôlés et un protocole, avec l'objectif de répondre aux questions suivantes concernant l'utilisation de la tDCS comme outil thérapeutique : Dans quel environnement stimuler ? Où ? À quel moment ?

Quel environnement – Dans un essai randomisé contrôlé de faisabilité et d'efficacité, nous avons étudié les effets de la tDCS du cortex préfrontal gauche, **prodiguée à domicile** pour une période

prolongée de 20 jours, chez des patients en ECM chronique à la suite d'une lésion traumatique ou non. Il y avait un effet comportemental significatif du traitement au niveau du groupe, pour autant qu'au moins 80% des sessions prévues étaient effectivement administrées.

Où – La première étude pilote a permis d'étudier les effets d'une session de tDCS du **cortex moteur** chez dix patients en ENR et ECM à la suite de lésions traumatiques ou non-traumatiques. Nous n'avons pas identifié un effet comportemental du traitement au niveau du groupe. Au niveau individuel, en revanche, deux patients ont répondu à la tDCS en montrant un nouveau signe de conscience pour la première fois après la stimulation active et non placebo. Ensuite, dans un essai randomisé contrôlé incluant 46 patients en ENR, ECM ou EECM, à la suite d'une lésion traumatique ou non, nous avons utilisé la tDCS multifocale afin de stimuler le **réseau frontopariétal**, également connu comme étant le réseau de la conscience externe. À nouveau, aucun effet comportemental du traitement n'a été identifié au niveau du groupe, alors qu'au niveau individuel, sept patients ont répondu positivement à la tDCS. En revanche, sept autres patients ont répondu négativement à la stimulation en perdant un signe de conscience qui était présent auparavant. Ces patients se démarquaient électrophysiologiquement par une complexité du signal EEG significativement plus élevée dans la bande thêta.

Quand – Finalement, nous avons développé un nouveau protocole utilisant un mode d'**application dépendant de l'état cérébral**, dans un modèle en boucle fermée. En se fondant sur des marqueurs entropiques de vigilance identifiés par électroencéphalographie, nous allons comparer les effets comportementaux et électrophysiologiques de la tDCS appliquée à

un niveau de vigilance élevé ou faible, en émettant l'hypothèse que cette approche aura un impact significatif sur la réponse individuelle à la tDCS.

Globalement, ces résultats montrent que les patients en ECA ont un bon potentiel de récupération au stade subaigu de leur lésion et qu'un faux désespoir devrait être évité lors de la prise en charge initiale. Ces patients peuvent bénéficier de la tDCS ; elle a démontré son efficacité lorsqu'elle est appliquée au niveau du cortex préfrontal, et ce lors de sessions répétées, et il est également sécuritaire d'impliquer activement les soignants et les proches dans cette approche thérapeutique. Enfin, déterminer le moment d'application en se basant sur l'activité cérébrale spontanée semble prometteur.

Abbreviations

AE	Adverse Event
CMD	Cognitive Motor Dissociation
CRS-R	Coma Recovery Scale-Revised
DBS	Deep Brain Stimulation
DLPFC	Dorsolateral Prefrontal Cortex
DRS	Disability Rating Scale
DOC	Disorders of Consciousness
DOCS	Disorders of Consciousness Scale
EEG	Electroencephalography
EMCS	Emergence from the Minimally Conscious State
ES	Effect Size
FC	Functional communication
FDG-PET	Fluorodesoxyglucose positron emission tomography
fMRI	Functional Magnetic Resonance Imaging
FOUR	Full Outline of UnResponsiveness
GCS	Glasgow Coma Scale
GLS	Glasgow-Liège Scale
GPI	Globus Pallidus Interna
IC	Intentional Communication
IQR	Interquartile Range
ITB	Intrathecal Baclofen
IQBA	Individualized Quantitative Behavioral Assessment
LTD	Long-Term Depression
LTP	Long-Term Potentiation
M1	Primary Motor Cortex

MAS	Modified Ashworth Scale
MCS	Minimally Conscious State
mITT	Modified Intention to Treat
MRI	Magnetic Resonance Imaging
MTS	Modified Tardieu Scale
NCS-R	Nociception Coma Scale-Revised
NMDA	N-methyl-D-aspartate
PCI	Perturbational Complexity Index
POC	Percentage of Change
PP	Per Protocol
REDCap	Research Electronic Data Capture
REM sleep	Rapid Eye Movement sleep
rTMS	Repeated Transcranial Magnetic Stimulation
SCS	Spinal Cord Stimulation
TBI	Traumatic Brain Injury
tACS	Transcranial Alternating Current Stimulation
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation
tRNS	Transcranial Random Noise Stimulation
UMN	Upper Motor Neuron
UWS	Unresponsive Wakefulness Syndrome
VNS	Vagal Nerve Stimulation
VS	Vegetative State
WHIM	Wessex Head Injury Matrix

1. Introduction

"*Consciousness is only the surface of the mental ocean*."

Swami Vivekananda

The present section is based on the following articles:

Martens, G., Deltombe, T., Foidart-Dessalle, M., Laureys, S., & Thibaut, A. (2018). Clinical and electrophysiological investigation of spastic muscle overactivity in patients with disorders of consciousness following severe brain injury. *Clinical Neurophysiology*, 130(2), 207-213.

Martens, G., Laureys, S., & Thibaut, A. (2017). Spasticity management in disorders of consciousness. *Brain sciences*, 7(12), 162.

Martens, G., Foidart-Dessalle, M., Laureys, S., & Thibaut, A. (2018). How Does Spasticity Affect Patients with Disorders of Consciousness? In *Coma and Disorders of Consciousness* (pp. 119-135). Springer, Cham.

Bodien, Y., **Martens, G.**, Ostrow, J., Sheau, K., Giacino, J. (2019). Cognitive Impairment, Clinical Symptoms and Functional Disability in Patients Emerging from the Minimally Conscious State. *NeuroRehabilitation*. In press

Bonin, E., **Martens, G.**, Cassol, H., Chatelle, C., Laureys, S., & Thibaut, A. PET Imaging in Altered States of Consciousness: Coma, Sleep, and Hypnosis. In *PET and SPECT in Neurology*. In press

1.1. Consciousness and its altered states

Defining consciousness has been a matter of debate for a very long time, both within and beyond the scope of neurosciences. Originally, John Locke proposed a definition related to the modern concept of consciousness in 1690 as “the perception of what passes in a man’s own mind” (Locke, 1841). Since then, definitions have flourished, borrowing alternately concepts from philosophical, psychological or religious fields. The clinical approach, widely used by neuroscientists, researchers and care practitioners, uses two main components to define different levels of consciousness: arousal and awareness. Arousal means wakefulness while awareness refers to the content of consciousness (Plum and Posner, 1972; Laureys et al., 2002). A natural conscious experience is therefore characterized by maximum levels of both arousal and awareness: the person is awake in the sense that the eyes are spontaneously open; the person is also aware in the sense that he/she is mindful of his/her own environment. While one could think a person needs to be awake in order to be aware, lucid dreaming or Rapid Eye Movement sleep (REM sleep) are conditions that prove the contrary. This example shows that various combinations of high or low levels of wakefulness and awareness are possible and that a continuum of modified states of consciousness exists. Figure 1 is a commonly used two-dimensional representation of this continuum using the two axes (i.e., arousal and awareness) to integrate these modified states of consciousness. Besides different stages of sleep or the medical intervention of general anesthesia, this diagram includes a clinical population of interest: patients with disorders of consciousness

(DOC). DOC include the unresponsive wakefulness syndrome (UWS; previously coined as 'vegetative state' – VS – (Laureys et al., 2010)) and the minimally conscious state (MCS); later subcategorized between MCS *plus* and *minus* (Bruno et al., 2011).

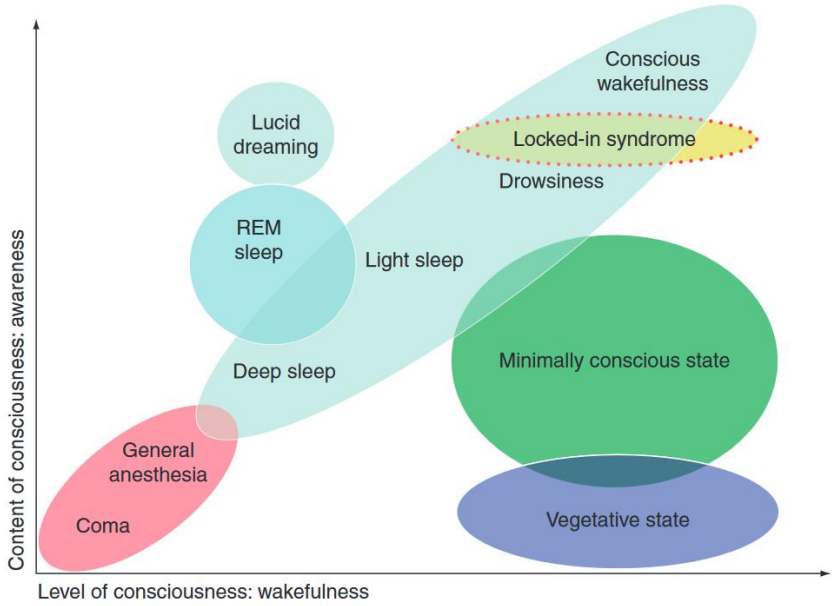


Figure 1 – Simplified diagram of the two main components of consciousness: the level of consciousness (i.e., arousal or wakefulness) and the content of consciousness (i.e., awareness of the experience). From *Laureys S (2009) Coma. Encyclopedia of Neuroscience 2: 1133-1142*. With the authors' permission.

DOC usually occur after a severe brain injury leading to coma. The etiologies vary from hypoxic/ischemic incidents, traumatic brain

injury, or infection; but are commonly subdivided into non-traumatic and traumatic brain injuries (TBI).

In the **comatose state**, the patient is completely unresponsive to the environment, as assessed at the bedside by the sustained absence of eye-opening, even in response to painful stimulation (Plum and Posner, 1972). This is due to the alteration of the arousal systems (reticular activation system) located in the brainstem, the basal ganglia and projecting up to cortical areas. As opposed to other states of transient unconsciousness (e.g., concussion, syncope), coma means the period of unconsciousness lasts for at least one hour. Similarly, in contrast to popular beliefs, the coma period does not extend over four weeks. Indeed, it then evolves either to brain death, to UWS or to MCS. In **brain death**, vital functions such as respiration, homeostatic regulation or cardiac function are irreversibly down (Bernat, 1998). The diagnosis is made within six to 24 hours post-injury (Barclay, 1981). For the UWS, the first formal definition brings us back to 1972, when Jennett and Plum introduced the "**Persistent Vegetative State**" (PVS) to define patients who awoke from coma (i.e., opened their eyes) but were unable to show responses other than reflexive (e.g., withdrawal to painful stimulation) (Jennett and Plum, 1972). They proposed the term vegetative with reference to the preservation of autonomic nervous functions (e.g., sleep-wake cycles, digestion, respiration) and are insured by the vagus nerve. The authors also remind the term vegetative suggested relates to the Oxford English Dictionary definition of "to vegetate"; "to live a merely physical life, devoid of intellectual activity or social intercourse" (1740) and of "vegetative"; "an organic body capable of growth and development but devoid of sensation and thought" (1764). The term "persistent" was introduced

in opposition to the existing terms around that period that had an irrecoverable connotation such as “permanent” or “irreversible”. Later on, in 1994, the US Multi-Society Task Force on PVS extended the medical description of these patients by describing seven diagnostic criteria: (1) no evidence of self or environmental awareness; (2) no evidence of voluntary responses to external stimuli; (3) no evidence of language abilities; (4) intermittent wakefulness; (5) preservation of autonomic functions; (6) incontinence and; (7) variable preservation of cranial-nerve and spinal reflexes (The Multi-Society Task Force on PVS, 1994a). They also described, for the first time, a time frame for consciousness recovery based on prospective data collected on more than 750 post-comatose patients. According to their statement, recovery of consciousness after a non-traumatic brain injury (non-TBI) is unlikely after three months while after a TBI, this period extends to a year (The Multi-Society Task Force on PVS, 1994b).

While the term vegetative was initially chosen to describe preservation of vegetative functions, it appeared later on that an increasing number of care practitioners felt uncomfortable using that terminology, partly given the pejorative connotation perceived by most of the lay public and the media. Indeed, the ongoing confusion with the concept of vegetable felt unacceptable for many medical and scientific authors as well as social, political and religious groups. The fact that patients could be incorrectly referred to as vegetable-like was perceived as a violation of their right to be considered as human beings. To respond to the need for a new name, the European Task Force on Disorders of Consciousness presented the **Unresponsive Wakefulness Syndrome** (UWS): a neutral descriptive term depicting patients showing numerous clinical symptoms (hence

the use of syndrome) of wakefulness without responsiveness (i.e., the inability to show purposeful behaviors or command following while being awake as shown by spontaneous or induced eye opening) (Laureys et al., 2010).

The Minimally Conscious State (MCS) was in turn introduced in 1997 as a replacement for the term Minimally Responsive State (Giacino et al., 1997) to better discriminate patients lacking any sign of consciousness (i.e., coma and UWS) from those presenting with some preservation of conscious awareness. However, further diagnostic criteria were needed: they were released in 2002 and based on an expert panel consensus (the Aspen Neurobehavioral Conference Workgroup) (Giacino et al., 2002). The following behavioral features were described to diagnose MCS: following simple commands, ability to gesture or verbalize yes/no responses (regardless of accuracy), intelligible verbalization, and showing purposeful behaviors occurring following relevant stimuli that are distinguishable from reflexive responses (e.g., appropriate smiling or crying, reaching for objects and/or using them, eye pursuit or fixation to moving stimuli).

Once the criteria were well established and applied, it appeared that the MCS clinical entity was quite heterogeneous in terms of range of behaviors observed. Therefore, it was later subdivided into two clinical entities: the *MCS plus* (MCS+) and the *MCS minus* (MCS-). Initially, Bruno and colleagues defined MCS+ based on the presence of either command following, intelligible verbalization or intentional communication while MCS- included the rest of the MCS features, such as automatic movement, object manipulation or visual pursuit (Bruno et al., 2011). This initial dichotomous classification was based on the level of complexity of

the observed behaviors. Later neuroimaging studies assessed the difference between MCS+ and MCS-, using however a different classification. Indeed, another inaugural study led by Bruno et al used [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET) and functional magnetic resonance imaging (fMRI) to assess cerebral glucose metabolism and functional connectivity in patients in MCS, but they characterized MCS+ by the presence of command following only. This study showed a significantly greater preservation of metabolic and functional activity (at rest) in the language network (i.e., Broca's and Wernicke's regions, left premotor, left caudate and post- and precentral cortices) of patients behaviorally diagnosed as MCS+, as compared to the MCS- ones (Bruno et al., 2012). Later neuroimaging studies however relied on the first criteria proposed by Bruno et al (i.e., command following, intelligible verbalization and/or intentional communication) and showed similar results (Zheng et al., 2017; Aubinet et al., 2018). There seems to be a lack of consensus about the exact diagnostic criteria for MCS+, as confirmed by authors using either their own criteria (e.g., object recognition, command-following and intelligible verbalization (Schnakers et al., 2015)) or simply generally referring to "preserved language functions" or "high-level behavioral interactions" (Estraneo et al., 2016; Guldenmund et al., 2016). As it was the case for the definition of MCS before 2002, standardized consensus-based criteria still need to be set up.

Like the vegetative state, the term minimally conscious is currently subject to some debate as it represents a too large and heterogeneous set of states. The term "minimally" raises confusion, notably for caregivers and relatives and can also be seen as pejorative. On the contrary, the "conscious state" is usually perceived

as positive, assuming the patient is conscious and thereby able to self-report which is contradictory with the typical clinical picture. For these and other reasons, Naccache suggested to reframe this definition and proposed the term 'cortically mediated state'. This terminology is mainly based on the CRS-R, in which signs of MCS actually depict cortically mediated behaviors (Naccache, 2018). While a consensus still needs to be reached on this interesting reconceptualization, the present work will continue to use the term MCS, as it is also encouraged by recently updated guidelines from both American and European Academies of Neurology (Giacino et al., 2018b; Kondziella et al., 2020).

When the patient regains the ability to show some even more complex behaviors such as functional communication (i.e., the ability to answer correctly six out of six situational questions) or functional use of objects (i.e., the ability to appropriately use two different objects), he is considered to have emerged from the MCS (Giacino et al., 2002). **Emergence from the MCS** (EMCS) lies therefore between the MCS and the fully conscious state but the precise boundary is ill-defined. Recent research emphasizes, however, the important disability and the cognitive alterations (in orientation, memory and attention) associated with the EMCS state, that are similar with the acute confusional state (Bodien et al., 2019). To sum up, after a severe brain injury leading to a coma, a patient can evolve either to brain death or to VS or MCS. From there, the path to full recovery passes by the EMCS and severe disabilities, as shown in Figure 2.

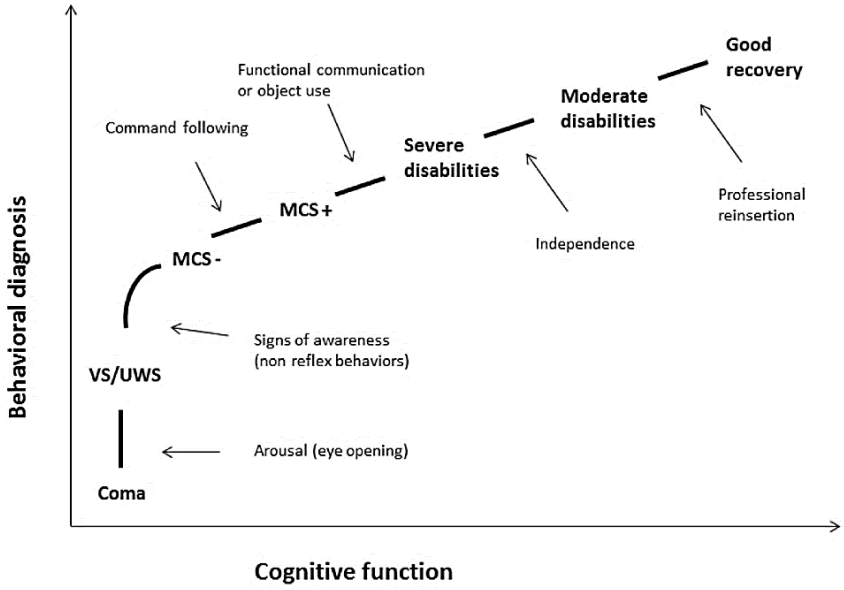


Figure 2 – Path to recovery from coma with gradually increasing levels of behavioral and cognitive output. From (Chatelle and Laureys, 2011), with the authors' permission.

However, a full recovery is never guaranteed. This progression path is not fixed in the sense that a patient can evolve from UWS to MCS and then decline back to UWS. Not only the evolution after brain injury is a highly dynamical situation, but also these patients are known to present fluctuation of their state over time, in different time scales (from one hour to another as well as from one day to another or a week). This makes diagnosing them a challenging task for clinicians.

From an epidemiological perspective, patients with DOC present a low prevalence and fall within the definition of a rare disease. Overall, UWS prevalence estimation varies from 0.2 to 6.1 patients per 100 000 individuals as reported by a systematic review including data from Europe, Asia and the USA (van Erp et al., 2014). Most of the studies retrieved in this review however predated the clinical definition of MCS (Giacino et al., 2002) and thereby most probably merge UWS and MCS cases. Another review focusing on the USA reports prevalence estimates ranging between 1.78 and 15 per 100 000 individuals for UWS and 40 and 99 per 100 000 individuals for MCS (Giacino et al., 2018a). Given the local economic and health insurance factors, these figures are presumably underestimated as many patients are not tracked in the healthcare system. In Belgium, initial censuses from the Health Federal Public Service report 359 UWS and MCS patients in 2004 and an average of 250 new cases yearly. It is difficult to obtain the exact prevalence because of the heterogeneity of the diagnostic criteria applied, especially regarding the MCS.

As DOC classically occur following a severe brain injury, it is relevant to know the incidence of DOC in patients who sustained such injury. Again data is lacking but a Norwegian study focused on prospectively computing the rate of DOC in adults after a TBI using the CRS-R. They found that 2% of patient admitted with TBI remain in UWS or MCS three months after injury and that this rate was reduced by half after one year (Løvstad et al., 2014). All these studies acknowledge that epidemiological data for DOC is difficult to accurately collect, mainly because of methodological flaws in existing publications and the challenge of accurately diagnosing these patients.

1.2. Diagnosing disorders of consciousness

Obtaining an accurate diagnosis has tremendous implications regarding several aspects. First of all, from an ethical perspective, diagnosing a coma from an UWS or a MCS will have consequences regarding end-of-life decisions, since the prognosis differs for each state. Furthermore, clinicians' opinion toward treatment withdrawal is not the same for UWS and MCS: they tend indeed to remove treatment less frequently in MCS (Demertzi et al., 2011). Second, the pain management may vary as patients in UWS and MCS do not process painful stimuli in similar ways. Neuroimaging studies showed a cortical activation in MCS patients following a painful stimulus that is similar to healthy controls, while UWS patients showed way less activation (Laureys et al., 2002; Boly et al., 2005). To obtain a diagnosis regarding the level of consciousness of these patients several tools are available. We can distinguish the diagnosis performed at the bedside, where a clinician will obtain behavioral information; and the complimentary diagnostic information provided by neuroimaging, where the clinician will obtain some additional data, but often delayed due to the demanding processing the collected signals require.

1.2.1. At the bedside

Naccache accurately described the "bipedal approach" of the neurologist when evaluating a patient presenting neurological

symptoms (Naccache, 2018). The first component of this approach is the so-called “behaviorist foot”, which consists in the observation of spontaneous and elicited behaviors (e.g., reflex testing). The second one, the “psychologist foot”, concerns the collection of patient’s subjective reports (e.g., symptoms description). By combining both approaches, the neurologist maximizes his chances to establish the correct diagnosis. However, as mentioned is his work, when working with patients who have trouble understanding and communicating (e.g., dementia, newborn babies), we cannot rely on the patient’s subjective report and have to focus on the objective assessment approach alone. This includes of course DOC patients, for whom the neurologist or clinician aims to assess the level of consciousness they have (UWS, MCS or EMCS), but without subjective report. This makes the situation paradoxical since consciousness itself is defined as the ability to formulate internal thoughts and to report them (e.g., feelings, perception, actions) (Dehaene and Naccache, 2001). To help the “one-legged” neurologist making the most of his behaviorist foot, standardized behavioral scales were developed to evaluate the level of consciousness in DOC patients. Initially, the **Glasgow Coma Scale** (GCS) was developed to “assess the depth and duration of impaired consciousness and coma” (Teasdale and Jennett, 1974). It consists of three subscales assessing motor responsiveness, verbal output and eye opening and can be performed in a couple of minutes. The total score ranges from 3 (deep coma) to 15 (normal consciousness). Later in the 1990s, a new generation of scales designed specifically for DOC patients were developed, with varying levels of structure and standardization. This proliferation of new DOC scales led Seel and colleagues to perform a systematic review in 2010 and to evaluate the diagnostic utility (i.e., differentiating UWS, MCS and EMCS), the

interrater reliability, the validity and the prognostic value of these instruments (Seel et al., 2010). They identified 13 different assessment scales for DOC and rated seven aspects based on expert consensus between paired reviewers: (1) standardized administration and scoring guidelines; (2) content validity; (3) reliability (i.e., internal consistency, interrater reliability, test-retest reliability); (4) criterion validity; (5) construct validity; (6) diagnostic validity and; (7) prognostic validity. The experts' ratings ranged from unacceptable to excellent. The scale that best survived the ranking is the **Coma Recovery Scale-Revised** (CRS-R) (Giacino et al., 2004), given its excellent content validity, acceptable standardized administration and scoring procedures, as well as good to excellent reliability. Its criterion validity is however unproven. It was also the only scale recommended for use to assess patients with DOC with minor reservations. Other scales such as the Sensory Modality Assessment and Rehabilitation Technique (SMART) (Gill-Thwaites and Munday, 1999), the Wessex Head Injury Matrix (WHIM) (Shiel et al., 2000) or the Disorders of Consciousness Scale (DOCS) (Pape et al., 2005) were recommended with moderate reservations, while some were simply not recommended (e.g., Full Outline of UnResponsiveness; FOUR (Wijdicks et al., 2005), Glasgow-Liège Scale; GLS (Born, 1988)).

The CRS-R presented by Giacino and colleagues in 2004 is therefore currently the gold standard for behavioral assessment of patients with DOC. It consists in 23 items, hierarchically organized within six different subscales. Every subscale is designed to interrogate a function: auditory, visual, motor, oromotor/verbal, communication and arousal. The list of the items and their administrating procedures can be found in Appendix 1. Within each

subscale, the items range from high-level cortically mediated behaviors (e.g., response to command) to lower-level reflexive movements (e.g., auditory startle). The use of the CRS-R requires some training, but is an essential tool for clinicians and researchers working with patients with DOC. Since its development, there has been ongoing research on how to best use the administration guidelines. For instance, the CRS-R recommends to use a "a brightly colored or illuminated object" to assess visual fixation, but Di and colleagues showed that using a mirror is the most efficient, as compared to a ball or a light (Di et al., 2014). Likewise, using the patient's own name to evaluate localization to sound, is more efficient than using a meaningless sound such as a ringing bell, given the high saliency of the personal stimulus (Cheng et al., 2013). In the same vein, using patient's preferred objects to evaluate functional object use (e.g., cigarette or paper instead of the comb or cup recommended by the CRS-R) elicits more responses in patients in EMCS (Sun et al., 2018). Finally, a key study regarding CRS-R evaluation in DOC patients concerns the repetition of the assessments to tackle the behavioral fluctuation, that is a well-known feature of this population (Sherer et al., 2005; Candelieri et al., 2011; Piarulli et al., 2016). Wannez and colleagues indeed showed that the number of consecutive assessments had a significant impact on the clinical diagnosis: up to the fourth evaluation, the risk of misdiagnosis is still 17%, while the risk is reduced to 10% at the fifth assessment and onwards (with no significant difference anymore between misdiagnosis rates). The authors therefore recommend performing at least five CRS-R assessments in DOC patients to obtain an accurate diagnosis (Wannez et al., 2017b).

1.2.2. Using neuroimaging

Neuroimaging methods allow for structural, metabolic and electrophysiological investigation of the brain and may complement the behavioral diagnosis. This is of particular interest in specific cases where executive functions are impaired and/or the deficit in motor abilities prevent the patient to show any behavioral response while having, at least partly, preserved signs of consciousness. This situation, coined cognitive motor dissociation (CMD) or covert consciousness (Laureys and Schiff, 2012; Fernández-Espejo et al., 2015), can raise the rate of misdiagnosis up to 32% (Stender et al., 2014). Therefore, the role of neuroimaging to detect responses invisible at the bedside is of paramount importance. **Magnetic resonance imaging** (MRI) uses strong magnetic fields to form tridimensional representations of the brain's structure. It is therefore widely used in clinical settings to objectify swelling, bleeding and other injury processes concerning white and grey matter in patients with brain injury (Kampfl et al., 1998; Giacino et al., 2014). For white matter specifically, **diffusion tensor imaging** (DTI) uses the diffusion of water molecules to reveal the structural integrity of axon tracts in the brain and thereby map white matter tracts. The decrease in water diffusion would reflect a diminished myelination of white matter and is negatively correlated with functional outcome (Newcombe et al., 2011).

Functional MRI (fMRI) uses the blood oxygen level to monitor neuronal activity and therefore enables measurement of cerebral processes with a high temporal resolution. At rest,

spontaneous blood oxygen level fluctuations allow to assess functional connectivity between regions of interest. Within the default mode network, for instance, the connectivity decreases with the level of consciousness and can discriminate between conscious, MCS, UWS and comatose patients (Demertzi et al., 2015; Di Perri et al., 2016). In so-called active paradigms, cortical activation can be measured with fMRI following application of visual, auditory and/or somatosensory stimuli and is known to encompass associative cortices in patients in MCS, similar to what is observed in healthy controls (Di et al., 2008). While patients in UWS show, in contrast, activation only in primary sensory areas following these stimuli (Di et al., 2007). fMRI is particularly interesting in the detection of CMD and could even, in some cases, be used to establish communication using « yes-no » active paradigms (Monti et al., 2010).

From a metabolic standpoint, **FDG-PET** studies the glucose consumption of cerebral areas and can therefore quantify the global and regional brain metabolism, based on ^{18}FDG uptake. In patients in UWS, the decrease in global brain metabolism can go up to 40% as compared to healthy controls (Laureys, 2005). As opposed to previous beliefs, however, global brain metabolism does not accurately discriminate between conscious and unconscious states. Some areas are indeed more crucial than others in consciousness recovery processes (i.e., frontoparietal network, posterior parietal cortex and anterior cingulate cortices (Nakayama et al., 2006; Silva et al., 2010)). Diagnostic-wise, FDG-PET seems to present better sensitivity and agreement with behavioral diagnosis as compared to fMRI (Stender et al., 2014).

Recently, emphasis has been placed on more affordable, user-friendly bedside neuroimaging tools such as

electroencephalography (EEG). High-density resting-state EEG (256 channels) derived metrics, for instance, are able to accurately discriminate between UWS, MCS and EMCS (Chennu et al., 2017) and event present prognostic value. Indeed, patients with high connectivity (i.e., strong connections between different cortical areas) in the delta frequency band (slow wave activity) tend to have a negative outcome at one year, meaning dead or chronic UWS. In contrast, patients with positive outcomes (i.e., severe disability up to good recovery) had lower delta connectivity (Chennu et al., 2017). Low-density clinical EEG (19 channels) also confirm poor CRS-R outcome when important delta activity is observed and better outcome when alpha rhythms are present (Bagnato et al., 2015). Beyond resting-state, recording the EEG brain response when exposed to external perturbation (e.g., transcranial magnetic stimulation – TMS) can also contribute to characterize patients' levels of consciousness. Again, patients in UWS and in MCS present different type of responses to the magnetic trigger: a stereotypical slow wave that mostly remains local and short lasting for the first; a widespread, differentiated and long lasting wave for the second (Rosanova et al., 2012). The **perturbational complexity index** (PCI) allows to quantify these patterns, by calculating the spatial and temporal brain responses to the TMS perturbation. PCI successfully differentiates between conscious and unconscious states, with a clear-cut difference at the individual level (Casali et al., 2013; Casarotto et al., 2016). The calculation of the PCI uses a specific lossless data compression algorithm based on providing an upper bound to the data compression ratio: the Lempel-Ziv compression (Ziv and Lempel, 1978). The range of sensitive techniques complementing the bedside behavioral diagnosis is thus wide (Figure

3). But we should keep in view the limits inherent to their use, including poor affordability, high expertise required for both acquisition and analysis of the signals as well as the probability of false-negative/positive findings.

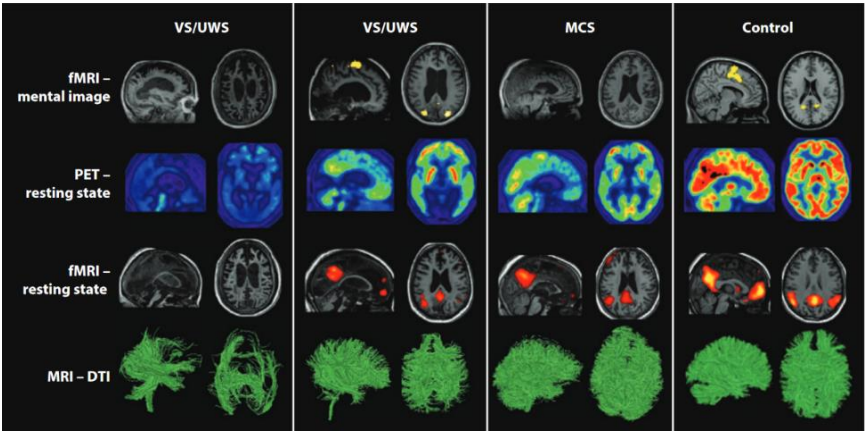


Figure 3 – Illustrative summary of some of the neuroimaging techniques available for DOC patients across different diagnoses. Brain activity typically decreases with the level of consciousness. From (Gosseries et al., 2014), with the authors' permission.

1.3. Clinical management of disorders of consciousness

1.3.1. Active treatment

The field of treatment options for patients with DOC suffers from a scarcity of evidence due to the small amount of studies and/or the low class of evidence they provide. To date, both pharmacological and non-pharmacological options haven been investigated in either randomized controlled trials, open-label and/or case report studies. The aim of these studies is to improve patients' level of consciousness and functional recovery, while understanding the cerebral mechanisms of the interventions.

1.3.1.1. Pharmacological treatments

Only one pharmacological agent has been investigated in a large randomized controlled trial providing class II evidence: **amantadine hydrochloride**. Initially used as an antiviral agent and in order to treat Parkinson, it acts as a dopamine agonist and N-methyl-D-aspartate (NMDA) antagonist (Peeters et al., 2002). Its efficacy for DOC patients has been shown in subacute (4 – 16 weeks after injury) patients with TBI; as compared to the placebo arm, the amantadine arm had a significantly faster recovery over 4-week treatment and 2-week follow-up, as measured by the Disability Rating Scale (DRS) and the CRS-R (Giacino et al., 2012).

Zolpidem is a GABA-agonist hypnotic agent that occasionally induces paradoxical responses in patient with DOC. Dramatic, yet

transient, improvements have been described (e.g., recovery of functional communication, reading abilities) but the rate of responders is extremely low. A double-blind crossover randomized controlled trial performed with 84 patients with DOC reported 5% (n=4) of responders only (Whyte et al., 2014). A higher proportion (20%) was described in a placebo-controlled trial that included 60 patients with DOC; improvements included recovery of response to command or objet localization (Thonnard et al., 2014). Further studies are needed to determine why some patients respond so well and others do not.

An increasing interest has been growing around **apomorphine** as a new treatment option. This non-selective dopamine agonist has a relatively short half-life (30 – 90 minutes (Kolls and Stacy, 2006)) and it is therefore recommended to administer it continuously through a subcutaneous pump (Katzenschlager et al., 2005). For DOC patients, one case report and one prospective open-label trial (including 8 patients) performed by the same team reported fast and nearly complete cognitive and functional recovery, with enduring effects after the end of the 3-month treatment period (Fridman et al., 2009, 2010). Given the promising results, proper randomized controlled trials are on their way (Sanz et al., 2018). Other drugs such as levodopa or midazolam have been investigated but in case reports only (Carboncini et al., 2014; Herrold et al., 2014), larger randomized controlled trials are needed to confirm their effects.

To sum up, some drugs appear somewhat efficient for treating patients with DOC. However, as for many pharmacological agents, some undesired side effects can be observed (e.g., drowsiness, emesis), the rate of response is inconsistent, and the

habituation effect hinders the clinical efficacy. Therefore, non-pharmacological interventions have been investigated as well.

1.3.1.2. Invasive brain stimulation

Deep brain stimulation (DBS) is probably one of the most famous interventions in this category following the renowned case report of Schiff and colleagues in 2007 (Schiff et al., 2007). This team surgically implanted electrodes targeting the intralaminar nuclei of the thalamus in a patient who was in MCS for six years after a TBI and evaluated the effects using the CRS-R, in a double-blind alternating crossover fashion (stimulator turned on and off every 30 days for 6 months). When the stimulator was on, important clinical improvements were observed, such as consistent response to command, functional communication and oral feeding. The clinical state of the patient decreased when the stimulator was off and statistical logistic regression modelling showed a significant link between the improvements and the stimulation. Two other prospective open-label studies were conducted in a total of 19 patients with DOC and reported moderate clinical improvements (CRS-R score increase of 1-3 points) (Magrassi et al., 2016) and 29% of responders (i.e., emergence from MCS, response to command recovery), respectively (Chudy et al., 2018). This appears encouraging but several challenges are reported too, such as poor enrollment due to strict inclusion criteria, scalp infection and legal issues. Besides, no sham-controlled trial is available yet.

Spinal cord stimulation (SCS) is classically used to treat refractory neuropathic pain by masking pain signals before they

reach the brain. It modulates the spinal neurons' excitability and firing rate toward excitation or inhibition depending on the frequencies used (Yampolsky et al., 2012). Aiming at reaching cortical neural networks, SCS has been investigated for patients with DOC too. To date however, only two sham-controlled studies using SCS in DOC were performed by the same team, and neither of them measured behavioral changes after stimulation; they focused on EEG only. The first one was performed on 11 MCS patients and found altered band power and synchronization in delta and gamma bands after active and not sham SCS session. The authors suggest SCS does modulate brain function, particularly the frontal region, in MCS patients through thalamo-cortical connections including the reticular formation (Bai et al., 2017b). In the second one, using a similar design with 16 MCS patients, they focused on frontal connectivity in the gamma band (high frequency; 30 – 45 Hz) and showed decreased connectivity in the frontal regions after active stimulation (Bai et al., 2017c). These findings thus pertain to the mechanisms of SCS (i.e., alteration of thalamo-cortical connections via the frontal cortex) more than its potential therapeutic use.

Vagal nerve stimulation (VNS) is another neuromodulation technique that can be applied invasively (surgical implantation) or not. To date, however, only one case report of invasive VNS performed with a chronic (15 years) UWS patient is available and showed diagnostic improvement to MCS (Corazzol et al., 2017). Stimulating the vagal nerve would induce compensatory responses from the central thalamus and hypothalamus to distal fronto-parietal and striatal networks through basal forebrain or brainstem projections. VNS can also be administered in a non-invasive way through afferent branches located in the ear concha. Again, a single

case report is the only mention of using this technique in another chronic (3 months) UWS patient who showed new motor and oromotor signs of consciousness and thereby evolved to MCS within the next few weeks (Yu et al., 2017).

1.3.1.3. Non-invasive brain stimulation

The most widely studied method with DOC patients certainly is **transcranial direct current stimulation** (tDCS). tDCS is a non-invasive neuromodulation technique using weak electrical currents (1-2 mA) applied on the scalp to modify the excitability of targeted cortical areas, with stimulation durations classically varying between five to 40 minutes. The direct current circulating between the anode and the cathode can modulate brain activity and thereby improve the functions underpinned by the stimulated brain area (Stagg and Nitsche, 2011). The direction of the current is responsible for the excitability changes of the neural membrane. Sending current through the anode will indeed induce a slight depolarization which lowers the threshold for membrane depolarization and action potential generation (Purpura and McMurtry, 1965). On the contrary, cathodal stimulation will induce a hyperpolarization of the neural membrane and thus increase the threshold for depolarization and action potential generation. These electrical changes are responsible for the immediate effects of tDCS and are related to ion channels activity. Latest findings in human studies confirm the major contribution of membrane potential alterations over synaptic changes for the direct effects of tDCS. When inactivating voltage-gated ion channels involved in neural membrane depolarization, the

motor effects of tDCS disappear while blocking glutamate receptors and GABA receptor has no effect (Nitsche et al., 2003a; Stagg et al., 2018). The so-called long term effects (lasting a few hours after a single session) involve long-term potentiation (LTP)- and long-term depression (LTD)-like synaptic pathways through glutamatergic synapses and especially NMDA receptors (Nitsche and Paulus, 2001). Blocking NDMA receptors using an antagonist such as dextromethorphan indeed abolish motor tDCS effects as assessed by motor evoked potentials with single-pulse TMS (Liebetanz et al., 2002). On the contrary, using a NMDA agonist such as D-cycloserine prolongs these tDCS effects (Nitsche et al., 2004). These longer-lasting after effects can be enhanced by stronger and longer stimulation (Stagg et al., 2018). One should however keep in view that most of the animal and human studies focused on the motor cortex and that the results should not be directly translated to other areas.

Many other factors further affect the functioning of tDCS: intensity and duration of the applied current, surface of the sponges, underlying neural activity and orientation of the neurons themselves (Stagg and Nitsche, 2011). The physiological effects are indeed optimal when the current flows in the same direction of the neuron, along its axis; as opposed to when the current flows perpendicularly to the neuron orientation and the effects are decreased as demonstrated by *in vivo* and *in vitro* animal studies (Bindman et al., 1964; Bikson et al., 2004). Another important modulator of tDCS effects is the intracellular calcium (Ca^{2+}). Intracellular Ca^{2+} levels control LTP and LTD mechanisms in animal models (Lisman, 2001) and in humans, blocking calcium Ca^{2+} channels inhibits plasticity induced by tDCS (Nitsche et al., 2003a). On top of that, Ca^{2+} signalling

in astrocytes, the most represented glial cell type surrounding neurons, also supports synaptic plasticity and can be modulated by tDCS as shown by recent *in vivo* animal studies (Monai et al., 2016; Monai and Hirase, 2018).

Traditional montages use one anode and one distant cathode (typically sponge electrodes between 25 and 35 cm^2) and it has been reported that only 10 to 50% of the current sent reach the cortex with this kind of setting and thereby only cortical areas could be stimulated (Miranda et al., 2006; Coben and Evans, 2011). However, recent advancements in both montage optimization and modelling challenge this premise. Indeed, with multi-electrode montages amplifying current sources and optimizing configuration directed toward specific targets, deep subcortical structures such as basal ganglia can be reached (Gomez-Tames et al., 2020). This applies especially to areas located close to the ventricles and their cerebro-spinal fluid which has important conductive properties (Huang and Parra, 2019). As a matter of fact, it is important to keep a comfort-efficacy balance and even though multifocal montages allow applying higher amounts of total current (above 4 mA), the intensity should be limited at 1 mA per electrode. This limit allows decreasing the risk of discomfort or burning since these electrodes are usually smaller (3-4 cm^2).

Given its numerous advantages (inexpensive, painless, safe, easily applicable), this tool has been investigated as a therapeutic option for various neurological diseases (Lefaucheur, 2016). It has been shown to improve cognitive functions (i.e., working memory, attention) in Alzheimer's disease (Khedr et al., 2014), Parkinson's disease (Boggio et al., 2006) and stroke (Schlaug et al., 2008), when applied over the left dorsolateral prefrontal cortex (DLPFC). It also

has strong indications for depression (Ferrucci et al., 2009) and chronic pain (Valle et al., 2009) and could thereby even prevent and treat opioid-dependence (Gallucci et al., 2019). For studies aiming at improving cognitive functions, the left DLPFC is the preferential target because most of the functions underpinned by this brain area mainly relate to executive functions including attention and working memory. A majority of tDCS studies performed both in healthy volunteers and pathological populations therefore used stimulation over the left DLPFC. This area integrates a high amount of inputs from associative cortices and is a key component of motor control, planning and behavior (Devinsky and D'Esposito, 2004; Heekeren et al., 2006). The right DLPFC has also an important role to play in arousal and attention (Sturm and Willmes, 2001) but is far less involved in higher level cognitive functions.

For severely brain-injured patients with DOC, tDCS also emerged as a potential candidate in their unsatisfyingly small therapeutic arsenal for severely brain-injured patients with DOC. It could indeed be used to improve the level of consciousness in these patients. To investigate this, Thibaut and colleagues conducted a first of its kind double-blind randomized controlled trial, evaluating the effects of tDCS applied for 20 minutes at 2 mA on the left DLPFC on both acute and chronic patients with disorders of consciousness (UWS or MCS) following severe acquired brain injury (Thibaut et al., 2014). Fifty-five patients were included (with both traumatic and non-traumatic etiologies) and, while at the whole group level the treatment effect (based on the CRS-R total score) was not significant, it was in the MCS subgroup of 30 patients ($p=0.003$, Cohen's effect size; ES =0.38). This was the first study showing that MCS patients appear to respond better to tDCS as compared to UWS patients, with

also a higher proportion (43% against 8%) of tDCS responders (i.e., a patient showing a new sign of consciousness after active stimulation that was never observed before or before/after sham stimulation). This does not mean that UWS patients are not a suitable population to target, since individual responders are identified within this population as well. In a case report, for instance, a chronic UWS patient was able to follow commands only following the application of a tDCS session over the DLPFC (Thibaut et al., 2018a). It simply implies that one would expect a lesser proportion of responders in the UWS population as compared to the MCS population. These inaugural results still paved the way for further trials to favor inclusion of MCS patients and to enhance the duration of tDCS related effects (which are usually transient and vanish within an hour). Several teams therefore opted for increasing the tDCS dose by repeating the amount of sessions received. In another randomized controlled trial, Thibaut and colleagues applied prefrontal tDCS for five consecutive days, stimulating 16 MCS patients (acute and chronic, traumatic and non-traumatic etiologies) daily (Thibaut et al., 2017b). This time, not only significantly greater clinical improvements were observed in favor of the active stimulation after five days ($p=0.013$, Cohen's ES=0.43), but these effects remained up to one week after the end of the stimulation sessions. This study showed that for five of the nine patients who responded to tDCS, the new sign of consciousness appeared after two, three, four days of stimulation, meaning individual response to tDCS cannot be predicted from the application of a single session; at least several sessions are needed. Another team also tested the effects of five days of tDCS in 10 UWS or MCS subacute and chronic patients in a non-randomized controlled fashion. The target area was either the

DLPFC or the primary sensorimotor cortex and the MCS patients behaviorally improved for both montages while the UWS patients did not (Angelakis et al., 2014). The five consecutive tDCS sessions were further tested by another group on 13 patients (7 UWS and 6 MCS) in a double-blind randomized controlled design and enabled clinically relevant behavioral improvements in five patients that were paralleled with enhancement of EEG background (Estraneo et al., 2017). Other studies investigated different areas than the DLPFC but showed less important behavioral effects. For instance, stimulating the posterior parietal cortex for five days targeting the precuneus, that is a critical area for consciousness recovery, in 33 MCS patients showed a significant behavioral improvement at the group level, but with a lesser Cohen's ES (0.31 against 0.43 previously reported) than for the DLPFC as well as fewer responders identified (18% against 56%) (Huang et al., 2017). The orbitofrontal cortex was targeted in another prospective open-label study and did not elicit any clinically relevant behavioral change in 22 patients with DOC (Naro et al., 2015). Finally, Wu et al checked the behavioral and electrophysiological differences between stimulating the left and right DLPFC (controlled with sham conditions), given the role of the right DLPFC in arousal mentioned above, that could be particularly relevant for DOC (Wu et al., 2019). They included 15 patients with DOC (5 in each stimulation group) and showed that left DLPFC stimulation significantly increased EEG functional connectivity between the stimulation site and central and parietal cortices while for right DLPFC stimulation, no such changes were observed and connectivity even tended to decrease. Two patients behaviorally improved by increasing their CRS-R score (2 and 7 points gained, respectively) in the left stimulation group while in the right

stimulation as well as in the sham groups, no behavioral changes were observed.

Based on a recent scoping review (Thibaut et al., 2019b) and an ongoing systematic review (Martens et al., 2019a), a summary of all tDCS randomized controlled trials performed with DOC patients and reporting behavioral outcome (i.e., CRS-R) is presented in Table 1. It clearly appears that tDCS represents a valuable therapeutic option for patients with DOC (ES ranging from 0.31 to 2.22). However, montages targeting other areas than the DLPFC could still be explored and the individual response to tDCS seems variable.

Table 1 – List of randomized controlled trials assessing the behavioral effects of tDCS in patients with DOC, up to March 2019.

Study	tDCS Intervention	Study design	N (etiology)	Diagnosis	Time since injury	Procedure	Results	Effect sizes
(Thibaut et al., 2014)	Prefrontal (DLPFC), 1 session, 20 minutes, 2 mA	Double blind, crossover	55 (25 TBI, 30 non-TBI)	30 MCS 25 UWS	1 week to 19 years	Comparison of a single session (20 minutes) of active and sham stimulation over the left DLPFC, separated by 2 days of washout, with CRS-R before and after tDCS	13/30 patients in MCS and 2/25 patients in UWS clinically improved (recovery of visual pursuit or command following). At the group level, clinical improvement (2 points on the CRS-R) for MCS patients. No side-effects observed.	For MCS (n=30): d=0.38
(Thibaut et al., 2017b)	Prefrontal (DLPFC), 5 sessions, 20 minutes, 2 mA	Double-blind, crossover	16 (11 TBI, 5 non-TBI)	16 MCS	5 months to 30 years	Comparison of 5 sessions of active and sham tDCS (20 minutes a day)	9/16 responders. Clinical improvement (2 points on the CRS-R) maintained up to	After tDCS: d=0.43 ; at 1week follow-

						over the DLPFC, separated by a week of washout. CRS-R performed before, after 5 days of tDCS and at 1-week follow-up	one week after the end of the stimulation. No side-effects observed.	up: d=0.57
(Estraneo et al., 2017)	Prefrontal (DLPFC), 5 sessions, 20 minutes, 2 mA	Double-blind, crossover	13 (1 TBI, 12 non-TBI)	7 UWS 6 MCS	3 months to 7 years	Comparison of 5 days of active and sham stimulation over the DLPFC for 20 minutes a day. EEG and CRS-R performed at baseline, after 5 days of tDCS and up to 3-month follow-up	Behavioral (CRS-R total score) and EEG changes in 5/13 patients (3 in MCS and 2 in UWS). At the group level, no statistical difference between the two groups. No information on side-effects.	/

(Zhang et al., 2017)	Prefrontal (DLPFC), 20 sessions, 20 minutes, 2 mA	Double-blind, parallel	26 (12 TBI, 14 non-TBI)	11 UWS 15 MCS	1 to 18 months	Comparison of 20 sessions of active or sham tDCS over DLPFC for 20 minutes twice a day for 10 days	CRS-R improvement in MCS in the active group, coupled with increase in P300 amplitude. No information on side-effects.	For MCS (n=15): d=2.22
(Huang et al., 2017)	Posterior parietal cortex, 5 sessions, 20 minutes, 2 mA	Double blind, crossover	33 (20 TBI, 13 non-TBI)	33 MCS	5 weeks to 1 year	Comparison of active or sham tDCS applied for 5 days over the PPC (20 minutes a day), separated by a week of washout. CRS-R performed before, after 5 days of tDCS and at 5 days follow-up	9/33 responders. Clinical improvement immediately after the 5 days of active tDCS (1 point on the CRS-R). No effects at 1-week follow-up. No side-effects observed.	After tDCS: d=0.31

(Martens et al., 2018b)	Prefrontal (DLPFC), 20 sessions, 20 minutes, 2 mA	Double-blind, crossover	27 (12 TBI, 15 non-TBI)	27 MCS	10 months to 14 years	Comparison of 20 sessions of active and sham tDCS (20 minutes per day) over DLPFC, separated by 8 weeks. CRS-R before, after 4 weeks of tDCS (20 sessions) and at 8-week follow-up.	No improvement at the group level. For patient group who received at least 80% of the stimulation sessions, increase in CRS-R total scores. No difference between active and sham tDCS at 8-week follow-up. No tDCS related side-effects observed.	Group level: d=0.47; subgroup of patients who received >80% of tDCS sessions: d=0.53.

Effect sizes were taken from the articles when available or calculated (Cohen's effect size – small: d=0.2; medium: d=0.5; large: d=0.8) based on data provided between active and controlled condition when a statistical difference was found (*). "/" refers to no statistical difference between groups. tDCS= transcranial Direct Current Stimulation, DLPFC= DorsoLateral PreFrontal Cortex; TBI= Traumatic Brain Injury, MCS= Minimally Conscious State; UWS= Unresponsive Wakefulness Syndrome; EEG= Electroencephalography; PPC= Posterior Parietal Cortex. Adapted from (Thibaut et al., 2019b), with the author's permission.

Another increasingly investigated non-invasive brain stimulation method is **repeated TMS** (rTMS); that uses an electromagnetic pulse to focally depolarize the neurons and induce firing. As with other brain stimulation methods, it allows for inhibition (low frequency – about 1 Hz) or activation (higher frequencies: 5-20 Hz) of neuronal populations (Thibaut et al., 2019b). The clinical effects seem however less remarkable than for DBS or tDCS, as reported by three different randomized controlled trials totaling 31 patients (21 UWS and 10 MCS) and reporting no behavioral improvement at all (Cincotta et al., 2015; Pisani et al., 2015a; Liu et al., 2016a). Other outcomes however seemed to be influenced by rTMS such as cerebral blood flow velocity (Liu et al., 2016b), EEG slow wave activity and power (Pisani et al., 2015b); that were increased only in MCS patients and not in UWS. On top of that, rTMS is expensive, requires a lot of training and is far from portable. Therefore, its therapeutic interest is limited, as opposed to its diagnostic use.

As a matter of fact, other non-invasive options are available such as VNS mentioned above, transcranial alternating current stimulation (tACS) or transcranial random noise stimulation (tRNS); but there is scarce evidence to date of their efficacy for DOC patients. An illustrative summary of available techniques is presented in Figure 4.

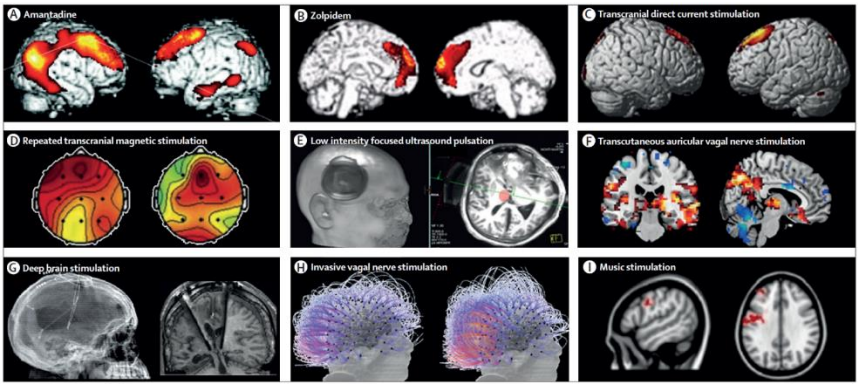


Figure 4 – Therapeutic options and their neuroimaging findings for patients with disorders of consciousness. From (Thibaut et al., 2019b), with the authors' permission.

A common framework for the mechanisms underlying treatment efficacy relies on the **mesocircuit hypothesis**. Initially proposed by Schiff (Schiff, 2010), it describes a model for neural mechanisms of impaired consciousness. It is based on the interactions between the thalamus, the frontal cortex, and the basal ganglia; forming a thalamo-cortical loop that can be affected by cerebral lesions and external therapeutic interventions. As shown in Figure 5, in a healthy brain, the frontal cortical areas activate the thalamus in a direct bidirectional fashion, as well as in an indirect manner through the basal ganglia. The striatum is indeed activated by the frontal cortex and, in turn, inhibits the globus pallidus interna (GPI) that inhibits the thalamus. This double inhibition leads to an excitation of the thalamus and sustains further bidirectional activation between the thalamus and associative fronto-parietal cortices. This cortico-thalamo-cortical loop that drives internal and

external awareness can be impacted in case of a brain injury. The striatal neurons have an important metabolic demand and are thereby particularly sensitive to oxygen deprivation (Grillner et al., 2005; Schiff, 2010). In case of focal traumatic/hemorrhagic or anoxic brain injury, these striatal neurons will be primarily affected and, since they project on the GPI, the GPI will be "free" to inhibit the central thalamus, in turn weakening its activation of the fronto-parietal cortical areas.

This model sheds light on mechanistic effects of several therapeutic interventions mentioned above. Neuromodulation using **tDCS** or non-invasive brain stimulation using **rTMS** allow, for instance, to directly target the frontal cortex and thereby increase the activation over the thalamus, but also over the striatum, with which the frontal cortex has many connections. Indirectly stimulating the striatum might decrease the inhibition over the thalamus and restore the damaged cortico-striato-thalamic loop (Fridman et al., 2014). **Zolpidem**, as a GABAergic agent, preferentially acts on the GPI, which expresses many GABA subunits. Its selective inhibitive action could substitute the normal inhibition from the striatum and unleash the thalamus (Schiff, 2010). **Amantadine**, in turn, would rely on dopaminergic modulation of the associative fronto-temporo-parietal areas, as suggested by a PET case report with a chronic MCS patient. This patient was given amantadine for two times six weeks separated by a 6-week washout period. There was a behavioral improvement when amantadine was on, marked by recovery of response to command and automatic motor responses. PET investigations at baseline, during amantadine and washout showed increased regional metabolism in the temporo-parietal, mesiofrontal and right sensorimotor areas during amatandine as compared to

baseline and sham (Schnakers et al., 2008). Other treatment interventions directly target the central part of the loop, the thalamus, such as **DBS** or low intensity focused ultrasound pulse; a novel technique showing promising effects (recovery of communication and spatio-temporal orientation) in a case report with an acute TBI patient (Monti et al., 2016). The thalamus can also be indirectly stimulated through the brainstem using **VNS**.

This mesocircuit model therefore provides a natural common ground for all treatment interventions aiming at improving the level of consciousness in patients with DOC. It can also pave the way for future research in treatments development.

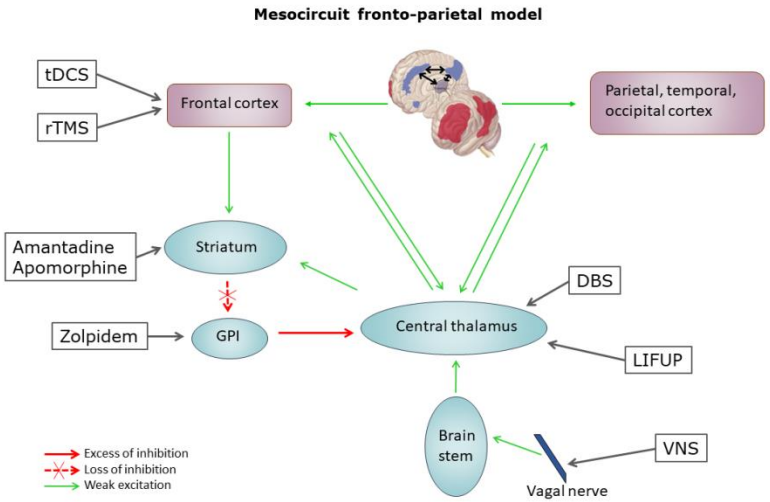


Figure 5 – The mesocircuit model proving neuroanatomical and connectivity rationale for the effects of various therapeutic interventions. tDCS= transcranial direct current stimulation; rTMS= repeated transcranial magnetic stimulation; DBS= deep brain stimulation; LIFUP= low-intensity focused ultrasound pulsation; VNS= vagal nerve stimulation. Adapted from (Thibaut et al., 2019b), with author's permission.

To sum up, several therapeutic interventions are available for patients suffering from disturbances in consciousness following severe brain injury. Invasively, DBS or VNS could be considered, with the associated surgical risks. Non-invasively, both pharmacological and brain stimulation options are available with relative efficacy. This field still suffers from a scarcity of evidence, especially for pharmacological studies. There clearly is a growing interest in the field of neuromodulation, as shown by the increasing amount of randomized controlled trials. tDCS, in particular, represents a promising treatment option, especially for patients in MCS. As a matter of fact, there is still plenty of work to be achieved, such as better characterizing treatment responders and optimizing stimulation interventions, both from a spatial (i.e., montage) and temporal (i.e., timing) perspective.

1.3.2. Palliative treatment

After a severe brain injury leading to a DOC, several primary and secondary complications may arise depending on the extent and the localization of the cerebral lesions. These lesions may affect upper motor neurons (UMN) including the pyramidal tract which is the neural pathway responsible for voluntary movements. The axonal fibers of this tract originate from the primary, the secondary and the supplementary motor cortices, cross the internal capsule to the brainstem where about 80% of the fibers cross the median line and continue to contralateral section of the spinal cord arriving at the dorsal horn. At the spinal level, the fibers project on the secondary motor neuron, which leaves the spinal cord through the ventral horn

on to the neuromuscular junction. Other UMN fibers run closely to fibers of the pyramidal ones and are therefore called parapyramidal fibers. They are responsible for tone and movement modulation (Gladson, 2010). Involuntary motor commands such as anti-gravity reflexes and postural balance are managed by the extrapyramidal system, that modulates motor activity but without directly projecting on the secondary motor neuron. The extrapyramidal tracts mainly originate from the brainstem, with higher influence from basal ganglia and sensory cortical areas. When any part of the UMN is damaged, there is a risk for **spasticity** to arise because of the disturbed balance between supraspinal excitatory and inhibitory inputs (Martens et al., 2018a). However, parapyramidal fibers (and especially the dorsal reticulospinal tract) are thought to be responsible for most of the spastic features (Balakrishnan and Ward, 2013). Indeed, an isolated lesion of the pyramidal tract does not cause spasticity however because of their anatomical proximity a single lesion often affects both pyramidal and parapyramidal tracts and the clinical picture thereby reflects the combined lesion (Sheean, 2002). Spasticity is a motor disorder arising from anarchic reorganization of the central nervous system and is clinically characterized by increased velocity-dependent stretch reflexes (Gracies, 2005). Another definition depicts spasticity as “a disordered sensory-motor control, resulting from an UMN lesion, presenting as intermittent or sustained involuntary activation of muscles” (Pandyan et al., 2005). The latter one better represents the clinical picture of patients suffering from spasticity, shown in Figure 6.

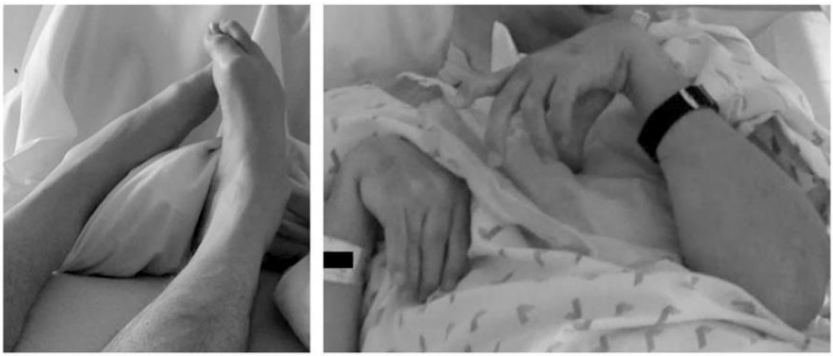


Figure 6 – Spastic joints after brain injury. Imbalance between activation of the flexing muscles and of the stretching muscles creates a classical pattern of equinovarus feet (left) and internal rotation of the shoulder with flexion of the wrist and fingers (right). From (Thibaut et al., 2013), with the author's permission.

Since this motor trouble arises after a central lesion affecting the UMN responsible for inhibitory and excitatory supraspinal drive, it is classically observed in patients with stroke, spinal cord injury, multiple sclerosis or TBI. However, while its occurrence and pathophysiology are well described in these populations, little is known about the cases involving more complex lesions leading to DOC. In most of cases, DOC patients are bedridden and suffer prom paresis or paralysis, which favors the apparition of spasticity (by disuse and immobilization) and can provoke loss in range of motion, pain and bed sores (Martens et al., 2018a).

A retrospective study performed by Nakase-Richardson et al collected all medical complications arising during rehabilitation in a sample of 122 veterans and active duty military individuals with DOC following severe brain injury. Spasticity was, by far, the most common complication, affecting 70% of the study participants. Next

in line were autonomic nervous system dysregulation (e.g. autonomic storming, fever, tachycardia) for 34% of the sample, epileptic seizure (30% – more frequent for blast-related etiologies), hydrocephalus (25%) and intracranial infection (22%) (Nakase-Richardson et al., 2013). Two other prospective studies documented the prevalence of spasticity in patients with DOC and found rates of 57% spastic patients (in a sample of 68 patients (Ganesh et al., 2013)) and 89% (sample of 65 patients (Thibaut et al., 2015a)). Even though the prevalence is known to be that high, few studies investigating treatment options for DOC patients are available. A systematic review performed in 2017 retrieved only four interventional studies primarily targeting spasticity in patients with DOC (Martens et al., 2017): two clinical trials investigating soft splints (Thibaut et al., 2015b) and acupuncture (Matsumoto-Miyazaki et al., 2016) as well as two case reports about intrathecal baclofen (ITB) (Francois et al., 2001; Shrestha et al., 2011). All these techniques led to a clinically significant decrease in spasticity and thereby in the level of disability. They should therefore be considered in the palliative/comfort care arsenal for patients with DOC. **Soft splints** are polyurethane materials designed in the form of a roller splint that fits in the palm of the hand and promotes its (passive) opening. The softness of the splint allows for muscle contraction and grasping reflex, as opposed to conventional rigid splints that are less tolerated and can even induce skin injuries or pain. However, for now, they are only applicable to the hand. **Acupuncture**, a medical practice far more used in Asia than in Western Europe or North America, relies on ancient traditions only and is not truly backed up by modern scientific works. The mechanisms for spasticity reduction would involve modulation of motor cortex excitability, potentially associated with a reduced spinal

motor neuron activity (Matsumoto-Miyazaki et al., 2016). **Baclofen**, on the contrary, is part of the conventional treatment package for spastic disorders and can be administered either *per os* or by an intrathecal pump. This GABAergic compound enhances presynaptic inhibition at the spinal level and thereby reduces spastic overactivity (Richard and Menei, 2007).

Interestingly, when spasticity was considered as a secondary outcome, further treatment options could be identified such as tilt table therapy (Krewer et al., 2015) or invasive thalamic stimulation (Magrassi et al., 2016). These approaches initially focused on improving the level of consciousness but proved their efficacy in reducing spasticity as well. It is also interesting to note that, for ITB therapy, the primary indication is to decrease spasticity but it further enables to increase the level of consciousness, with case series reporting recovery of conscious cortically mediated behaviors such visual pursuit, object recognition and verbalization (Margetis et al., 2014; Pistoia et al., 2015).

Unfortunately, none of these studies formally evaluated the effect of a multidisciplinary program that would combine pharmacological, orthopedic and rehabilitation approaches; while this is the approach used on the field. This still has to be investigated in future studies. In the meantime, pharmacological and non-pharmacological treatments usually used for post-stroke spasticity are administered for patients with DOC too. A summary of these interventions and where they act is presented in Figure 7. However, patients with DOC differ from stroke patients in several aspects, the main one being the extent of the brain lesions. In DOC patients, numerous cortical and subcortical areas are damaged most of the time, leading to an atypical clinical presentation of spastic features.

Indeed, in stroke patients, the clinical spastic component assessed by bedside standardized scales evaluating resistance to passive movement such as the Modified Ashworth Scale (MAS) is often reflected by the electrophysiological component assessed by the so-called Hmax/Mmax ratio. This ratio represents the percentage of excited afferent fibers through spinal reflexes (H response) over the direct activation of efferent fibers (M response), following electrical stimulation of the motor nerve (Katz et al., 1992). In DOC patients however, we did not find such correlation between clinical and electrophysiological measures of spasticity in a sample of 21 patients (Martens et al., 2019c). This discrepancy could be due to the localization of the lesions; the ratio was more increased when they were both cortical and subcortical lesions (objectified by MRI), while it was in a normal range when the lesions were subcortical only. This suggests that cortical lesions may be partly obscured by subcortical ones and therefore less electrophysiologically expressed. Furthermore, oral antispastic medication such as baclofen did not significantly influence the clinical or the electrophysiological component of spasticity in this study. This is concerning since it means the pharmacological management of spasticity in DOC is still inappropriate. Future trials need to focus on treating spastic DOC patients only and on consistently reporting follow-up data.

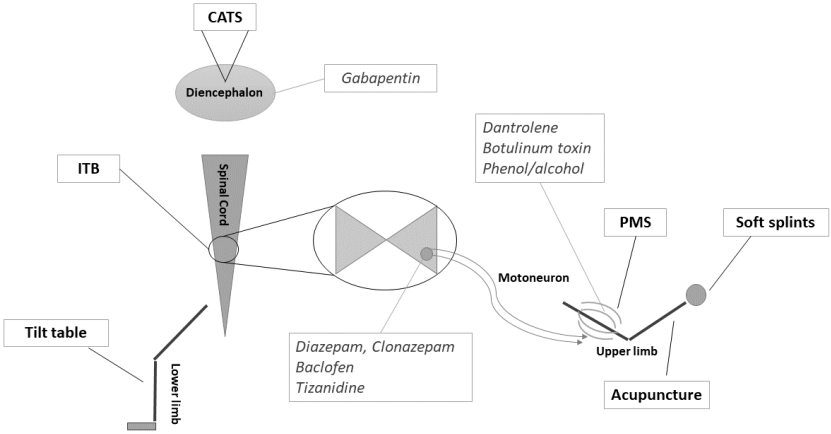


Figure 7 – Available treatments for spasticity in DOC patients. CATS= cortical activation by thalamic stimulation; ITB= Intrathecal baclofen; PMS= passive muscle stretch. Conventional pharmacological treatments are shown in italics. From (Martens et al., 2017).

Finally, spasticity does not only impact the motor status of the patient; it also induces pain. Indeed, in a sample of 65 chronic DOC patients, Thibaut et al showed that 58 of them presented with spasticity and there was a significant correlation with the Nociception Coma Scale-Revised (NCS-R) scores, a clinical scale specifically designed and validated to evaluate pain in patients with DOC (Chatelle et al., 2012). This further highlights the need for appropriate spasticity and pain management for DOC specifically. The lack of available well-designed studies, however, forces clinicians to rely on empirical approaches.

1.4. Objectives of this work

The work presented in this dissertation aims to improve the multidisciplinary management of patients with DOC. This population is indeed often neglected by care practitioners and researchers, due to assumed poor prognosis and low prevalence. These patients present with an intricate clinical picture and a challenging therapeutic strategy. The primary objectives of this doctoral thesis are thus two-fold: first, to contribute to the behavioral diagnosis at the bedside and second, to investigate alternative administration methods for therapy using tDCS.

The first part focuses on early detection of consciousness recovery at the bedside to improve the initial diagnosis and to provide quantitative behavioral data regarding the months that follow the initial brain lesion, in a rehabilitation program setting. Emphasis is placed on a particular milestone highly valued by both medical personnel and relatives: recovery of meaningful communication. Providing clinicians and families with objective and realistic expectations hopefully contributes to a better care for these patients.

In the second part, the optimal use of tDCS is investigated. Indeed, it is known as a potentially efficient treatment option for patients with DOC, but its application is still subject to a lot of variations (i.e., environment, montage, timing). We first focus on the environmental setting for tDCS therapy and investigate the feasibility, the safety and the efficiency of a long-term tDCS protocol delivered at home or in rehabilitation centers. We then focus on stimulating other areas than the prefrontal cortex based on

neuroanatomical and functional hypotheses. The motor cortex plays a key role in expressing signs of consciousness and is therefore our first target. Second, based on pre-identified crucial networks for consciousness recovery, we investigate the effects of network-based stimulation and particularly the frontoparietal network involved in external awareness. Eventually, interested as well in the timing of delivering tDCS, we hypothetically explore the use of brain-state dependent stimulation and provide directions for future research.

2. Part One: Toward enhanced diagnosis at the bedside, a field approach

"The spirit of a man can endure his sickness, But as for a broken spirit who can bear it?"

Proverbs 15

The present section is based on the following articles:

Martens, G., Bodien, Y., Thomas, K., & Giacino, J. Temporal profile of recovery of communication in patients with disorders of consciousness following severe brain injury. *Submitted*

Martens, G., Bodien, Y., Sheau, K., Christoforou, A., & Giacino, J. T. (2019). Which behaviours are first to emerge during recovery of consciousness after severe brain injury? *Annals of physical and rehabilitation medicine*. doi:10.1016/j.rehab.2019.10.004

2.1. Context

A critical challenge in the appropriate care of patients with DOC pertains to the diagnosis and, specifically, the misdiagnosis. Diagnostic error between UWS and MCS is unfortunately common and it has been shown that about 40% of patients clinically diagnosed as UWS were actually MCS when evaluated with standardized behavioral features (Childs et al., 1993; Andrews et al., 1996; Schnakers et al., 2009) or with an active fMRI paradigm (Monti et al., 2010). As mentioned above, misdiagnosing conscious and unconscious patients can have major consequences regarding admission to rehabilitation, pain management and end of life decisions (Giacino et al., 2014). Patients with DOC are also prone to exhibit confounding factors that make the diagnosis even more challenging. Indeed, sensory deficits, neuromuscular dysfunction, subclinical seizure activity or fluctuations in vigilance will make it easy to miss signs of consciousness at the bedside (Giacino et al., 2009).

It is therefore important to detect the **transition from unconscious** (i.e., coma, UWS) **to conscious** (i.e., MCS, EMCS) **states** as early as possible. Some previous studies detailed below focused on quantifying the prevalence of MCS signs at various stages post-injury. Little is known, however, about the initial manifestation of MCS signs: which one(s) tend to reappear first and thereby signal the transition from an unconscious state to a conscious state? Which one(s) appear later on and might suggest they are more difficult to recover or to assess?

Among these signs, an item that appears more valuable than the other ones in the eyes of the close relatives is the ability to

communicate again. Consistent **recovery of communication** is not only a critical milestone during the rehabilitation period, it is also the most anticipated one. Indeed, both relatives and caregivers want to know which needs, thoughts or emotions the patient is experiencing. Objective data extracted from similar situations may guide both clinicians and relatives through their expectations.

2.2. 'Which behaviors should I track?' – a clinicians' perspective

Monitoring recovery of consciousness in the subacute setting is of paramount importance for any clinician: for the physician in charge of the patient's treatment and management, for the rehabilitation interventions (physical therapy, occupational therapy, speech therapy) and for the nursing team. While major decisions concerning end of life or discharge disposition are often addressed in the intensive care units in the days or weeks following the incident, some patients remain unconscious (i.e., comatose or UWS) for longer than that. It is therefore not rare that these patients get discharged to rehabilitation facilities still being unconscious. These patients obviously represent a challenge for rehabilitation interventions and there are a lot of unknowns as to whether they are going to regain consciousness during their stay or not. Physicians in charge of these patients have no quantitative data to rely on while they are responsible for the accuracy of the diagnosis, appropriate treatment planning and family counseling. From the clinician's perspective, recovery of consciousness is marked by the transition from unconscious states (i.e., coma and UWS) to conscious states (i.e., MCS and EMCS); but which signs can they first expect to see at the bedside? It is known that visual behaviors such as pursuit or fixation are most often observed, as suggested by previous studies, including the inaugural one from Noé and colleagues, who prospectively followed 32 patients with severe acquired brain injury (20 MCS and 12 in UWS). It appeared that, on admission, patients in MCS were diagnosed as such based solely on visual abilities (relying on the CRS-

R visual subscale) (Noé et al., 2012). The high prevalence of visual conscious behaviors to diagnose MCS was later confirmed by a larger multicenter cross-sectional study conducted by Estraneo and colleagues and identifying 52 patients in MCS using the CRS-R, both in the intensive, rehabilitation and long-term care settings. In most patients (43/52), the diagnosis of MCS was, again, captured by the CRS-R visual subscale (Estraneo et al., 2015). Finally, a recent retrospective study led by Wannez and colleagues and focusing on documenting the prevalence of MCS signs on a large sample of patients diagnosed as MCS at various times post-injury revealed that, among the 282 chronic MCS patients assessed with the CRS-R, visual fixation and visual pursuit were the two most frequently observed conscious behaviors (57%, and 52% of cases, respectively), preceding reproducible movement to command (51%) (Wannez et al., 2017a). This is however still not informative enough regarding the time and the nature of the initial emergence of conscious behaviors. Bagnato and colleagues attempted to characterize the clinical signs denoting the first occurrence of conscious behavior in 31 patients in UWS (both TBI and non-TBI) admitted to rehabilitation about two months (mean of 54 ± 35 days) after their injury and followed them with the CRS-R at month 1, 2, 3, 6 and 12 post-admission (Bagnato et al., 2016). Interestingly, they found that 21 patients regained consciousness during the study period and that in 42.9% of the cases, this was objectified with only the visual CRS-R subscale, while the motor subscale alone accounted for 9.5% of cases. Timewise, they observed that 90.5% of the patients who regained consciousness did so within the first three months post-admission. Another interesting finding pertains to the influence of etiology: patients with TBI showed significantly more signs of consciousness as compared to the

non-TBI group. The authors concluded that visual pursuit and fixation are the commonest early behaviors denoting MCS. However, since CRS-R evaluations were performed on a monthly basis, early signs of consciousness might have been missed between consecutive evaluations and more frequent evaluations could capture this transition more precisely.

To address these questions, we conducted a retrospective observational study focusing on capturing the transition from unconsciousness to consciousness, from a behavioral perspective, using the gold standard CRS-R. We aimed at answering the following questions: 1) How long does it take for patients admitted as unconscious to a rehab program to regain consciousness? 2) Which behavior(s) mark the transition to consciousness? 3) Is there any significant influence of etiology on consciousness recovery (TBI *versus* non-TBI)?

We extracted data from a REDCap (Harris et al., 2009) database housing demographic and clinical metrics collected by trained rehabilitation therapists on all patients admitted to the specialized DOC program at Spaulding Rehabilitation Hospital in Boston, MA. This program includes bi-weekly assessments of patients with DOC using the CRS-R until the patient emerges from the MCS. We extracted our data from this database using the following criteria: (1) at least 17 years old, (2) documented acquired brain injury with medical diagnosis of coma or CRS-R-based diagnosis of UWS/VS on admission to the DOC program and (3) evidence of transition to consciousness during the inpatient rehabilitation stay, defined as two consecutive CRS-R assessments obtained within seven days indicating a new MCS or eMCS diagnosis. As presented in Figure

8 below, 79 out of 323 patients screened met these criteria and were included in the study.

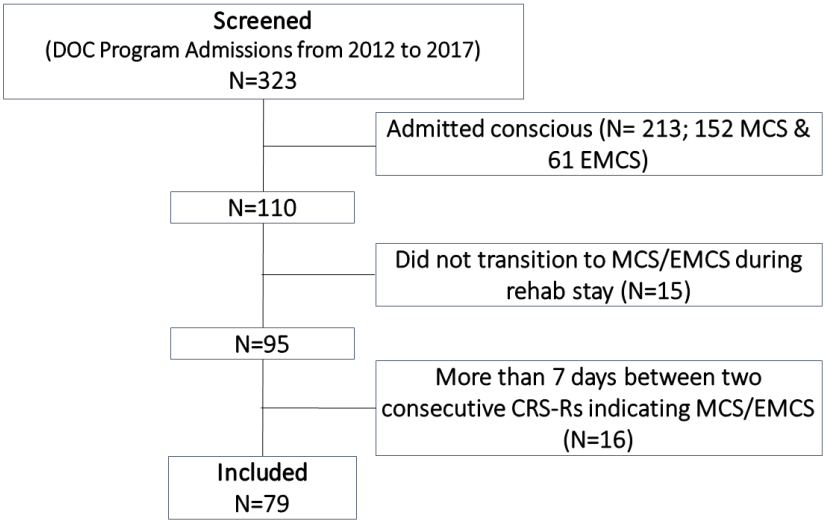


Figure 8 – Participant flow diagram. MCS= Minimally Conscious State; EMCS= emergence from the MCS; CRS-R= Coma Recovery Scale-Revised

According to the CRS-R, 11 items denote MCS (i.e., consistent movement to command, reproducible movement to command, object recognition, object localization, visual pursuit, visual fixation, automatic motor response, object manipulation, localization to noxious stimulation, intelligible verbalization, intentional communication) while two other ones denote EMCS (i.e., functional object use, functional communication). We used these 13 behavioral markers to characterize consciousness recovery. These 13 items and their operational definitions can be found in Appendix 2.

Regarding the analyses, we used descriptive statistics to summarize the study sample characteristics. We then computed incidence rates along with 95% confidence intervals for the first behavioral signs of consciousness to reemerge. To compare the time to recovery of consciousness, the CRS-R total score and the number of conscious behaviors observed at transition between TBI and non-TBI patients, we used the Wilcoxon Rank Sum Test. Results were considered significant at $p < 0.05$. To further evaluate the influence of etiology on the nature of conscious behaviors recovered, we clustered them into three categories: 1) language abilities (i.e., consistent and reproducible command following, intelligible verbalization, intentional and functional communication); 2) motor abilities (i.e., functional object use, automatic movement, object manipulation, localization to pain) and; 3) visuoperceptual abilities (object recognition, object localization, visual pursuit, visual fixation). Differences between the TBI and non-TBI subgroups were tested using Fisher's exact test, with a Bonferroni correction for multiple comparisons (3 comparisons; $p < 0.016$).

The demographic data and clinical characteristics of the study sample are presented in Table 2. It can be noted that the TBI group was significantly younger than the non-TBI one, which is not surprising as it has been reported in similar studies (Bagnato et al., 2016; Bodien et al., 2019). Otherwise, these two subgroups presented no other difference. Patients were admitted within the month following their injury and six patients were still in a comatose state while the rest of the sample was in UWS.

Table 2: Demographic and clinical characteristics of the study sample on admission.

	Total	TBI	Non-TBI	p value
N (male)	79 (51)	34 (25)	45 (26)	0.147 [a]
Age, median [IQR]	48 [26 – 61]	33 [23 – 53]	57 [33 – 64]	0.002 * [b]
Days from injury to admission	26 [20 – 36]	29 [20 – 36]	25 [20 – 36]	0.454 [b]
Initial CRS-R total score	4 [3 – 6]	4 [3 – 6]	4 [3 – 6]	0.869 [b]

TBI= Traumatic Brain Injury; IQR= Interquartile Range; CRS-R= Coma Recovery Scale-Revised; a = Fisher's exact test TBI vs. non-TBI; b = Wilcoxon Rank Sum test TBI vs. non-TBI; *= significant statistical difference ($p<0.05$).

Regarding the CRS-R monitoring, patients were assessed twice a week and were followed for a median [IQR] time of 61 [42 – 98] days before either the discontinuation criteria for the CRS-R were met (i.e., the patient emerged from MCS) or the patient was discharged. The initial CRS-R exam, on which the initial diagnosis was based, took place upon admission (median time of one day post-admission). The median time between consecutive CRS-R assessments was 4 days [3– 5].

Time to consciousness recovery

Patients admitted as unconscious recovered their first signs of consciousness in a median [IQR] time of 44 [33 – 59] days (about 6 weeks) after the injury and 14 [6 – 26] days after admission. This is consistent with previous findings reporting consciousness recovery within 12 weeks after injury but provides more granular data. Indeed,

given the present study is the first of its kind using bi-weekly follow-up, behavior-specific estimates of time to recovery of consciousness are provided and suggest the transition to conscious states can actually be expected within a shorter time window in this type of patients. We should however bear in mind that it concerns about 85% of the patients followed. Indeed, for the purpose of this study, we excluded 15 patients who did not transition during their rehabilitation and remained unconscious.

Nature of signs of consciousness recovered

When plotting the most common behavioral signs of consciousness (MCS or EMCS) observed on the very first assessment denoting consciousness recovery, visual pursuit clearly ranks first (see Figure 9). It was observed in 41% (95% CI 30.2 – 51.8) of our sample while the next most commonly-observed behaviors concerned 25% of cases or less. The second was reproducible movement to command (25%; 95% CI [15.5 – 34.6]) and the third was automatic movement (24%; 95% CI [14.6 – 33.4]). The remaining 10 behavioral markers of consciousness emerged first in less than 16% of the sample.

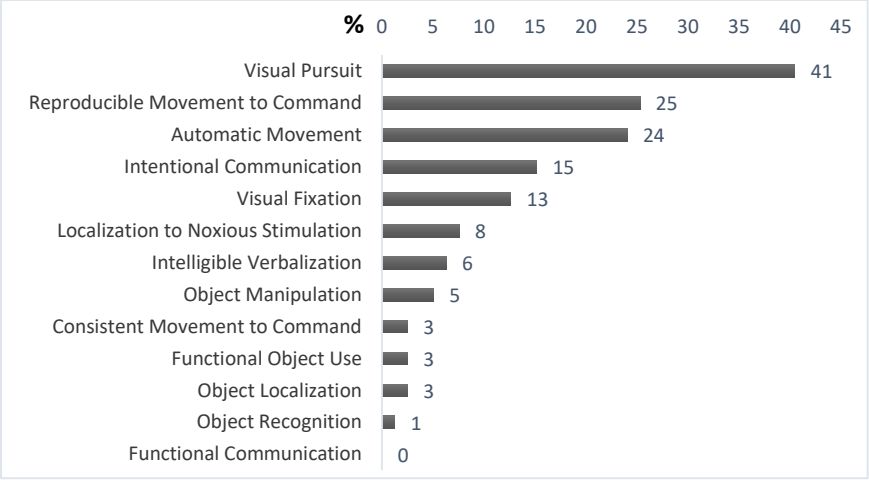


Figure 9 – Proportion of patients (n=79) presenting with each behavior as the first sign of consciousness. Bars indicate the percentage of the sample that recovered each behavior.

It is unsurprising that **visual pursuit** was the most prevalent initial sign of consciousness recovery, as it has already been well documented as an early indicator of consciousness (Dolce et al., 2008; Candelieri et al., 2011; Noé et al., 2012). Visual fixation, however, was less observed as compared to previous studies. Two hypotheses might explain this. First, from a methodological perspective, CRS-R guidelines state that when an item is successfully passed within a subscale, the examiner moves on to the next subscale and does not asses the lower-level behaviors underneath the successful item. However, in the study from Wannez et al reporting a higher prevalence of visual fixation in MCS (52%), each single item was assessed, meaning a patient could obtain both visual

pursuit and fixation. Second, from a neuroanatomical perspective, the reoccurrence of visual pursuit likely reflects some preservation of connectivity between the brainstem and the cortex, supporting not only basic arousal functions but also complex eye movements (complex enough to present visual pursuit as an output, and not only fixation). Since the inputs from the vestibular nuclei to the pons are reactivated, they mediate arousal but further activate downstream frontal and parietal cortices responsible for eye movement control, reflected by visual pursuit.

Reproducible command-following (i.e., the ability to follow a simple one-step command at least three out of four trials) was the second most observed behavior denoting consciousness recovery, which is reassuring since it is widely used during routine bedside examination as a definitive sign of conscious awareness (Teasdale and Jennett, 1974). The incidence we found (25%) falls within the range previously reported from 14 to 51% (Estraneo et al., 2015; Bagnato et al., 2016; Wannez et al., 2017a) but there seems to be a wide variability for which it is unclear what the contributing factors are. It could, again, be due to the administration of this item during the CRS-R assessment. Indeed, even though the CRS-R guidelines are unequivocal, there is no standard expliciting the type of commands administered and the amount of trials for different commands. What is consistent across studies is the lower occurrence of consistent command following (i.e. the ability to clearly answer at least two different commands on four out of four trials each), ranging within 0 to 6% and probably reflecting a way more cognitively demanding task, especially regarding working memory and attention capacities.

Automatic motor movement ranks closely after reproducible command following. These over-learned, often repetitive, behaviors

(e.g., nose-scratching, bedrail gripping) are triggered either by interoceptive or exteroceptive stimuli and are supposed to reflect at least partial preservation of self and environmental awareness. They can also be a prognostic sign of better outcome, as suggested by Rémi and colleagues who prospectively followed a cohort of 120 patients after severe acute stroke and found that the ones presenting with automatic movement (n=34), specifically leg-crossing, had a better functional outcome one year after the injury than a matched control group (n=34) who did not (Rémi et al., 2011).

The remaining ten signs of MCS or EMCS emerged first in 15% of cases or less. This lower prevalence can be explained either because these behaviors depend upon well-preserved network connectivity (often absent at this stage of recovery) or because they require an important participation of motor abilities, often significantly impaired in patients with DOC.

An interesting observation is that in 72% (95% CI [62.1 – 81.9], n=57) of the sample, recovery of consciousness was signaled by the emergence of a behavior in only one of the CRS-R subscales. This implies that the procedures used to detect behavioral signs of consciousness should be designed to reliably detect them, with a specific attention to visual pursuit, command-following and automatic movements. For visual pursuit, it is strongly recommended to use a mirror, as the high saliency of this stimulus (probably due to its auto-referential aspect) has been emphasized by previous works (Vanhaudenhuyse et al., 2008; Wannez et al., 2017c). Command-following should be evaluated using standardized procedures such as those described in the CRS-R (Giacino et al., 2004), the WHIM (Shiel et al., 2000) or the SMART (Gill-Thwaites and Munday, 1999). In a more selective fashion, individualized quantitative behavioral

assessment (IQBA) methods use statistical comparisons between volitional (target command), noise (other command) and rest (absence of command) conditions to reliably discriminate command-following from random behavior on a single-subject level (Whyte et al., 1999). Regarding automatic movement, they are by their nature difficult to elicit at the bedside but assessment methods using passive observation or active alternating commands using familiar gestures (as offered by the CRS-R) to capture spontaneous or induced automatic motor responses can be used (Giacino et al., 2004).

An important aspect of capturing these early behavioral signs of consciousness pertains to the fluctuations in arousal and vigilance specific of this population. Serial assessment is therefore essential to reduce the diagnosis error rate and to avoid missing critical behaviors (Wannez et al., 2017b; Giacino et al., 2018b). Finally, it is interesting to note than in very few cases, the transition of recovery did not follow the typical course of recovery (i.e., coma to UWS to MCS to EMCS). Two patients indeed transitioned from UWS directly to EMCS within several days. This could either be a very fast recovery or a misdiagnosis on the first baseline assessment.

Influence of the etiology

When comparing the TBI (n=34) and the non-TBI (n=45) subgroups, it appeared that there were no significant difference in terms of time to recovery of consciousness, CRS-R total score at transition and number of conscious behaviors recovered at transition, as presented in Table 3.

Table 3 – Clinical characteristics of the study sample (n=79) at the time of transition from coma or UWS to MCS or EMCS.

	Total	**TBI**	**Non-TBI**	**p value** [a]
Days to recovery of consciousness, median [IQR]	44 [33 – 59]	41 [29 – 50]	46 [35 – 63]	0.517
CRS-R total score	9 [8 – 11]	9 [7 – 11]	9 [8 – 10]	0.317
Number conscious behaviors recovered	1 [1 – 2]	1 [1 – 2]	1 [1 – 1]	0.250

TBI= Traumatic Brain Injury; IQR= Interquartile Range; CRS-R= Coma Recovery Scale-Revised; a = Wilcoxon Rank Sum test TBI vs. non-TBI

This is in agreement with Bagnato's findings, with the exception of the number of behaviors recovered, that was reported to be significantly higher in TBI patients as compared to the non-TBI in their study (Bagnato et al., 2016). The sample in the present study is more than three times larger than Bagnato's one meaning this difference did not survive in a larger population. This divergence could also be explained by the difference in patients' time post-admission between the two studies, the patients presented here being in a more acute stage. Concerning the type of behaviors recovered, there was a significant influence of etiology only for the motor behaviors, and not for the language-related or visual ones, as depicted in Figure 10. Patients with TBI showed motor function compatible with consciousness recovery significantly more often than non-TBI patients at time of transition to consciousness (Fischer's p=0.011) while there was no difference in the frequency of recovery of language (p=0.99) or visual (p=0.066) signs of MCS. This might be due to the pathophysiological differences between TBI and non-TBI

insults. It is indeed known that non-traumatic lesions arising from severe hypoxic-ischemic events preferentially damage brain areas with high oxygen consumption demands (Cervós-Navarro and Diemer, 1991; Busl and Greer, 2010). The basal ganglia, for instance, which have a high metabolic activity and oxygen demand, play a key role in motor control and execution. This may participate in the lower frequency of automatic movements observed in the non-TBI patients. For the visual cluster, a trend can be noted toward non-TBI patients showing initial visual conscious behaviors more frequently than the TBI ones, both from a graphical and statistical perspective (p value close to significance). A larger sample of patients might confirm this trend and thereby suggest patients with anoxic and vascular injuries present a better preservation of visual pathways.

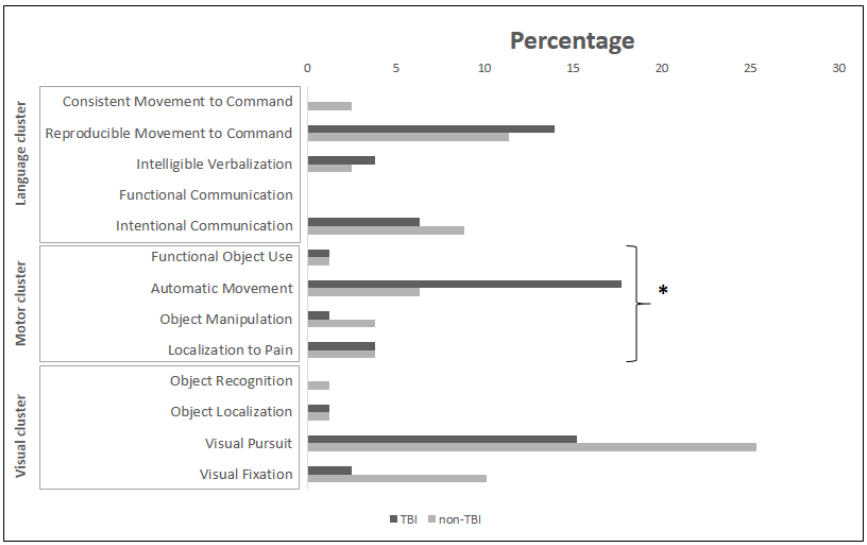


Figure 10 – Comparison of behavioral recovery by domain in patients with TBI and non-TBI. *= Statistically significant difference between TBI and non-TBI ($p<0.05$).

Some limitations hindering the generalizability of the results should be mentioned. First, the setting of the rehabilitation facility may represent a selection bias in the sense that only patients discharged to that facility were included, between three to five weeks from injury. This means patients transitioning to MCS or EMCS earlier, in the intensive care for instance, have not been captured and may present with a different pattern for behavioral recovery. Therefore, prospective studies performed in the acute setting should be conducted to fill in this knowledge gap. Second, since this was a single-site study, the sample size is limited and the characteristics of the sample (demographics and behavioral) might be slightly different from one site to another. Multi-center or replication studies would address this issue. Last but not least, a more general issue pertains to the aim of the study. We indeed used the behavioral output observed at the bedside, however, as stated above, we cannot infer the presence or absence of consciousness based solely on behavior. It has been raised previously that behavioral testing without subjective report from the patient does not provide the full picture of conscious awareness (Bernat, 2002; Giacino et al., 2009; Naccache, 2018). There is, however, no existing way yet to fully address this issue, as it is related to the conception of consciousness itself.

To sum up, this study shows that patients who have been behaviorally unconscious for weeks due to a severe acquired brain injury and recovered consciousness during their rehabilitation stay, did so within approximately six weeks post-injury. The first behavioral signs of consciousness typically recovered were visual pursuit, command-following and automatic movement, with a single subscale depicting transition in most of the cases (72%). The etiology (traumatic or non-traumatic) did not influence the time to recovery

or the amount of behaviors initially recovered but it did influence the type of behaviors recovered. Motor behaviors were indeed more frequently observed in TBI patients, as compared to the non-TBI. The take-home message for clinicians working with these cases would be to use neurobehavioral methods sensitive enough to detect these three behaviors and to perform repeated exams, as the diagnosis of the patient might change in a few days' span. It would be interesting to investigate further whether the early emergence of these specific behaviors is associated with long-term functional outcome.

2.3. 'When will we be able to communicate?' – a relatives' perspective

The clinicians working with patients with DOC are undoubtedly facing many challenges on a daily basis, as depicted by the high amount of burnout in caregivers (Gosseries et al., 2012). But one should keep in view the biggest burden is on the family and relatives, dealing with a lot of anxiety and unanswered (or unanswerable) questions: "Can he/she hear me?", "Is he/she in pain?", "Will he/she recover? When?". And while the clinician is sometimes focusing on the tiniest improvements (e.g., recovering visual fixation or localization to pain), one critical step prioritized by the relatives is "When will I be able to communicate again with him/her?". Recovery of communication is, as a matter of fact, a highly anticipated milestone for the patient and for all the parties directly or indirectly involved. It means indeed the patient can reliably express his/her own needs, participate actively in the care with autonomous decision-making and have meaningful social interaction. It is known to be part of the most important anticipated behavior to be recovered in related conditions such as locked-in syndrome and stroke (Wallace and Bradshaw, 2011; Krishnan et al., 2017; Lugo et al., 2017; Bucki et al., 2019). However, the time course to recovery of this crucial behavior has not been properly investigated yet, to the best of our knowledge, while it would be useful to assist with early decision-making regarding treatment planning but also with legal questions such as guardianship.

Previous early studies report recovery of communication in patients with chronic DOC between four months and several years post-injury (Najenson et al., 1978; Andrews, 1993). Qualifying this range as wide would be an understatement. Furthermore, these studies are limited by low sample size and non-standardized nor validated communication assessment methods. Indeed, according to the gold standard CRS-R (Seel et al., 2010; Giacino et al., 2018b), communication should be assessed using six consecutive situational orientation questions requiring "yes/no" verbal or gestural responses (e.g., "Am I clapping my hands right now?") (Giacino et al., 2004). The scoring criteria describe three items hierarchically organized by cognitive complexity. The lower level is the absence of communication, determined by the absence of discernable responses to the questions, or the presence of only one tentative answer. The next item, intentional communication (IC), is scored when there are clearly discernible yes/no responses to at least two out of the six questions, regardless of accuracy. Finally, functional communication (FC) means the patient is able to respond accurately to the six consecutive questions. The latter has a high level of cognitive complexity and is therefore considered as a sign of EMCS. A prospective study used the CRS-R on a monthly basis to follow 32 patients with DOC admitted to rehabilitation about five months post-injury and showed that eight patients (25%) were EMCS within one year after admission (Noé et al., 2012). Unfortunately, no distinction was made between emergence based on functional object use or functional communication so no data can be extracted regarding time-course to communication recovery. A larger multicenter study followed 52 patients with DOC both in intensive care and rehabilitation settings for six weeks and showed that 30% of the

patients recover FC (based on the CRS-R administered at enrolment and at week 6) within approximately three months post-injury (Giacino et al., 2019). Replicating these results on a larger sample with closer CRS-R assessments would provide more granular quantitative data for clinicians and caregivers to rely on.

We therefore conducted another retrospective observational study using the same REDCap database described above with the following inclusion criteria: 1) acquired brain injury; 2) at least 16 years old at admission; 3) admitted to rehabilitation with no evidence of communication on initial CRS-R administration 4) at least three valid CRS-R assessments within two-weeks of rehabilitation admission; 5) at least an eight-week rehabilitation length of stay, or recovery of functional communication prior to the eighth week. We chose the eight-week cut-off as it is the standard length of the specialized DOC program on site. However, in a secondary analysis, we looked at the patients who stayed longer than eight weeks as well.

Of the 323 patients screened, 175 patients met the inclusion criteria (see Flowchart – Figure 11), their demographic characteristics are presented in Table 4. We identified four different patterns of communication recovery when sampling time to recovery of IC or FC over the eight weeks post-admission: 1) patients who did not recover IC nor FC (Group 1: -IC-FC); 2) patients who recovered IC, but not FC (Group 2: +IC-FC); 3) patients who recovered IC then FC (Group 3: +IC+FC) and; 4) patients who recovered FC, without prior recovery of IC (Group 4: -IC+FC).

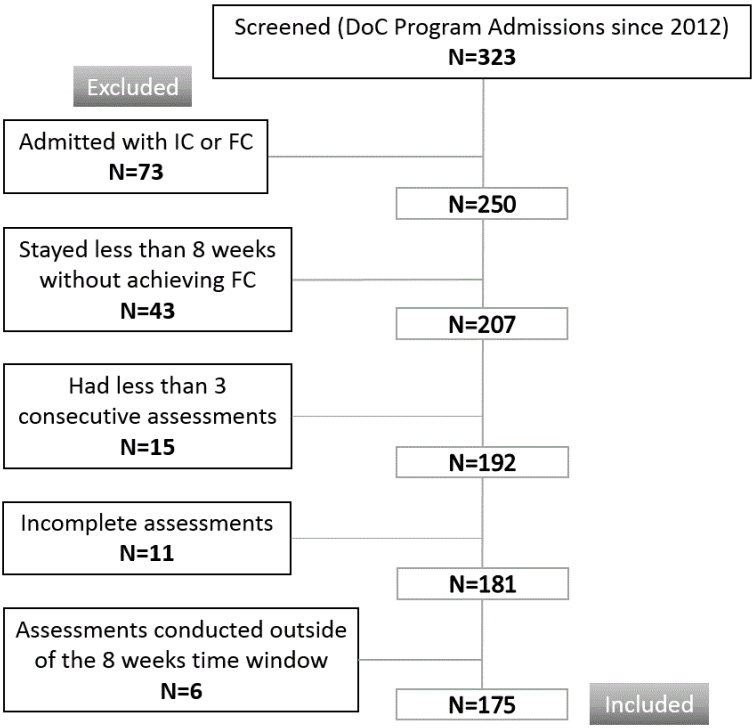


Figure 11 – Flow diagram and days to recovery of communication for each group. IC= Intentional Communication; FC= Functional Communication; CRS-R= Coma Recovery Scale-Revised. Results are presented as median [IQR].

We described the time to recovery to IC and FC (i.e., days between injury and the first CRS-R indicating IC or FC) using medians and IQRs in each of these groups as well as in the whole study sample. We checked any significant difference between the four groups regarding gender, age, days post-injury on admission, etiology and length of rehabilitation stay using non-parametric analyses. We used Pearson's Chi-squared test for dichotomous variables and

Kruskal-Wallis Rank Sum Test for continuous variables. When a significant result was obtained ($p < 0.05$), we conducted post-hoc pairwise comparisons using Fisher's test for dichotomous variables and Wilcoxon Rank-sum test for continuous variables. We applied a Bonferroni correction to adjust for multiple comparisons (n=6; $p<0.0083$). The results are presented in Table 4.

Table 4 – Demographics, clinical characteristics, and time to recovery of communication.

	Study sample n=175	**Group 1 (-IC, -FC) n=54**	**Group 2 (+IC, -FC) n=30**	**Group 3 (+IC, +FC) n=72**	**Group 4 (-IC, +FC) n=19**	**p value [c]**
Gender (% male)	60%	31	12	53	9	**p=0.008**
Age (years)	48 [27 – 61]	34 [25 – 52]	55 [37 – 64]	52.5 [27 – 66]	53 [40 – 59]	**p=0.023**
Days between injury and admission	28 [21 – 38]	33.5 [27 – 51]	29 [20 – 34]	26 [20 – 33]	23 [21 – 29]	**p=0.0004**
Etiology (% TBI)	57%	32	11	48	9	**p=0.034**
Days of rehabilitation admission	94 [67 – 152	131 [87 – 198]	100 [78 – 157]	75 [57 – 113]	98 [58 – 121]	**p=0.00003**
Days from injury to recovery of IC	40 [34 – 54] [a]	*NA*	52 [38 – 67]	37 [32 – 47]	*NA*	**p=0.0004**
Days from injury to recovery of FC	49 [41 – 61] [b]	*NA*	*NA*	50 [42 – 61]	43 [32 – 63]	p=0.106
			Post-hoc pairwise comparisons [d]			
Groups	1 vs. 2	1 vs. 3	1 vs. 4	2 vs. 3	2 vs. 4	3 vs. 4
Gender	p=0.136	p=0.99	p=0.629	p=0.018	p=0.650	p=0.99
Age	**p=0.007**	p=0.020	p=0.032	p=0.557	p=0.538	p=0.953

Days between injury and admission	p=0.016	**p=0.00009**	**p=0.0029**	p=0.515	p=0.366	p=0.513
Etiology	p=0.466	p=0.775	p=0.370	p=0.450	p=0.141	p=0.370
Days of rehabilitation admission	p=0.157	**p=0.000004**	p=0.041	**p=0.005**	p=0.388	p=0.353

Data are median [IQR] unless indicated. Group Definitions- Group 1: patients who did not recover communication within 8 weeks or prior to discharge from rehabilitation, Group 2: patients who recovered IC but not FC within 8 weeks, Group 3: patients who recovered IC and then FC within 8 weeks, Group 4: patients who recovered FC (without prior evidence of IC) within 8 weeks. [a] includes 102 patients in Group 2 + Group 3 who recovered IC; [b] includes 91 patients in Group 3 + Group 4 who recovered FC; [c] Pearson's Chi-squared test for dichotomous variables and Kruskal-Wallis Rank Sum Test for continuous variables; [d] Fisher's test for dichotomous variables and Wilcoxon Rank-sum test for continuous variables. Bonferonni's corrected threshold for multiple comparisons= 0.05/6 ($p<0.0083$). p values in bold depict significant differences at $p<0.0083$

At the group level, the 102 patients (58% of the sample) who recovered IC did so in a median time of 40 days following injury. The 91 patients (52%) who went on to recover FC, did so at 49 days post-injury. Within each of the four above-identified subgroups, the demographic and clinical characteristics have different patterns, as underlined by the significant differences between the groups for all our variables of interest but the time to recovery of FC. Post-hoc comparisons revealed, most notably, that patients in Group 1 (-IC-FC) were younger than Group 2 (+IC-FC), had longer acute length of stay than Groups 3 (+IC+FC) and 4 (-IC+FC) and longer rehab length of stay than Group 3 (+IC+FC). Moreover, patients who recovered IC but not FC within eight weeks (Group 2: +IC-FC) had longer rehab lengths of stay than patients who recovered IC and then FC within eight weeks (Group 3: +IC+FC). The time from injury to recovery of IC and FC for each group is illustrated in Figure 12.

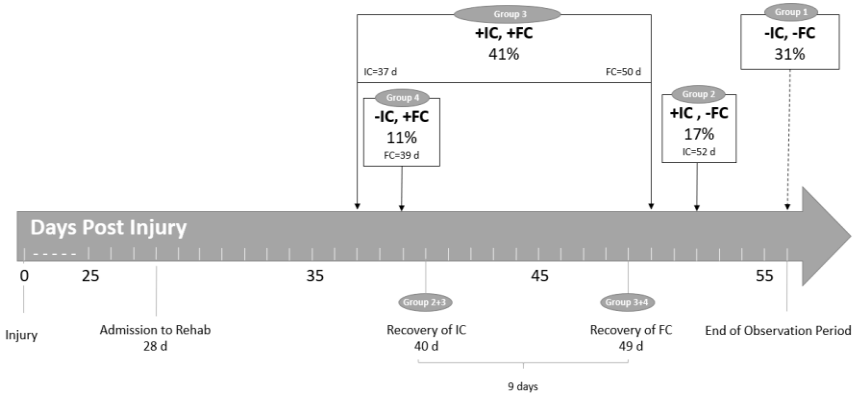


Figure 12 – Timeline of the recovery of communication after injury. Days are reported using medians. Group 1 (-IC-FC): Patients who did not recover communication within the eight-week primary observation period (31% of

the study sample); Group 2 (+IC-FC): Patients who recovered IC but not FC within eight weeks (17% of the sample); Group 3 (+IC+FC): Patients who recovered IC and then FC within eight weeks (41% of the sample); Group 4 (-IC+FC): Patients who recovered FC (without prior evidence of IC) within eight weeks (11% of the sample).

Secondary analyses were performed on 49 patients who did not recover IC or FC by week 8 after admission and 26 who recovered IC but not FC. Among these 75 patients, 16 (21%) recovered FC within 15 [13 – 19] weeks; nine (12%) recovered only IC within 16 [13 – 18] weeks and 50 (67%) recovered neither IC nor FC by discharge. This means that, overall, 52% (n=91) of the whole sample recovered FC within the 8-week rehabilitation program (in a median time of seven weeks post-injury). When taking into account these patients as well as those presenting later recoveries of FC (i.e., past 8 weeks), 61% (n=107) of the patients recovered FC with a median time of 15 weeks after injury. These findings have several implications.

First of all, it was somewhat surprising to identify four different **patterns of communication recovery**. Indeed, without any *a priori* hypothesis, we would expect to see patients either recovering IC and then FC during their rehabilitation, or not. This binary perspective was challenged here by the emergence of groups recovering IC but not FC or recovering FC without any prior evidence of IC. The transition to communication therefore does not appear to be a long calm (and predictable) river. In patients following the 'classic' transition path (i.e., Group 3; +IC+FC), FC emerged about nine days following IC, suggesting IC might be a harbinger of FC. Second, our findings suggest that patients with shorter acute and post-acute rehab **length of stay** are more likely to recover FC by

discharge. This confirms previous results showing shorter acute length of stay in patients who recovered communication as compared to those who did not (Noé et al., 2012). This might be due to the severity of the initial lesions. Shorter acute and post-acute lengths of stay may indeed reflect a less severe initial injury that can reasonably be associated with an increased likelihood of recovering cognitive abilities (such as communication) early on. Third, another surprising finding was the significantly younger **age** in the group of patients who did not recover any evidence of communication during these eight weeks, which contradicts previous works associating younger age with better prognosis (The Multi-Society Task Force on PVS, 1994b). This suggests that in spite of being a prognostic factor, age does not specifically influence recovery of communication. An important fact to consider at this stage is the setting of the study and the local healthcare policies applied. In the USA where this study took place, healthcare is privatized and thereby not every patient has access to the intensive care and rehabilitation facilities. Admission and care in these structures highly depend on the patient's socio-economic status. In practical terms, this means that there is an important selection bias on the front end. Likewise, as hospitals depend on their survival and outcome statistics to obtain funding and maintain a certain level of reputation, they preferentially admit patients with better prognosis in order to report good outcomes for the admitted populations. This further reinforces the selection bias and leads to admitting younger patients in intensive settings. This could partly explain our findings about younger patients not recovering communication: there are probably far more young people admitted to start with, as compared to studies conducted in a setting with public healthcare and inclusive admissions (e.g.,

European countries). Nonetheless, despite this selection process, nearly two thirds overall went on to recover FC, which is encouraging for clinicians and relatives in the early stages of the injury and contributes to refining outcomes for patients admitted to rehabilitation.

Regarding our primary aim, the time range from injury to communication recovery reported here (seven weeks) falls within the broad range reported by previous studies. Interestingly, some patients presented a late recovery of communication (about four months after injury), which has also already been observed previously and has important clinical implications. Indeed, decisions regarding palliative care or referral to rehabilitation are taken at the acute stage, when the clinical picture and prognosis seem hopeless. It turns out however that even patients who are unable to communicate in this post-acute setting on admission to rehabilitation, later improve to communication recovery in more than half of the cases. Referral to rehabilitation should therefore be supported early on. We were unable to include acute patients in an intensive care setting in the present study and overcoming this limitation would provide a comprehensive picture of the prevalence and the time course to communication recovery in severely brain-injured patients. Other limitations include a monocentric retrospective design and strict criteria for presence of communication based on the CRS-R. The scale recommends indeed assessment of communication with situational orientation questions while other administration criteria (e.g., using autobiographical questions) could elicit more responses (Nakase-Richardson et al., 2009).

Nevertheless, this study shows that patients with severe brain damage suggestive of a poor prognosis may achieve this highly relevant recovery milestone during rehabilitation, which should be initiated and encouraged as much as possible. The data presented here provides inpatient rehabilitation clinicians with quantitative parameters and caregivers with realistic expectations for communication recovery. Contributing to a better initial behavioral diagnosis will further guide treatment interventions, as they may differ depending on the patient's level of consciousness.

3. Part Two: Transcranial direct current stimulation as a therapeutic option

"Do not fear failure but rather fear not trying."

Roy T. Bennett

The present section is based on the following articles:

Martens, G., Ibanez-Soria, D., Soria-Frisch, A., Barra, A., Piarulli, A., Gosseries, O., Salvador, R., Rojas, A., Nitsche, M., Laureys, S., Ruffini, G., & Thibaut, A. A novel closed-loop EEG-tDCS approach to promote responsiveness of patients in minimally conscious state: a study protocol. *Submitted*

Martens, G., Kroupi, E., Bodien, Y., Cassol, H., Barra, A., Martial, C., Annen, J., Soria-Frisch, A., Gosseries, O., Ruffini, G., Laureys, S., & Thibaut, A. Behavioral and electrophysiological effects of network-based frontoparietal tDCS in patients with severe brain injury: a randomized controlled trial. *Submitted*

Martens, G., Fregni, F., Carrière, M., Barra, A., Laureys, S., & Thibaut, A. (2019). Single tDCS session of motor cortex in patients with disorders of consciousness: a pilot study. *Brain Injury*, 1-5.

Martens, G., Lejeune, N., O'Brien, A. T., Fregni, F., Martial, C., Wannez, S., Laureys, S. & Thibaut, A. (2018). Randomized controlled trial of home-based 4-week tDCS in chronic minimally conscious state. *Brain stimulation*, 11(5), 982-990.

3.1. Context

While patients with DOC face a critical lack of treatment options, tDCS appears as a valuable adjuvant to their therapeutic management. As opposed to pharmacological options, it does not present a risk of inducing adverse effects such as sleepiness, emesis or agitation. Amantadine, for instance, is the drug presenting the highest level of evidence for post-acute TBI patients with DOC but can reduce the epileptogenic threshold, thereby increasing the risk of seizure. Drugs can also be contraindicated in some cases, partly because of their metabolic interactions. This is not a concern with tDCS, as its action mainly affects cortical areas. Since tDCS is also affordable and easy to administer, it is an optimal candidate for therapeutic applications in patients with DOC.

Prefrontal tDCS has been shown to be efficient to transiently improve the level of consciousness for some patients, especially those in MCS, while repeating the amount of sessions could prolong the duration of its effects (Bourdillon et al., 2019). This latter aspect is not easy to comply with in classic clinical or research settings and **alternative applications** in other environments have not been investigated in patients with DOC.

When reviewing the literature, it appears that a majority of the interventional studies using tDCS focused on stimulating the left DLPFC, partly because of its involvement in many different functions such as attention, working memory and its integrative role in motor control and behavior (D'Esposito et al., 1995, 1998; Heekeren et al., 2006). However, other regions are crucial in consciousness recovery processes. The precuneus, for instance, located in the posterior

parietal cortex and part of internal awareness network is known as a critical hub for consciousness recovery (Laureys et al., 2004). Therefore, Huang et al targeted this area by stimulating the posterior parietal cortex in 33 patients in prolonged MCS using repeated 2 mA tDCS (applied for 20 minutes over five consecutive days). This led to significant clinical improvements as measured by the CRS-R but to a lesser extent than the studies stimulating the DLPFC (Huang et al., 2017). Since **other brain regions** are also important for consciousness recovery or motor output, we want to investigate montages targeting other critical zones.

Likewise, most tDCS studies with DOC patients use conventional unifocal montages with two electrodes: an anode and a cathode, allowing to stimulate a single cortical area. Recent technologic advancements however allow to target larger areas using **multifocal stimulation**. This type of administration enables to target specific cortical areas with a high-definition or to stimulate entire brain networks. This method has however not been applied to patients with DOC, yet.

3.2. Translation into clinical and home use

As for any type of device or drug investigated in clinical research, the endpoint is "Is this efficient, safe and usable in the patient's daily life?". Indeed, tDCS, as many other techniques has been widely investigated in research and clinical settings, but less is known about the applicability of the method in patients' day-to-day routine. The fact that tDCS has to be applied by trained researchers or care practitioners limits its use outside of a hospital or a research facility. This means that since most of the chronic patients with DOC are discharged from hospital or rehabilitation centers to nursing homes or at home, they are unable to benefit from the potential long-lasting effects that characterize repeated stimulations sessions (Boggio et al., 2007; Ulam et al., 2014). In patients with DOC, the repetition of tDCS sessions leads indeed to higher rates of responders and greater amplitude of clinical improvement (Thibaut et al., 2017b; Zhang et al., 2017).

A way to overcome both of these issues of routine applicability and repetition of the sessions is **remotely supervised tDCS**. A controlled remote application could indeed reduce the burden of travelling to a specialized facility, allowing for time gain and larger sample sizes (leading in turn to enough powered studies and diminished dropout rates). However, major aspects have to be addressed on the front end such as safety, compliance and feasibility. To that end Charvet and colleagues provided the scientific community with clearly established guidelines for using remotely-supervised tDCS (Charvet et al., 2015). These are as such: 1) Appropriate training of the persons in charge of applying the

stimulations; 2) Continuous evaluation of the subject's compliance with study inclusion and exclusion criteria; 3) Support with training procedures and manuals of operating procedures; 4) User-friendly electrode preparation and montage; 5) Fixed stimulation parameters; 6) Ongoing assessment of the subject's compliance with the treatment intervention; 7) Continuous monitoring of potential adverse events; 8) Strict procedures for discontinuation of a session or for the study, adapted to each population of patients. These guidelines apply for all types of patients (e.g., attention deficit hyperactivity disorder, depression, multiple sclerosis) but endorse a particular importance for our population of patients with DOC, as these patients are non-communicative in a majority of cases and are therefore unable to provide any feedback on how the stimulation sessions are tolerated. They are also unable to apply the stimulations themselves for obvious reasons; the major cognitive and motor disabilities they face hinder their ability to participate actively in conventional therapies. We still decided to address all these challenges and to conduct a first of its kind home-based tDCS study, with the aim of bringing the tDCS technique that is known to be efficient for a proportion of patients with DOC, directly at their bedside. We used a randomized double-blind sham-controlled crossover design to include patients according to the following criteria: established diagnosis of MCS following an acquired severe brain injury; at least 16 years old; chronic state (i.e., more than three months post-injury); stable vital condition (i.e., no infection, intubation, recent hospitalization). Exclusion criteria were: presence of intracerebral metallic material, pacemaker, uncontrolled epilepsy, central-acting medication and introduction any new kind of treatment during the study period. Patients were screened during a

one-week hospitalization to assess their level of consciousness and their prognosis using advanced neuroimaging and electrophysiological techniques. The study protocol was presented to their relatives and if they were interested and could identify a person responsible for applying the stimulations (e.g., a member of the family, a therapist, a nurse), the patient was included in the trial. The first phase consisted in training this dedicated person (i.e., the stimulation referent) to apply the stimulations independently. The training was composed of 1) watching a video with theoretical introduction and placement instructions; 2) receiving a user guide for placement instructions (available in Appendix 4); 3) observe and video-tape the investigator applying the device and; 4) applying the whole set-up themselves. We used a customized device provided by Cefaly Technology (Belgium) designed for an ease of use (see Figure 13). It consists in a constant battery-driven stimulator, with preprogrammed stimulation settings (i.e., 2 mA, 20 minutes) and an internal clock monitoring the usage (i.e., amount of sessions applied and total time of each session). A set of two devices, one active and one sham was assigned to every new patient included. Only one device was provided at a time to the stimulation referent, in a randomized order. Since the firm was responsible for treatment allocation, patients, stimulation referents and investigators were blinded to the active/sham order.

Figure 13 – Device designed for applying tDCS in a home environment (image provided by Cefaly Technology®)

The protocol consisted in applying the stimulation daily for four consecutive weeks, five times a week for a total of 20 stimulation sessions per period. The two periods, active and sham, were spaced by eight weeks of washout, as presented in Figure 14. The active tDCS sessions used 2 mA direct current for 20 minutes (with 5 seconds ramp-up and ramp-down periods) while the sham ones used 2 mA current for 5 seconds only, with the same ramping scheme. As shown in Appendix 4, the anode had to be placed over F3 corresponding to the left DLPFC according to the 10-20 international EEG placement system (Herwig et al., 2003) while the cathode was placed over the supraorbital contralateral area. During the 4-week stimulation periods, the stimulation referent and the relatives and caregivers had to fill in a detailed questionnaire regarding potential adverse events (AE) as well as anything else they would consider abnormal. An example of this questionnaire, extracted from the Case Report Form, is available in Appendix 5. The investigators performed

CRS-R assessments at baseline (week 0), after the end of the first stimulation period (week 4), after eight weeks of washout (week 12), after the end of the second stimulation period (week 16) and after the last eight weeks of washout (week 24).

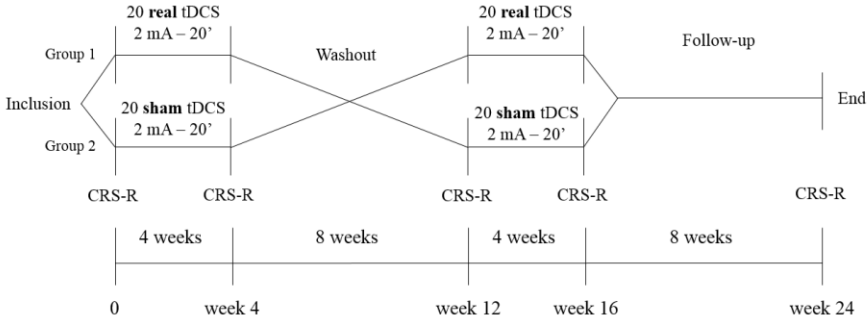


Figure 14 – Study protocol with timeline of assessments. tDCS= transcranial direct current stimulation; CRS-R= Coma Recovery Scale-Revised. From (Martens et al., 2018b)

Our primary outcomes included the change in CRS-R total score after four weeks of stimulation (active *versus* sham). It also included the safety estimation of this type of home-based study assessed by our AE questionnaire (i.e., amount of AEs reported) and the patients' adherence to the treatment, recorded by the device (i.e., ratio between effective stimulations and planned stimulations). Our secondary outcome was the change in CRS-R total score at 8-week follow-up.

For the statistical analyses, we first compared the baseline characteristics of the two groups (active-sham and sham-active) in

terms of age, time since injury, and baseline CRS-R score; using Wilcoxon Rank-sum tests. For the tDCS treatment effect, we checked a potential carryover effect between active and sham conditions using a Wilcoxon match-paired test to compare the CRS-R total scores before active and before sham stimulations. In the absence of a significant difference, the treatment effect was calculated by comparing the CRS-R score difference following four weeks of active tDCS (ΔCRS-R active) and the difference following four weeks of sham (ΔCRS-R sham). The same procedure was applied for the secondary outcome, after the eight weeks of washout using the score difference at week 12 *minus* baseline (active *versus* sham). All the statistical analyses were performed on Stata 13 (StataCorp LP) and results were considered significant at $p < 0.05$. When looking at our other primary outcome, the adherence, we noted that some of the patients did not receive all of the planned stimulations and this accounts for our decision to conduct modified intention to treat (mITT) and per protocol (PP) analyses separately for the tDCS treatment effect. For the mITT, we used all available data meaning the CRS-R scores of the subjects who underwent all the CRS-R assessments (Raine et al., 2005). For the PP, we considered only the scores of the patients who received at least 80% of the planned sessions (Leuchter et al., 2015; Thibaut et al., 2017a). For the two types of analyses, the Cohen's d effect size was calculated as the difference in means and standard deviations between baseline and post-treatment comparing active with sham tDCS. Regarding the other primary outcomes, every AE extracted from the CRF was expressed as a percentage of the total amount of sessions delivered. For the adherence, the effectively applied sessions were expressed as a percentage of the total duration of planned stimulations (i.e., 6 hours and 40 minutes).

After screening 86 patients, 37 were deemed eligible and included in the study after legal representatives agreed and signed informed consent. The study flowchart is presented in Figure 15. During the study, ten patients were excluded due to one of the above-mentioned exclusion criteria. These patients did not significantly differ from the final study sample in terms of age, time since injury and baseline CRS-R (all p's > 0.05). Upon study completion, when consulting the adherence data, five patients were excluded from the PP analysis because they received less than 80% of the planned sessions. The final study sample therefore consisted of 27 patients for the mITT analysis and 22 patients for the PP analysis. No significant differences between the active-sham and sham-active groups were observed (all p's > 0.05).

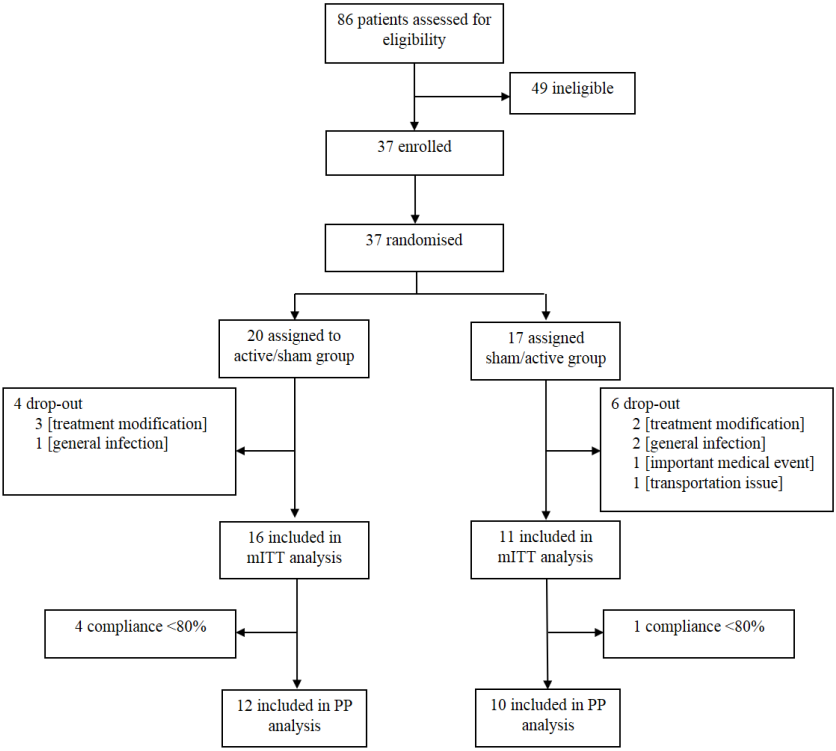


Figure 15 – Study participants flow diagram. mITT= modified intention to treat; PP= per protocol. From (Martens et al., 2018b).

Overall, the stimulations were applied at home by the family for 17 patients and in rehabilitation or nursing homes by the nursing team for 10 patients. All the patients tolerated the tDCS sessions well and no severe AE (i.e., threatening the patient's life) was reported. A total of 13 mild AE were reported: skin redness for 10 patients and sleepiness for three patients; this represents 1% of the total amount of sessions performed (n=946). Regarding adherence, the mean ± SD

of stimulation duration was 94 ± 14% (range: 48 – 130%), as presented in Figure 16. Five patients received less than 80% (16 sessions) of active treatment sessions; three of them were in rehabilitation centers and two were at home. On the other hand, five patients at home received more than the 20 treatment sessions planned.

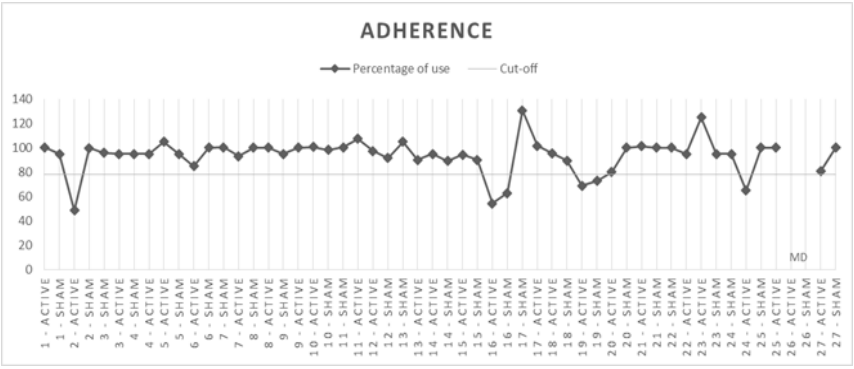


Figure 16 – Percentage of adherence for each patient, for active and sham conditions. Percentage of use expressed over the total time of stimulation planned (i.e., 6 hours and 40 minutes – 20 sessions). The cut-off line represents 80% of the planned stimulation time. MD= missing data. From (Martens et al., 2018b).

Regarding the evolution of the CRS-R total score, no carryover effect was identified between baseline active and sham conditions for both mITT (n=27; Z=1.506; p=0.132) and PP (n=22; Z=0.893; p=0.372) analyses. There was no significant treatment effect at four weeks in the mITT analysis (Z=1.934; p=0.053) while there was one for the PP analysis (Z=2.029; p=0.043). In terms of effect sizes in favor of the active treatment, it was small for the mITT (ES=0.47) and medium for the PP (ES=0.53). For our secondary outcome at 12

weeks (long term effects), there was no significant treatment effect for both mITT (Z=1.263; p=0.207; ES=0.38) and PP analyses (Z=1.884; p=0.060; ES=0.67). The score variation for both types of analyses and both conditions is presented in Figure 17.

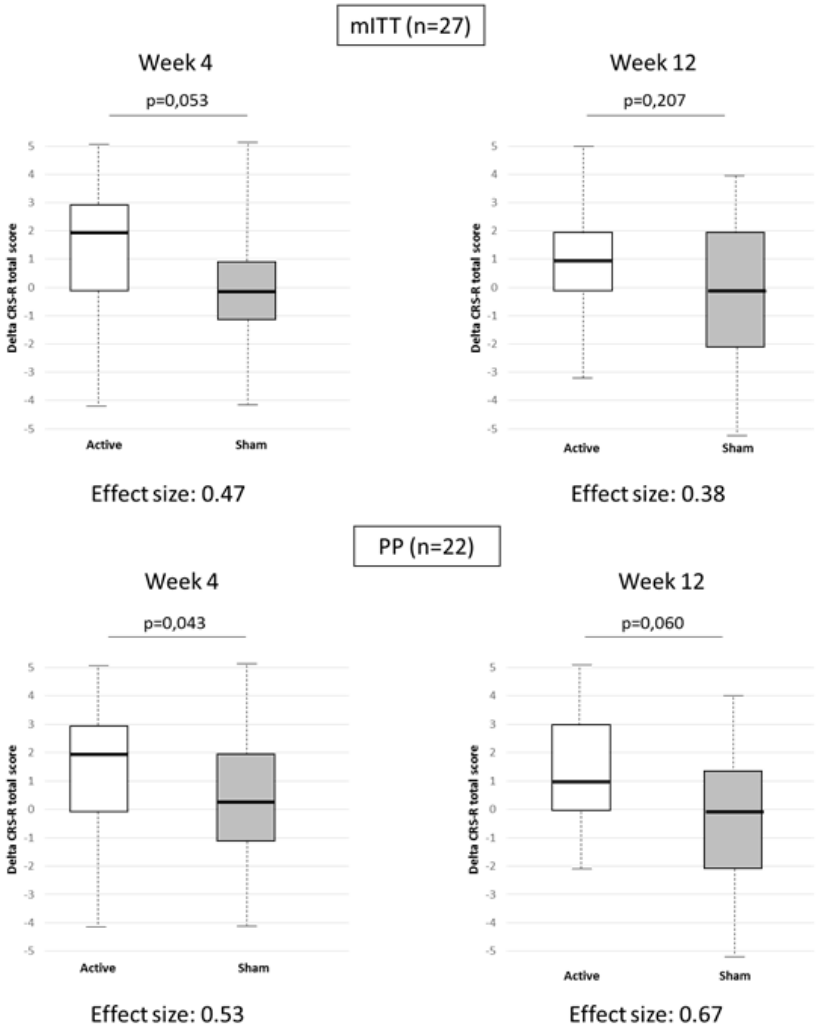


Figure 17 – Boxplots of the CRS-R total score variation for active (in white) and sham (in grey) tDCS, after four weeks of treatment and after 8 weeks of washout. Delta represents post minus pre conditions CRS-R total score. Black lines represent the medians of the delta CRS-R, boxes represent the

interquartile range and dashed lines represent minimum and maximum values. mITT= modified intention to treat analysis; PP= per protocol analysis; CRS-R= Coma Recovery Scale-Revised. From (Martens et al., 2018b).

Individual data for CRS-R scores and adherence is presented in Table 5.

Table 5 – Demographic data, Coma Recovery Scale-Revised scores and adherence data.

ID	Age (gender)	Etiology	Time since injury	Session	CRS-R total score (subscores)			Adherence (%)
					Time 1	Time 2	Time 3	
1	36 (F)	TBI	12 years, 5 mo.	active	10 (3-3-2-1-0-1)	15 (3-4-4-1-1-2)	10 (1-3-2-2-1-1)	100
				sham	10 (1-3-2-2-1-1)	6 (1-1-2-1-0-1)	8 (3-1-2-1-0-1)	95
2	75 (M)	ISCHEMIC STROKE	11 years	active	9 (1-3-1-2-0-2)	11 (2-3-2-2-0-2)	8 (3-0-2-2-0-1)	48
				sham	8 (3-0-2-2-0-1)	8 (1-2-2-1-0-2)	5 (0-1-0-2-0-2)	100
3	35 (M)	TBI	14 years	sham	10 (1-4-4-0-0-1)	11 (3-1-5-1-0-1)	10 (1-1-5-1-0-2)	96
				active	10 (1-1-5-1-0-2)	14 (3-4-5-1-0-1)	15 (3-4-5-1-0-2)	95
4	35 (F)	TBI	5 years, 3 mo.	sham	13 (3-5-1-1-1-2)	15 (3-3-5-1-1-2)	13 (3-3-4-1-0-2)	95
				active	13 (3-3-4-1-0-2)	15 (3-3-5-1-1-2)	16 (3-5-4-1-1-2)	95
5	37 (M)	CARDIAC ARREST	13 years, 11 mo.	active	9 (1-3-2-1-0-2)	5 (1-0-1-1-0-2)	9 (1-3-1-2-0-2)	105
				sham	9 (1-3-1-2-0-2)	6 (1-0-1-2-0-2)	9 (1-3-1-2-0-2)	95
6	32 (M)	TBI	15 years, 4 mo.	active	18 (4-5-5-2-1-1)	19 (4-5-6-2-1-1)	16 (3-5-4-2-1-1)	85
				sham	16 (3-5-4-2-1-1)	19 (4-5-6-2-1-1)	18 (4-5-5-2-1-1)	100
7	33 (F)	TBI	15 years	sham	14 (3-3-5-1-0-2)	14 (3-3-5-1-0-2)	12 (3-3-3-1-0-2)	100
				active	12 (3-3-3-1-0-2)	12 (3-3-3-1-0-2)	13 (3-4-3-1-0-2)	93
8	45 (M)	TBI	33 years, 5 mo.	sham	7 (0-3-2-1-0-1)	8 (1-3-2-1-0-1)	6 (1-1-2-1-0-1)	100
				active	6 (1-1-2-1-0-1)	6 (1-1-2-1-0-1)	9 (1-3-2-1-0-2)	100

9	31 (M)	TBI	5 years, 2 mo.	sham	5 (1-0-1-2-0-1)	4 (0-0-1-2-0-1)	5 (0-0-1-2-0-2)	95
				active	5 (0-0-1-2-0-2)	8 (3-0-1-2-0-2)	8 (3-0-1-2-0-2)	100
10	63 (F)	ANEURYSM	14 years	active	14 (3-1-5-3-0-2)	14 (3-1-5-3-1-1)	15 (3-1-5-3-1-2)	100
				sham	15 (3-1-5-3-1-2)	11 (3-0-5-2-0-1)	12 (3-0-5-3-0-1)	98
11	45 (M)	CARDIAC ARREST	3 years, 10 mo.	sham	10 (2-2-2-2-0-2)	6 (1-1-2-0-0-2)	9 (2-1-2-2-0-2)	100
				active	9 (2-1-2-2-0-2)	10 (2-2-2-2-0-2)	8 (1-1-2-2-0-2)	107
12	55 (M)	ANEURYSM	2 years, 11 mo.	active	11 (1-3-5-1-0-1)	13 (2-3-5-1-0-2)	12 (2-3-5-1-0-1)	97
				sham	12 (2-3-5-1-0-1)	13 (2-3-5-1-0-2)	14 (2-3-5-2-0-2)	91
13	40 (M)	CARDIAC ARREST	10 years	sham	6 (1-1-1-1-0-2)	8 (1-3-1-1-0-2)	6 (1-1-1-1-0-2)	105
				active	6 (1-1-1-1-0-2)	6 (1-1-1-1-0-2)	5 (1-1-1-0-0-2)	90
14	60 (M)	ANEUVRYSM	4 years, 1 mo.	active	2 (1-0-0-0-0-1)	5 (1-1-1-0-0-2)	3 (1-0-1-0-0-1)	95
				sham	3 (1-0-1-0-0-1)	3 (1-0-1-0-0-1)	5 (1-0-1-2-0-1)	89
15	57 (M)	CARDIAC ARREST	8 years, 8 mo.	active	4 (0-0-2-1-0-1)	9 (1-1-5-1-0-1)	9 (1-1-5-1-0-1)	94
				sham	9 (1-1-5-1-0-1)	12 (1-3-5-1-0-2)	9 (1-1-5-1-0-1)	90
16	46 (F)	CARDIAC ARREST	1 year, 5 mo.	active	3 (1-1-0-0-0-1)	4 (1-1-0-1-0-1)	5 (1-1-0-2-0-1)	54
				sham	5 (1-1-0-2-0-1)	4 (1-1-0-1-0-1)	8 (2-2-1-2-0-1)	63
17	33 (M)	TBI	8 years, 7 mo.	sham	8 (2-3-1-0-0-2)	9 (2-3-2-0-0-2)	9 (2-3-1-1-0-2)	130
				active	9 (2-3-1-1-0-2)	12 (2-4-2-2-0-2)	10 (2-3-2-1-0-2)	101
18	55 (M)	CARDIAC ARREST	8 years, 2 mo.	active	9 (1-3-1-2-0-2)	13 (1-3-5-2-0-2)	11 (3-3-1-2-0-2)	95
				sham	11 (3-3-1-2-0-2)	12 (1-3-5-1-0-2)	9 (1-3-1-2-0-2)	89

19	48 (F)	ISCHEMIC STROKE	10 mo.	active	10 (0-3-5-1-0-1)	9 (1-2-5-0-0-1)	7 (0-1-5-0-0-1)	69
				sham	7 (0-1-5-0-0-1)	8 (0-1-5-1-0-1)	10 (1-2-5-1-0-1)	73
20	30 (M)	CARDIAC ARREST	3 years, 4 mo.	active	9 (1-4-0-2-0-2)	7 (2-1-0-2-0-2)	9 (2-4-0-1-0-2)	80
				sham	9 (2-4-0-1-0-2)	9 (2-4-0-1-0-2)	10 (3-4-0-1-0-2)	100
21	38 (M)	CARDIAC ARREST	1 year, 2 mo.	active	5 (1-0-1-2-0-1)	7 (1-1-1-2-0-2)	5 (1-0-1-2-0-1)	101
				sham	5 (1-0-1-2-0-1)	5 (1-0-1-1-0-2)	8 (1-1-2-2-0-2)	100
22	23 (M)	TBI	2 years, 2 mo.	sham	10 (1-3-2-2-0-2)	9 (1-3-1-2-0-2)	5 (0-0-1-2-0-2)	100
				active	5 (0-0-1-2-0-2)	7 (1-0-2-2-0-2)	8 (1-1-2-2-0-2)	95
23	70 (F)	ANOXIA	4 years, 7 mo.	active	15 (3-3-5-2-0-2)	14 (3-3-4-2-0-2)	15 (3-4-4-2-0-2)	125
				sham	15 (3-4-4-2-0-2)	14 (3-3-5-2-0-1)	16 (3-4-6-2-0-1)	95
24	27 (M)	TBI	7 years, 11 mo.	sham	8 (1-3-2-1-0-1)	9 (1-3-2-1-0-2)	12 (3-3-2-2-0-2)	95
				active	12 (3-3-2-2-0-2)	9 (1-3-2-1-0-2)	10 (1-3-2-2-0-2)	65
25	26 (M)	TBI	7 years, 4 mo.	sham	7 (0-3-2-1-0-1)	12 (2-3-5-1-0-1)	11 (1-3-5-1-0-1)	100
				active	11 (1-3-5-1-0-1)	14 (3-3-5-1-0-2)	12 (3-4-2-1-0-2)	100
26	17 (M)	TBI	1 year, 9 mo.	active	13 (3-3-2-2-1-2)	13 (3-3-2-2-1-2)	15 (3-5-2-2-1-2)	MD
				sham	15 (3-5-2-2-1-2)	11 (2-3-2-2-0-2)	13 (3-3-2-2-1-2)	MD
27	42 (F)	CARDIAC ARREST	1 year, 9 mo.	active	7 (1-1-1-2-0-2)	9 (1-3-1-2-0-2)	8 (2-1-1-2-0-2)	81
				sham	8 (2-1-1-2-0-2)	13 (3-5-1-2-0-2)	6 (2-0-1-1-0-2)	100

Adherence (%) expressed as part of the expected total time of stimulation). Time 1= at baseline, Time 2= at 4 weeks (end of the treatment): Time 3= at 12 weeks (8 weeks after the end of the treatment). CRS-R= Coma Recovery Scale-Revised; mo.= months

This study is the first of its kind using a home-based design to deliver repeated tDCS during four weeks to patients in MCS in their daily environment and to investigate the long-term effects. It showed that this administration method is safe, feasible and efficient.

Safety

The low occurrence of AE reported here (1%) confirms the findings from previous works (Boggio et al., 2008; Sacco et al., 2016). If used in proper controlled conditions, according to established safety criteria, tDCS is a safe technique to use, even in patients with major cognitive deficits such as for DOC. These criteria mainly refer to the dose of tDCS received and conventional approaches establish a limit of 40 minutes a day at maximum 4 mA (Nitsche et al., 2003b; Bikson et al., 2016). Our results show that the safety in a home-based setting is similar, if not better, to research environments, were about 12% of AE are reported (itching in a majority of cases) (Russo et al., 2017). Regarding severe AE (i.e., threatening the patient's life), none have been reported here which is, again, in line with the tDCS literature. Bikson et al reviewed indeed the use of conventional tDCS in human trials and found no report of any type of severe AE in a total of 33200 sessions and 1000 subjects with repeated sessions, including vulnerable population such as children, elderly or home users (Bikson et al., 2016).

Feasibility

Delivering tDCS to patients with impaired consciousness already represents a challenge for a researcher, because they cannot

collaborate nor communicate. Involving non-professionals to apply the stimulations by themselves constituted an additional stake. An appropriate training of the stimulation referent was therefore essential. The data relative to the adherence showed us that the average compliance is excellent (94%) indicating that the sessions were correctly performed; but it also appeared that compliance was fairly low in some cases (48%). We therefore followed guidelines aiming at better tackling the issue of missed tDCS sessions during clinical trials for our analyses by conducting both mITT and PP analyses based on a 80% threshold (Thibaut et al., 2017a). The five patients for which the adherence was below this threshold were mostly in rehabilitation centers where the stimulation referents were part of the nursing team. Even though the setup and removal montage time is very short (~5 minutes), it might have been too burdensome to incorporate in the daily care routine. It might be more efficient, in future trials, to apply tDCS during rehabilitation interventions such as physical therapy. Indeed, as most of these sessions have to last at least 30 minutes, the therapist could easily apply the device at the beginning of the sessions and remove it at the end. Furthermore, applying tDCS during this kind of therapy can increase its benefits. Indeed, the addition of motor tDCS to conventional motor training programs in chronic stroke patients leads to a significant improvement in upper limb function; as well as increased grey matter volume in motor and premotor cortices as measured by structural MRI (Allman et al., 2016). Likewise, adding occupational therapy to controlesional cathodal tDCS over M1 leads to a significant motor improvement and functional recovery (Nair et al., 2011). Combining tDCS application with rehabilitation

interventions represents thus an efficient therapeutic approach and could reduce the number of missed sessions.

Unexpectedly, some relatives applied too many stimulations (up to 130% of the planned time). When investigating the devices logs, it appeared they tended to stimulate every day, without interruption, instead of five days a week. This could be due either to misinterpretation of the instructions or to deliberate protocol violation to increase the chances of recovery of their loved one. Even if comprehensible, the latter scenario raises concerns regarding compliance with established safety criteria. Indeed, the rationale for stimulating five times a week (besides feasibility in rehabilitation and nursing centers) relied on limiting the total dose of tDCS received, since a total of 20 tDCS sessions had never been investigated in patients with DOC at that time. The device we used had built-in safety features limiting the application of tDCS to 20 minutes a day; it did not limit its use over the course of a week. It is of course complex to perform ongoing monitoring of the protocol compliance but future trials should make additional efforts in that direction, by sending alerts to the investigators when there is an overuse of a tDCS device (on a daily, weekly or monthly basis), for instance.

Efficacy

Regarding the therapeutic benefits of our home-based repeated protocol, they appeared to be significantly greater for active tDCS than for sham, upon the condition to meet at least 80% adherence. The treatment effect was indeed significant for the 22 patients in the PP analyses, whereas only a trend could be noted for the 27 patients in the mITT group. This implies that continuous

neuromodulation through the DLPFC has moderate clinical effect in this subpopulation. tDCS mechanisms of action suggest long-term neuroplastic changes through modification of NMDA receptors (Nitsche et al., 2003a, 2004) and unmasking of cortical connections (Fregni and Pascual-Leone, 2006; Simis et al., 2014).

Noteworthy, the patients included in the study were extremely chronic; the median time since injury was eight years and maxed out at 33 years. It is still possible to observe improvements at that stage, as cases of late recovery, up to years post-injury, have already been described in the literature (Sara et al., 2007; Estraneo et al., 2010; Schiff, 2010). These cases rely on a common hypothesis of possible axonal regrowth leading to white matter plasticity and recovery of long range connectivity within white matter fiber tracts (Voss et al., 2006). This seems to be particularly true for patients in MCS, who also tend to have a better potential for late recovery overall, as compared to UWS patients (Luaute et al., 2010).

Regarding potential long-term effects of 20 sessions of tDCS (i.e., 8-week follow-up; secondary outcome), our results showed the treatment effect was not significant anymore. This is counterintuitive with regard to previous findings reporting tDCS-related enduring effects one week after tDCS in DOC patients (Thibaut et al., 2017b) and after one month in depression (Boggio et al., 2008) or pain (Fregni et al., 2006). However, MCS patients typically suffer from more extensive brain damage which might explain the need for continuous neuromodulation to maintain the neuroplastic changes. Taken together, these results suggest that, in this population of chronic MCS patients, tDCS should be maintained for the benefits to remain.

Inevitably, there are some limitations in this study that need to be considered before generalizing the results. Regarding the design of the protocol, we had to choose key time points for CRS-R assessments from a feasibility perspective. Therefore, the timeline of these assessments (i.e., at baseline, after four weeks of stimulations and eight weeks after the end of the stimulations) did not allow to evaluate the evolution of behavioral improvements during the four weeks of tDCS, neither how these faded out during the 8-week rest period. In the same vein, the duration of the complete protocol (i.e., six months) led to an important rate of dropout (27%) and a reduced sample size. Treatment amendments and general infections were the most common reasons for exclusion, yet these medical instabilities are likely to happen over such a long period given the frailty of these patients. Regarding home-based monitoring, strictly complying with each single one of the guidelines for remotely-supervised tDCS (Charvet et al., 2015) was tricky, from a feasibility and human resource perspective. There was therefore no daily monitoring of tDCS application and AE collection. This concern was mitigated by the previously established safety of both single and repeated sessions of tDCS (Bikson et al., 2016). Importantly, our aim was to investigate applicability of tDCS in ecological conditions; in the patient's daily routine where, most of the time, relatives have to apply a wide range of treatments themselves (e.g., medication, aerosol therapy, stretching). We thus tried to be as close as possible to clinical reality but balancing this requirement with a safety assessment is a delicate challenge.

Nevertheless, this inaugural home-based trial investigating 20 consecutive sessions of 20 minutes 2 mA tDCS applied over the left DLPFC by trained relatives or caregivers demonstrated that it is

safe, feasible and significantly improves the level of consciousness of patients who adhere to the protocol. These findings pave the way toward involving patients' relatives in the therapeutic management of their loved ones, beyond palliative care only.

3.3. Alternative targets and montages

3.3.1. The motor cortex: stimulate or inhibit?

The primary motor cortex, commonly referred to as "M1" is not only responsible for movement execution but also represents a cortical gateway to deeper structures such as the posterior cingulate cortex, part of the internal awareness network (Vanhaudenhuyse et al., 2011). A tDCS-fMRI study showed indeed increased connectivity in this area as well as in the right DLPFC and the left somatomotor cortex after 10 minutes of tDCS applied over the left M1 (Polanía et al., 2011). This suggests that stimulating M1 might indirectly increase the excitability of distant functionally related areas important for consciousness recovery. Moreover, the direct activation of M1 is of interest as well, given the important motor contribution in the clinical expression of signs of consciousness. There are some specific cases indeed where the patients are unable to show any sign of consciousness at the bedside, not necessarily because they don't have the cognitive capacities to do so but because they may lack the motor abilities to show such signs. This situation of CMD or covert consciousness may lead to a misdiagnosis with the now well-known consequences. An additional confounding issue is the strong reliance of the gold-standard CRS-R on motor abilities, as most of the items require a **preserved motor output** (Giacino et al., 2004). Therefore, stimulating M1 could be a critical option to increase the level of

consciousness and the patient's abilities to show signs of consciousness at the bedside.

To assess feasibility and to evaluate the short-term effect of M1 tDCS, we conducted a pilot randomized crossover sham-controlled trial. The inclusion criteria were as follows: 1) presenting a DOC following a severe brain injury as established by the international guidelines; 2) stable vital condition (i.e., no recent event requiring hospitalization, change in medication or intubation); 3) absence of documented neurological condition prior to the accident; 4) no medication comprising sedative agents, Na^+ or Ca^{2+} channel blockers or NMDA receptor antagonists; 5) absence of metallic cerebral material; 6) absence of craniectomy and; 7) absence of uncontrolled epilepsy.

For each patient, the most affected hemisphere was identified based on medical records and imaging review. The DC Stimulator Plus (Neurocare, Germany), that offers a built-in double-blind mode using code numbers, was used to deliver one active and one sham session of tDCS in a randomized 1:1 order with saline-soaked sponge electrodes (35 cm^2). The anode was placed over either C3 or C4 (the most affected side) based on the 10-20 international placement system (Herwig et al., 2003) while the cathode was placed over the contralateral supraorbital area. The active condition consisted in a ramp-up period of 30 seconds to 2 mA, applied for 20 minutes before ramping down. For the sham condition, the current was ramped up, applied for 5 seconds and then ramped down, to mimic the somatosensory effects of active tDCS (Palm et al., 2013). The two sessions were separated by at least 24 hours of washout. Behavioral effects were assessed at the group level using the CRS-R total score before and after stimulation (i.e.,

primary outcome: treatment effect). Individual response was investigated as well (i.e., responder patients defined as patients showing a new CRS-R sign of consciousness after active stimulation that was not present before, neither before or after sham). Secondary analyses included the assessment of any side effect, the treatment effect in each CRS-R subscale (n=6), computation of effect sizes and the influence of time since injury on the difference in CRS-R total score (i.e., ΔCRS-R) following active stimulation. For the calculation of the treatment effect, we first checked the absence of any carryover effect between active and sham sessions, by comparing the CRS-R total score before active and before sham tDCS (baseline conditions) using a Wilcoxon matched-pairs signed rank test. The treatment effect was then calculated using the same test but comparing ΔCRS-R active (i.e., CRS-R total score after active tDCS *minus* CRS-R total score before active tDCS) and ΔCRS-R sham (i.e., CRS-R total score after sham tDCS *minus* CRS-R total score before sham tDCS). The Wilcoxon's statistic Z was then used to calculate the effect size r using the formula:

$$r = \frac{Z}{\sqrt{2n}}$$

This procedure was applied for the CRS-R total score as well as for the score in every subscale (i.e., auditory /4, visual /5, motor /6, verbal /3, communication /2 and arousal /3). As exploratory analyses, the treatment effect was computed in patients in MCS only (n=6), and the correlation between time since injury and ΔCRS-R active was investigated using Spearman's Correlation test. All statistical analyses were performed using R 3.5.1 (R Core Team, 2008) and results were considered significant at $p < 0.05$.

We included 10 patients, 8 men, 6 MCS and 4 UWS, 49 ± 22 years old, 5 TBI, 7 ± 13 months post-injury. Individual demographic, CRS-R and MRI data are presented in Table 6. At the group level, no carryover was identified. No treatment effect was identified either ($p= 0.55$; $r= 0.1$). At the individual level, two responders were identified (P8 and P9) by newly showing visual pursuit and object localization, respectively, after active tDCS. P8 was a 19-year old man who had a TBI seven months earlier that caused damage in the frontal lobes and the hippocampi. As he was initially diagnosed as UWS, his diagnosis changed to MCS with the presence of visual pursuit following tDCS. P9 was a 64-year old man who suffered from a stroke 28 days before his inclusion in the study affecting the lest insula and the left basal ganglia. Some behavioral improvements were identified after sham stimulation too (e.g., object recognition, response to command), but no patient changed diagnosis. Regarding our secondary analyses, there was no significant treatment effect in any CRS-R subscale (all ps > 0.05), nor in the MCS patients only ($p=0.89$; $r= 0.06$). There was no further influence of time since injury on ΔCRS-R active ($t= –0.291$; $p= 0.78$).

Table 6 – Demographic data, tDCS allocation, CRS-R total scores and main MRI lesions of the study sample.

ID	Age (gender)	Etiol.	TSO (days)	BL Diag.	tDCS Alloc.	CRS-R Total Score (Sub-scores)				Main MRI lesions
						Before Active	After Active	Before Sham	After Sham	
P1	24 (M)	TBI	286	UWS	active/ sham	4 (1-0-0-1-0-2)	4 (1-0-0-1-0-2)	4 (1-0-0-1-0-2)	4 (1-0-0-1-0-2)	left temporo-parietal region
P2	32 (M)	non-TBI	150	MCS	sham/ active	20 (3-4-6-3-1-3)	20 (3-4-6-3-1-3)	18 (3-3-5-3-1-3)	22 (4-5-6-3-1-3)	left frontal subcortical region
P3	68 (M)	TBI	45	MCS	sham/ active	6 (0-1-3-1-0-1)	7 (0-1-3-1-0-2)	4 (0-0-1-1-0-2)	7 (3-1-1-0-0-2)	cerebellum, frontal lobes
P4	70 (M)	non-TBI	12	MCS	active/ sham	7 (0-3-1-1-0-2)	7 (0-3-1-1-0-2)	6 (0-1-2-1-0-2)	9 (0-3-3-1-0-2)	basal ganglia, posterior parietal region
P5	74 (M)	non-TBI	24	UWS	sham/ active	2 (0-0-0-1-0-1)	2 (0-0-0-1-0-1)	2 (0-0-0-1-0-1)	2 (0-0-0-1-0-1)	basal ganglia, left thalamus
P6	21 (M)	TBI	1332	MCS	sham/ active	9 (1-3-1-1-1-2)	9 (1-3-1-1-1-2)	13 (1-3-5-1-1-2)	8 (1-3-1-1-0-2)	frontal and temporal lobes, thalami, left parietal region

P7	51 (F)	TBI	42	MCS	active/ sham	18 (3-3-5-3-1-3)	18 (3-3-5-3-1-3)	15 (3-3-4-3-0-2)	17 (3-3-4-3-1-3)	right frontal lobe
P8 *	19 (M)	TBI	218	MCS	sham/ active	8 (1-3-1-1-0-2)	11 (1-**4**-**2**-2-0-2)	8 (1-3-1-1-0-2)	7 (1-2-1-1-0-2)	frontal lobes, hippocampi
P9 *	64 (M)	non-TBI	28	UWS	sham/ active	6 (1-1-1-1-0-2)	7 (1-**3**-1-1-0-1)	5 (1-1-1-1-0-1)	7 (2-1-1-1-0-2)	left insula, left basal ganglia
P10	68 (F)	non-TBI	39	UWS	active/ sham	4 (0-0-2-1-0-1)	4 (0-0-2-1-0-1)	4 (0-0-2-1-0-1)	4 (0-0-2-1-0-1)	bilateral fronto-parieto-temporal areas, right thalamus

Etiol. = etiology; TSO= Time Since Onset; BL Diag.= baseline diagnosis; CRS-R= Coma Recovery Scale-Revised; TBI= Traumatic Brain Injury; UWS= Unresponsive Wakefulness Syndrome; MCS= Minimally Conscious; *= Responders (i.e., patients showing a new sign of consciousness after active tDCS)

This study aimed at investigating the behavioral effects of M1 tDCS in patients with DOC. We included both patients in UWS and in MCS as they could hypothetically both benefit from this montage. While patients in MCS tend to betted respond to *prefrontal* tDCS as stated above, the effects of *motor* tDCS are still unknown in these subpopulations. Additionally, patients diagnosed as UWS at the bedside might present with CMD and therefore particularly benefit of motor neuromodulation with tDCS.

In our setting, this montage failed to show any significant treatment effect at the group level. This absence of effect can be explained by several hypotheses. First, the **low dose of tDCS** might be a limiting factor. Indeed, it is known that the effects of tDCS can be cumulative and that the number of applied sessions is an important factor for responsiveness in patients with brain injury (stroke, TBI and DOC) (Boggio et al., 2007; Ulam et al., 2014; Thibaut et al., 2017b). Applying more sessions could therefore have positively influenced the behavioral responsiveness of our sample. However, we have been constrained, for safety reasons, to start with investigating one session at a time before increasing the dose since this is a new type of montage and these patients are often unable to provide subjective report and express painful feelings. Given the absence of adverse events in our study, M1 tDCS sessions could be progressively increased in the future. Second, the **outcome measure** we used (the CRS-R), despite being the gold standard for behavioral assessment, might not be sensitive enough for motor related changes. Electromyography, motor evoked potentials or EEG might better reflect some neural changes following tDCS. The third and most important reason simply is the possible **absence of** M1 tDCS **effect** in DOC patients. Even though the dependency on motor behaviors is a

key issue in their management, targeting the motor cortex alone could fail to be an optimum choice. Given the extent of the brain damage that characterizes this population (Guldenmund et al., 2016), efficiently recruiting the motor cortex and distant functionally related areas might be impossible with such a montage.

We could also discuss the rationale of choosing the most affected area for stimulation. This choice was based on tDCS study models for patients with stroke. Indeed, a widely used approach consists in stimulating the affected hemisphere with the anode while decreasing the excitability of the unaffected hemisphere with the cathode to balance the **inter-hemispheric competition** (Murase et al., 2004; Schlaug et al., 2008). However, Thibaut and colleagues recently showed that, at least partial structural and metabolic preservation of the stimulated area would be needed to observe a greater behavioral response in patients with DOC, by comparing neuroimaging data of responders and non-responders (Thibaut et al., 2015c). This suggests that the stroke model cannot be used for our population, and that patients with DOC need optimized montages targeting cortical areas that are preserved in order to stimulate the local synaptic plasticity. When taking a look at the MRI data in our study, it appears a majority of our patients (70%) had lesions potentially involving the motor cortex, located in the frontal lobes, which might explain the low clinical effect overall. Although surprisingly, one of our responders (P8) suffered from structural damage in the frontal lobes, thereby being an exception that proves the rule.

We found another pilot study investigating the behavioral and electrophysiological effects of bilateral M1 tDCS (Straudi et al., 2019). This team applied 10 sessions of 40 minutes 2 mA tDCS with

two anodes over bilateral M1 (and the cathode over the nasion) over two weeks with 10 chronic patients in MCS following a TBI. Behavioral effects as measured by the CRS-R showed significant clinical improvements: median improvement of two points on the CRS-R at the group level and identification of eight tDCS-responders (80%) at the individual level. Electrophysiological effects as measured by low-density EEG showed significantly greater activity in the alpha band following stimulation. This study reports greater behavioral improvements than our trial and is in line with our first hypothesis regarding the lack of dosage. It is however limited by the design: it was an open label with no control condition, which significantly lowers the level of evidence. Moreover, the fact that they included only MCS patients with TBI makes the comparison with our study tricky as the clinical response to tDCS and the prognosis are, respectively, better in these subgroups. The better response to tDCS in this study might also be explained by the higher dose of tDCS applied; 40 minutes of 2 mA tDCS instead of the conventional 20 minutes. Since, again, no severe adverse events have been reported, increasing the dose of received tDCS appears as an interesting path to follow in future trials. Taken together these results suggest that M1 tDCS is a suitable option for DOC and especially MCS patients. While the clinical improvements might appear less significant than for prefrontal stimulation, this type of montages targeting the motor cortex also have been significantly less investigated in our population. A proper randomized controlled trial based on an *a priori* sample size estimation would allow an efficacy comparison.

With regards to our patient population, a crucial component neglected here but that should be considered is the **presence of spasticity**. As mentioned earlier, this motor trouble results from

lesions and anarchic reorganization of the motor neural pathways and is clinically expressed by a pathologically increased muscle activity. Therefore, stimulating the motor cortex might not be suitable for these specific cases and rather inhibiting it could represent a benefit. This hypothesis was tested in another randomized controlled trial using cathodal stimulation over the bilateral M1 to decrease its excitability and thereby potentially reduce spasticity, as measured by the MAS (Thibaut et al., 2019a). Fourteen patients received, both cathodal and sham tDCS with cathodes placed over the bilateral M1 and anodes over the bilateral prefrontal cortex. Spasticity-wise, reduced hypertonia was observed in the finger flexors at the group level, and four responders presented decreased hypertonicity in at least two joints after active and not sham stimulation at the individual level. From the level of consciousness perspective, no significant changes in the CRS-R total score were observed. This means that despite somewhat decreasing the spastic features, decreasing the excitability of M1 using cathodal tDCS did not lead to better expression of signs of consciousness in the end. Spasticity is therefore not the only component affecting motor responsiveness in patients with DOC, and this issue is clearly multifactorial.

Either stimulating or inhibiting the motor cortex to increase motor function or reduce spasticity, the above-presented studies showed that the level of consciousness was not significantly affected by these interventions, possibly because recruiting a cortical region and its functionally related areas requires a more complex setting than the ones used here. Targeting a single cortical area might indeed be too restrictive while recent technological advancements

made multi-site and network-based stimulation possible (Ruffini et al., 2014).

3.3.2. Multifocal stimulation: network-based approach targeting external awareness

A common specificity to all the previous studies using tDCS with DOC patients is that they targeted specific cortical regions with unifocal stimulation while recent technological advancements have made simultaneous multifocal stimulation available, paving the way for **network-based stimulation** (Ruffini et al., 2014). Targeting brain networks could be particularly relevant for DOC patients, not only because brain injury is a largely heterogeneous condition that involves a distribution of cortical and subcortical regions, but also because recovery of consciousness appears to be reliant on specific networks rather than individual regions (Laureys et al., 2000). Two distinct networks have been identified as potential mediators of conscious awareness, which during normal consciousness activate in an alternating fashion (Bodien et al., 2017) and in DOC patients gradually increase with the level of consciousness, in terms of functional connectivity (Threlkeld et al., 2018). The default mode network, encompassing bilaterally the precuneus, the temporo-parietal junction and the medial prefrontal cortex, is functionally related to internal awareness (i.e., stimulus-independent thought or self-related thoughts) (Vanhaudenhuyse et al., 2010). Conversely, the

executive control or external awareness network, located in the lateral frontoparietal regions, relates to external awareness processing (i.e., sensory perception of the environment and cognitive tasks) (Fox et al., 2005; Golland et al., 2007; Vanhaudenhuyse et al., 2011). Given the apparent role of network preservation and recovery in patients with DOC, simultaneous stimulation of multiple regions could result in a more drastic improvement in recovery of awareness than stimulating an isolated single node. To date, network-based stimulation has not been investigated in patients with DOC, and the external awareness network appears as an accessible and optimal target given its role in recovery of consciousness.

In addition to behavioral outcomes, tDCS-related EEG changes in patients with DOC have been investigated, but they focused on coherence (Bai et al., 2018; Guo et al., 2019) or P300 amplitude (Zhang et al., 2017). Although it has been proposed as a deterministic way to quantify consciousness based on algorithmic information theory (Ruffini, 2017), no study has investigated the complexity of the EEG signal following tDCS in DOC patients. The Lempel-Ziv-Welch (LZW) algorithm provides an estimate of brain algorithmic complexity and has been studied in aging (Anokhin et al., 1996; Fernández et al., 2012), as well as in neurological and psychiatric conditions (Li et al., 2008; Fernández et al., 2011; Méndez et al., 2012). It depicts the 'randomness' of the neural signal and thereby the integrity of inter-neural connectivity (Tononi and Edelman, 1998). For instance, LZW increases under ketamine, underlying its psychoactive properties (Li and Mashour, 2019) whereas it decreases under propofol general anesthesia (Schartner et al., 2015) or sleep (Schartner et al., 2017), suggesting a lower level of consciousness induces fewer simultaneous brain oscillations. In

patients with DOC, an EEG-TMS based index (the Perturbational Complexity Index (Casali et al., 2013)) calculated using the LZW algorithm is diminished as compared to healthy controls and can discriminate between UWS and MCS/EMCS (Casarotto et al., 2016). Since the LZW algorithm seems to quantify the level of consciousness, it could further be used to evaluate the effect of an external intervention on brain signal complexity and to better characterize patients responding to tDCS or to predict clinical response to tDCS in patients with DOC.

In light of this, we conducted a new study where we simultaneously stimulated four key regions of the external awareness network (i.e. left and right dorsolateral prefrontal and posterior temporo-parietal cortices) of patients with DOC (UWS and MCS) and EMCS following severe acquired brain injury. We evaluated the behavioral and electrophysiological effects using the behavioral gold standard CRS-R as well as EEG band power and LZW complexity in active and sham conditions.

For this randomized double-blind sham-controlled crossover study, our inclusion criteria were: 1) UWS, MCS or EMCS according to at least three CRS-R assessments conducted within a week; 2) acquired brain injury for more than 28 days before inclusion 3) medical stability (absence of infection, untreated epilepsy, ventilation); 4) free of sedative drugs, Na^+ or Ca^{2+} blockers and NMDA receptor antagonists. Exclusion criteria were: 1) premorbid neurological or psychiatric diseases; 2) metallic cerebral implant (e.g., aneurysmal clip, ventricular shunt) and; 3) craniectomy or cranioplasty. As presented in Figure 18, subjects participated in two sessions, one active (a-tDCS) and one sham (s-tDCS), spaced by 48 hours, in a randomized order. CRS-R assessments and EEG recordings

were performed before and after each session, following the order: CRS-R (~30 min) → EEG (10 min) → a-tDCS/s-tDCS [depending on randomization] (20 min) → EEG (10 min) → CRS-R (~30 min).

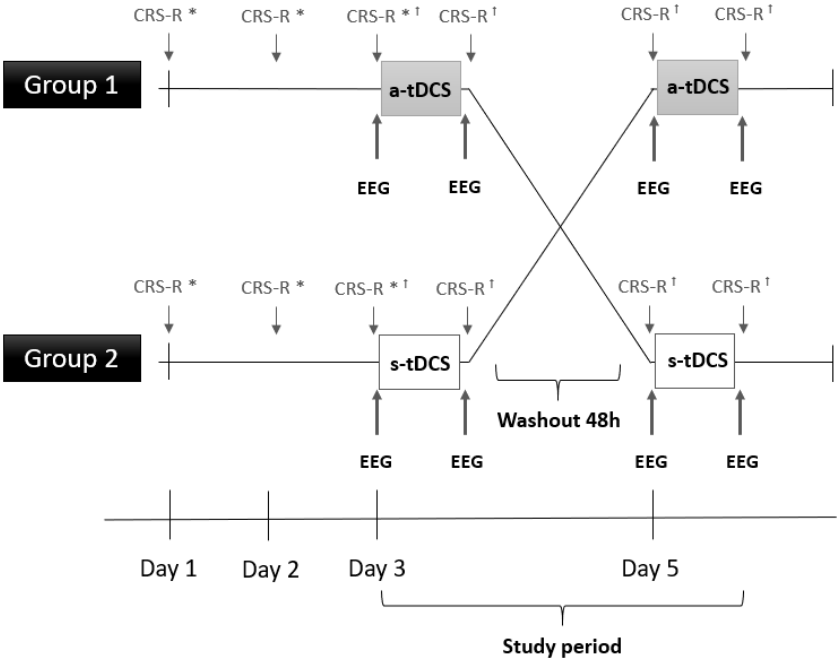


Figure 18 – Study protocol. CRS-R *= assessments taken into account for the baseline diagnosis. CRS-R†= assessments taken into account for the individual tDCS response. a-tDCS= active stimulation; s-tDCS= sham stimulation; CRS-R=Coma Recovery Scale-Revised; tDCS= transcranial direct current stimulation; EEG=electroencephalography

Direct current was applied with the Starstim 8 tDCS system (Neuroelectrics, Spain), a tDCS stimulator capable of measuring EEG

activity (Giovannella et al., 2018). The tDCS montage comprised eight gelled electrodes (3.14 cm² Ag/AgCl): four anodes and four cathodes. Stimulation was delivered over the bilateral frontoparietal areas through the anodes placed on F3-F4 and CP5-CP6 according to the international 10-20 EEG system (Herwig et al., 2003) while the cathodes were placed over the prefrontal and occipital areas on Fp2-Fpz and O1-Oz, as shown in Figure 19. This montage was based on an electrical field simulator and optimizer (Ruffini et al., 2014), targeting the highest field over the bilateral frontoparietal network. Intensity was set to 1 mA per anode, for a total of 4 mA of current delivered per session. For a-tDCS, current was applied for 20 min, preceded by a 30-second ramp-up period and followed by a 30-second ramp-down period for a total session time of 21 minutes. For s-tDCS, 1mA was applied through each anode for 30 seconds, preceded by a 30-second ramp-up and followed by a 30-second ramp-down and 19 min and 30 seconds of no stimulation. Impedances were monitored by the device and kept <10 kΩ and voltage <30 V.

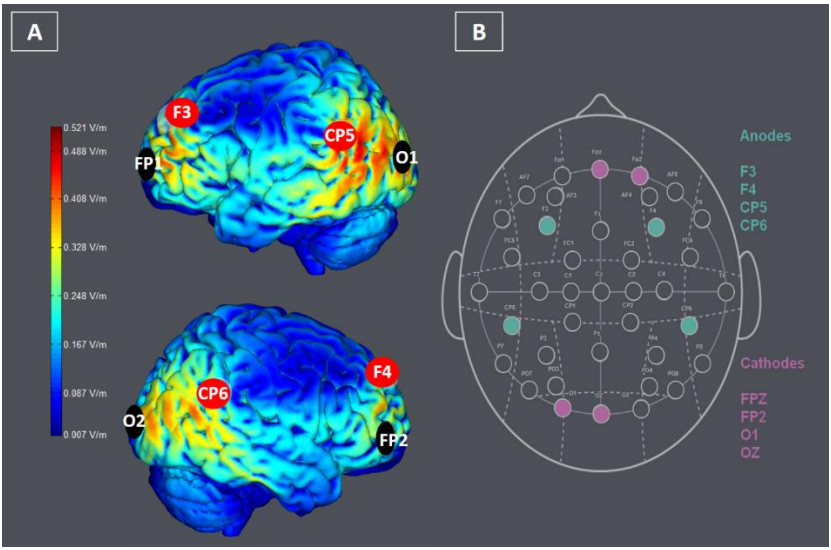


Figure 19 – E-field modelling with anodes in red and cathodes in black (A) and tDCS montage used (B).

The ten minutes resting state EEG was recorded with eyes open (patients were verbally or tactilely stimulated when drowsy) using the Startsim 8 with the same gelled electrodes as the ones used for the stimulation montage (i.e., Fp1, Fpz, F3, F4, CP5, CP6, O1, Oz). The sampling frequency was 500Hz. Two additional sticky electrodes were placed on both mastoids as reference. A random number generator was used for each new patient included to assign conditions in a 1:1 manner. Randomization was performed by a researcher who was not involved in any assessments; thus, the investigators and patients were blinded to the allocation. Behavioral assessments and EEG recordings were carried out by two blinded researchers and when the tDCS device was in “double-blind mode”,

the screen on the device did not display information regarding whether the device is set to a-tDCS or s-tDCS.

Our primary outcome measures concerned the behavioral effects of this new montage using the CRS-R total score at the group level, as well as the individual identification of new conscious behaviors (see Appendix 2) occurring for the first time after (a-tDCS), when taking into account the four CRS-R assessments conducted during the study period (see Figure 18). As described previously, after checking a potential carryover effect by comparing the median CRS-R total scores of the two baseline conditions (before a-tDCS and before s-tDCS) with a Wilcoxon matched-pairs test, we calculated the treatment effect using the same test but comparing the differences in CRS-R total score (i.e., ΔCRS-R; post tDCS *minus* pre tDCS) for both active and sham conditions. We used the same procedure to evaluate the treatment effect in subgroups stratified by diagnosis (i.e., UWS, MCS or EMCS) and by etiology (TBI or non-TBI), as part of our secondary outcomes. For the individual response to tDCS (primary outcome), we divided our sample into three categories, based on their individual responses: 1) “tDCS+”: patients who showed a new sign of consciousness for the first time following a-tDCS; 2) “tDCS=”: patients who neither gained nor lost a sign of consciousness following a-tDCS and; 3) “tDCS-”: patients who lost a sign of consciousness for the first time following a-tDCS. The four CRS-R assessments conducted during the study period were considered for this classification: before a-tDCS, after a-tDCS, before s-tDCS and after s-tDCS. Our secondary outcome measures also included tDCS electrophysiological effect on EEG relative power and LZW complexity, for which the processing is described below. We focused on changes in power and LZW complexity in several frequency bands

(i.e., delta, theta, alpha, beta1 and beta2) after a-tDCS vs s-tDCS in the whole sample, as well as in subgroups stratified by presence (i.e., MCS and EMCS) or absence (i.e., UWS) of consciousness. In order to estimate the difference between the active and the sham condition, the relative power and LZW complexity were estimated for both cases, as the POC with respect to the baseline condition, such as:

$$\frac{Post - Pre}{Pre} \times 100$$

where the Pre refers to the baseline (i.e., pre-stimulation/sham) and Post refers to after stimulation/sham.

For both relative band power and LZW complexity, we compared the median POCs for a-tDCS vs. s-tDCS using Wilcoxon matched-pairs test at the group level and for conscious (MCS and EMCS) and unconscious patients (UWS) separately. As exploratory analysis, we evaluated the potential relationships between our behavioral and electrophysiological outcomes. We first checked for a correlation between the ΔCRS-R and the POCs, in band power and complexity for each band using a Spearman's correlation test, at the group level and for conscious (MCS and EMCS) and unconscious patients (UWS) separately. We then checked for a significant difference between the three responders' groups (i.e., tDCS+, tDCS= and tDCS-) for the POC in power and complexity EEG metrics using a Kruskal-Wallis rank sum test with post-hoc pairwise comparisons using Wilcoxon rank sum test with Bonferroni correction ($p<0.016$), for both active and sham conditions. Finally, we investigated the potential relationship between the baseline EEG metrics (power and complexity before stimulation) and our behavioral outcomes by applying the exact same procedure as for the POC (i.e., check a significant difference between the three responders' groups using a

Kruskal-Wallis test and checked for a correlation between ΔCRS-R and baseline relative power/complexity, using Spearman's correlation at the group level and for conscious and unconscious patients separately). All the statistical analyses were performed on R 3.5.1 (R Core Team, 2008).

The EEG analyses were conducted on Matlab 2016b and Python 2.7. The acquired EEG signals were pre-processed in the following way: the signals were initially band-pass filtered into delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), low beta (beta1: 13-23 Hz) and high beta (beta2: 23-35 Hz) bands using an Infinite Impulse Response Butterworth filter. The data were segmented into 5-sec epochs with 50% overlap, as a compromise for a sufficient number of cycles for all bands and a sufficient number of clean epochs, while dealing with the non-stationary nature of the EEG data. Epochs with amplitude larger than 75uV in each frequency band were considered artifacts (muscular) and automatically excluded from the analysis. Additional channel rejection was performed for each frequency band, based on the median absolute deviation (MAD). Specifically, channels larger than 2.5 MAD values were considered noisy and automatically excluded from the analysis. Moreover, all channels with amplitudes less than 2uV were also considered artifacts and automatically excluded from the analysis. The final clean signals were demeaned and detrended as well as re-referenced to the common average of the clean remaining channels per epoch. After pre-processing the data, the relative band power (with respect to 1-35 Hz) was extracted by computing the power on the filtered signals and integrating over the discrete temporal domain. The LZW was estimated for each frequency band separately as each EEG rhythm is associated with different underlying cognitive functions (Buzsáki, 2006). It was

extracted as an approximate to describe the incomputable algorithmic complexity of the EEG signals under investigation. As described in (Lempel and Ziv, 1976), in LZW we consider a string of characters and alphabet with symbols (typically binary) of length n. The algorithm works by initializing the dictionary to contain all strings of length one and then it scans through the input string sequentially until it finds a string that does not belong to the dictionary and adds it to the dictionary. This process is repeated until all input string has been scanned through. Following this process, we end up with a set of words c(n) that make up the dictionary. The length of the compressed string is $L_{LZW} \leq n$ (an upper bound to Kolmogorov or algorithmic complexity). The description length of the sequence encoded by LZW would have length equal to the number of phrases times the number of bits needed to identify a seen phrase plus the bits to specify a new symbol (to form a new phrase), hence

$$L_{LZW} = c(n)log_2[c(n) + log_2 A] \simeq c(n)log_2[c(n)] \quad (1)$$

The L_{LZW} is normalized by the original string length leading to the final LZW. The input string is binary and is derived by taking the median of the input time series as the threshold as it is a robust metric against outliers, assigning zeros to all values below the threshold and ones to all values above the threshold. It was extracted for all channels of each epoch and frequency band concatenated, targeting to capture the spatially global brain complexity per frequency band, epoch, and patient. The LZW was then averaged across all epochs for each subject to get one complexity value for each subject and frequency band.

After screening 84 patients, we included 46 of them in the study (see Figure 20). The sample comprised 17 patients in UWS, 23

in MCS, and 6 in EMCS with both traumatic (n=22) and non-traumatic (n=24) etiologies. The median [IQR] age was 46 [35 – 59] years; median [IQR] time post-injury was 12 [5 – 47] months. Individual demographic data and CRS-R total scores of the stimulation conditions can be found in Table 7.

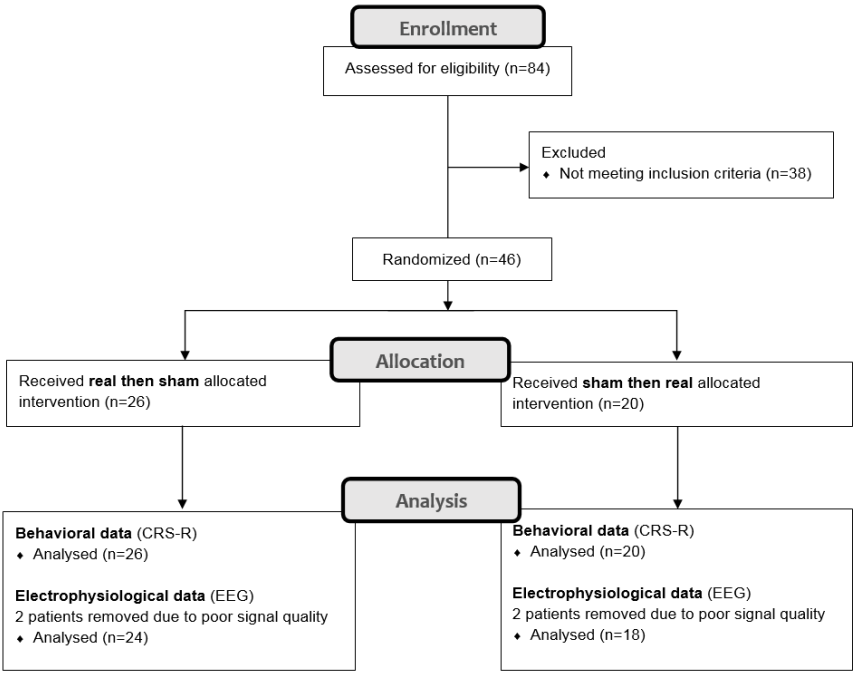


Figure 20 – Flow diagram of the study participants

Patients adequately tolerated all the tDCS sessions (i.e., no burns, skin damage or clinical signs of pain or discomfort) and no

patients dropped-out. We checked a potential significant difference between allocation groups (active-sham vs. sham-active) and there was none regarding age (p=0.308), gender (p=0.766), etiology (p=0.497), time since onset (p=0.317), baseline CRS-R score (p=0.680) and baseline diagnosis (p=0.172), as evaluated by Wilcoxon Rank Sum test for continuous variables, Fisher test for dichotomous variables and Chi-square test for categorical variables.

Table 7 – Individual demographic and clinical characteristics of the study sample

ID	Age (sex)	Diag.	TSO (days)	Etiol.	tDCS Alloc.	CRS-R pre sham	CRS-R post sham	Δ s-tDCS	CRS-R pre active	CRS-R post active	Δ a-tDCS	Behav. resp.
1	50(F)	UWS	188	nTBI	active/ sham	5 (1-0-1-1-0-2)	5 (1-0-1-1-0-2)	0	4 (1-0-1-1-0-1)	5 (1-0-1-1-0-2)	1	tDCS=
2	55(M)	MCS	2557	TBI	active/ sham	14 (2-4-5-1-0-2)	12 (0-4-5-1-0-2)	-2	13 (2-3-5-1-0-2)	13 (2-3-5-1-0-2)	0	tDCS=
3	38(M)	MCS	328	TBI	active/ sham	8 (0-0-5-1-0-2)	8 (0-0-5-1-0-2)	0	10 (0-3-5-1-0-1)	11 (0-3-5-1-0-2)	1	tDCS=
4	47(M)	UWS	32	nTBI	sham/ active	5 (1-0-1-1-0-2)	6 (1-1-1-1-0-2)	1	5 (1-0-1-1-0-2)	5 (1-0-1-1-0-2)	0	tDCS=
5	39(F)	UWS	338	nTBI	sham/ active	4 (1-0-1-0-0-2)	5 (1-0-1-1-0-2)	1	5 (1-0-1-1-0-2)	4 (1-0-1-1-0-1)	-1	tDCS=
6	36(F)	EMCS	2110	TBI	sham/ active	23 (4-5-6-3-2-3)	23 (4-5-6-3-2-3)	0	23 (4-5-6-3-2-3)	23 (4-5-6-3-2-3)	0	tDCS=
7	31(M)	MCS	2603	TBI	active/ sham	9 (2-3-1-1-0-2)	10 (2-3-2-1-0-2)	1	7 (0-3-1-1-0-2)	7 (0-3-1-1-0-2)	0	tDCS=
8	57(M)	EMCS	1401	TBI	active/ sham	16 (3-4-2-3-2-2)	16 (3-4-2-3-2-2)	0	8 (3-0-1-3-1-0)	11 (3-1-1-3-2-1)	3	tDCS+
9	35(F)	MCS	4053	TBI	active/ sham	5 (0-1-2-1-0-1)	7 (0-3-2-1-0-1)	2	7 (0-2-2-1-0-2)	7 (0-2-2-1-0-2)	0	tDCS=
10	35(M)	MCS	4600	nTBI	active/ sham	8 (1-3-1-1-0-2)	9 (2-3-1-1-0-2)	1	9 (2-3-1-1-0-2)	9 (2-3-1-1-0-2)	0	tDCS=

11	43(M)	MCS	11719	TBI	active/ sham	10 (2-3-2-1-0-2)	10 (2-3-2-1-0-2)	0	7 (0-3-2-1-0-1)	7 (0-3-2-1-0-1)	0	tDCS=
12	43(M)	UWS	41	nTBI	active/ sham	5 (1-0-2-1-0-1)	5 (1-0-2-1-0-1)	0	4 (1-0-1-1-0-1)	4 (1-0-1-1-0-1)	0	tDCS=
13	56(M)	UWS	85	nTBI	sham/ active	5 (1-0-2-1-0-1)	5 (1-0-2-1-0-1)	0	6 (1-0-2-1-0-2)	6 (1-0-2-1-0-2)	0	tDCS=
14	45(F)	UWS	43	nTBI	active/ sham	5 (1-0-1-1-0-2)	6 (1-1-1-1-0-2)	1	4 (1-0-1-1-0-1)	4 (1-0-1-1-0-1)	0	tDCS=
15	59(M)	MCS	104	TBI	active/ sham	7 (1-3-1-1-0-1)	7 (1-3-1-1-0-1)	0	5 (1-0-1-1-0-2)	8 (1-3-1-1-0-2)	3	tDCS+
16	20(F)	MCS	621	nTBI	sham/ active	11 (3-0-5-1-0-2)	6 (1-1-2-1-0-1)	-5	5 (0-0-1-2-0-2)	6 (1-1-2-1-0-1)	1	tDCS=
17	26(M)	MCS	3561	TBI	active/ sham	8 (1-3-2-1-0-1)	9 (2-3-1-1-0-2)	1	9 (2-3-1-1-0-2)	14 (4-5-0-2-1-2)	5	tDCS+
18	46(M)	MCS	1394	TBI	active/ sham	10 (3-3-2-1-0-1)	11 (3-3-1-2-0-2)	1	9 (3-3-1-1-0-1)	9 (3-3-1-1-0-1)	0	tDCS=
19	38(M)	MCS	201	nTBI	sham/ active	5 (1-0-2-1-0-1)	5 (1-0-2-1-0-1)	0	6 (1-1-2-1-0-1)	9 (1-3-2-1-0-2)	3	tDCS+
20	62(M)	MCS	170	nTBI	active/ sham	14 (2-1-5-3-1-2)	13 (2-0-5-3-1-2)	-1	17 (3-3-5-3-1-2)	15 (3-3-5-3-0-1)	-2	tDCS-
21	65(M)	UWS	129	nTBI	active/ sham	4 (1-0-1-1-0-1)	5 (1-0-1-1-0-2)	1	4 (1-1-0-1-0-1)	4 (1-0-1-1-0-1)	0	tDCS=
22	57(M)	MCS	246	nTBI	sham/ active	5 (1-1-1-1-0-1)	3 (0-0-1-1-0-1)	-2	6 (0-3-1-1-0-1)	6 (1-2-1-1-0-1)	0	tDCS-

23	46(F)	UWS	148	nTBI	sham/ active	6 (1-0-2-1-0-2)	5 (1-0-1-1-0-2)	-1	5 (1-0-1-1-0-2)	5 (1-0-1-1-0-2)	0	tDCS=
24	60(M)	MCS	2145	TBI	active/ sham	3 (0-0-2-1-0-0)	3 (0-0-2-1-0-0)	0	9 (2-0-5-1-0-1)	13 (2-4-5-1-0-1)	4	tDCS+
25	59(M)	UWS	29	nTBI	sham/ active	4 (1-0-1-1-0-1)	4 (1-0-1-1-0-1)	0	4 (1-0-1-1-0-1)	4 (1-0-1-1-0-1)	0	tDCS=
26	30(M)	MCS	1190	nTBI	active/ sham	5 (1-1-0-1-0-2)	6 (1-1-1-1-0-2)	1	10 (3-4-0-1-0-2)	6 (3-0-0-1-0-2)	-4	tDCS-
27	26(M)	EMCS	116	TBI	active/ sham	16 (4-5-4-1-0-2)	16 (4-4-5-1-0-2)	0	18 (4-5-5-1-2-1)	16 (3-5-4-1-2-1)	-2	tDCS-
28	48(F)	MCS	191	nTBI	sham/ active	9 (1-1-5-1-0-1)	15 (2-4-5-2-0-2)	6	10 (1-1-5-2-0-1)	10 (1-1-5-2-0-1)	0	tDCS=
29	60(M)	MCS	98	nTBI	active/ sham	11 (2-1-4-2-0-2)	8 (2-1-1-2-0-2)	-3	10 (1-3-2-2-0-2)	10 (1-3-2-2-0-2)	0	tDCS=
30	60(F)	EMCS	361	TBI	active/ sham	19 (3-5-6-1-2-2)	19 (3-5-6-1-2-2)	0	16 (3-3-5-1-2-2)	18 (4-4-6-1-1-2)	2	tDCS+
31	36(M)	UWS	806	nTBI	sham/ active	4 (1-0-1-1-0-1)	4 (1-0-1-1-0-1)	0	3 (0-0-1-1-0-1)	5 (1-0-2-1-0-1)	2	tDCS=
32	32(M)	MCS	557	TBI	active/ sham	8 (0-0-5-2-0-1)	8 (0-0-5-2-0-1)	0	11 (3-0-5-2-0-1)	11 (3-0-5-2-0-1)	0	tDCS=
33	20(M)	MCS	388	TBI	sham/ active	15 (3-3-5-2-0-2)	10 (3-3-1-1-0-2)	-5	9 (2-3-1-1-0-2)	9 (2-3-1-1-0-2)	0	tDCS=
34	32(F)	UWS	371	TBI	sham/ active	5 (1-0-1-1-0-2)	5 (1-0-1-1-0-2)	0	6 (1-1-1-1-0-2)	7 (1-1-1-2-0-2)	1	tDCS=

35	60(F)	UWS	304	nTBI	active/ sham	5 (1-1-1-1-0-1)	6 (1-1-1-1-0-2)	1	6 (1-1-1-1-0-2)	6 (1-1-1-1-0-2)	0	tDCS=
36	67(F)	UWS	283	nTBI	active/ sham	5 (1-1-1-1-0-1)	6 (2-1-1-1-0-1)	1	5 (1-1-1-1-0-1)	5 (1-1-1-1-0-1)	0	tDCS=
37	74(F)	UWS	47	nTBI	sham/ active	4 (1-1-0-1-0-1)	4 (1-1-0-1-0-1)	0	5 (1-0-2-1-0-1)	5 (1-0-2-1-0-1)	0	tDCS=
38	70(F)	MCS	1811	nTBI	sham/ active	8 (0-0-5-2-0-1)	14 (2-3-5-2-0-2)	6	16 (4-4-5-2-0-1)	12 (0-4-5-2-0-1)	-4	tDCS-
39	44(F)	UWS	586	nTBI	active/ sham	5 (1-0-1-2-0-1)	6 (1-1-1-2-0-1)	1	8 (1-1-2-2-0-2)	7 (1-1-2-2-0-1)	-1	tDCS=
40	48(F)	MCS	476	TBI	active/ sham	9 (1-3-2-2-0-1)	9 (1-3-1-2-0-2)	0	11 (3-3-1-2-0-2)	9 (1-3-1-2-0-2)	-2	tDCS-
41	59(F)	UWS	37	nTBI	active/ sham	4 (0-1-1-1-0-1)	4 (0-1-1-1-0-1)	0	4 (0-1-1-1-0-1)	4 (0-1-1-1-0-1)	0	tDCS=
42	21(F)	EMCS	169	TBI	sham/ active	14 (4-3-5-1-0-1)	17 (4-4-5-1-2-1)	3	16 (3-4-5-2-0-2)	18 (3-5-5-1-2-2)	2	tDCS+
43	77(F)	MCS	2069	TBI	active/ sham	12 (3-3-2-2-0-2)	18 (4-3-6-2-1-2)	6	14 (4-3-2-2-1-2)	11 (3-3-2-1-0-2)	-3	tDCS-
44	60(M)	EMCS	346	TBI	sham/ active	22 (4-5-6-2-2-3)	22 (4-5-6-2-2-3)	0	22 (4-5-6-2-2-3)	22 (4-5-6-2-2-3)	0	tDCS=
45	28(M)	MCS	1564	TBI	sham/ active	12 (3-5-2-1-0-1)	9 (2-3-2-1-0-1)	-3	8 (1-3-2-1-0-1)	10 (2-3-2-1-0-2)	2	tDCS=
46	31(M)	UWS	359	TBI	sham/ active	5 (0-0-2-1-0-2)	7 (1-1-2-1-0-2)	2	5 (0-0-2-1-0-2)	5 (0-0-2-1-0-2)	0	tDCS=

CRS-R scores are depicted as follows: Total Score (Auditory subscore – Visual subscore – Motor subscore – Oromotor/Verbal subscore – Communication subscore – Arousal subscore). Diag.= diagnosis based on 3 consecutive CRS-R assessments; F= Female; M= Male; diag.= diagnosis; UWS= Unresponsive Wakefulness Syndrome; MCS= Minimally Conscious State; EMCS= Emergence from the MCS; TSO= Time Since Onset; TBI= Traumatic Brain Injury: nTBI= non-Traumatic Brain Injury; CRS-R= Coma Recovery Scale-Revised; Δ = post – pre. In the last column, "tDCS+" = patients showing a new sign of consciousness after a-tDCS; "tDCS-" = patients losing a sign of consciousness after a-tDCS and "tDCS=" = patients not gaining nor losing a sign of consciousness a-tDCS, taking into account the 4 CRS-R assessments (pre and post a-tDCS and s-tDCS) conducted during the study period

Behavioral changes

The median [IQR] total CRS-R scores are reported in Table 7. Regarding the changes in the CRS-R total score, no carryover effect was observed between a-tDCS and s-tDCS (p=0.449). When comparing the ΔCRS-R (i.e., CRS-R total score post *minus* pre tDCS) of a-tDCS vs. s-tDCS at the group level (i.e., **treatment effect**; primary outcome), we did not find a significant difference (p=0.915). When stratified by diagnosis (i.e., secondary outcome), no significant treatment effect was observed for either UWS, MCS, or EMCS subgroups (Table 7). When subcategorizing by etiology (i.e., secondary outcome), we did not find a significant treatment effect for TBI or for non-TBI patients, even though patients with TBI did show an overall increase in CRS-R total score (median [IQR] improvement of 2 [0 – 2] points). The test statistics of these group comparisons can be found in Table 8.

Table 8 – Median CRS-R total scores for active and sham tDCS conditions

Sample	Median CRS-R total score						Wilcoxon match-paired	
	Active tDCS			Sham tDCS			Z value	p value
	Pre	Post	median Δ	Pre	Post	median Δ		
All (n=46)	7.5	7.5	0	7.5	7	0	0.107	0.915
UWS (n=17)	5	5	0	5	5	0	1.219	0.223
MCS (n=23)	9	9	0	9	9	0	-0.427	0.669
EMCS (n=6)	17	17	0	17.5	18.5	0	0.108	0.914
TBI (n=22)	9	11	0	10	10	0	-0.638	0.524
non-TBI (n=24)	5	6	0	5	5	0	0.810	0.418

UWS= Unresponsive Wakefulness Syndrome; MCS= Minimally Conscious State; EMCS= Emergence from the MCS; TBI= Traumatic Brain Injury; Pre= score before stimulation; Post= score after stimulation; Δ = Post minus Pre

At the individual level, we identified seven patients who behaviorally improved (i.e., **tDCS+**). Their clinical characteristics and individual behaviors gains can be found in Table 9. We also found out that seven patients behaviorally worsened by losing a sign of consciousness after a-tDCS that was present before, (i.e., **tDCS-** – Table 9). There were no significant differences between the three behavioral response groups (i.e., tDCS+, tDCS= and tDCS-) regarding age (p=0.44), gender (p=0.99), time since injury (p=0.99), etiology (p=0.27) or diagnosis (p=0.56), as assessed by Chi-square tests for categorical variables and Wilcoxon matched-pairs signed-rank test for continuous variables.

Table 9 – Individual clinically relevant behavioral changes

	tDCS+ (n=7)							
ID (allocation)	**Diag.**	**TSO (months)**	**Etiol.**	**Active**		**Sham**		**Behavioral changes (appearing for the first time after active)**
				CRS-R Before	**CRS-R After**	**CRS-R Before**	**CRS-R After**	
P8 (active/sham)	EMCS	47	TBI	8 (3-0-1-3-1-0)	11 (3-1-1-3-**2**-1)	16 (3-4-2-3-2-2)	16 (3-4-2-3-2-2)	Gained functional communication
P15 (active/sham)	MCS	3	TBI	5 (1-0-1-1-0-2)	8 (1-**3**-1-1-0-2)	7 (1-3-1-1-0-1)	7 (1-3-1-1-0-1)	Gained visual pursuit
P17 (active/sham)	MCS	119	TBI	9 (2-3-1-1-0-2)	14 (**4**-**5**-0-2-**1**-2)	8 (1-3-2-1-0-1)	9 (2-3-1-1-0-2)	Gained systematic response to command, object recognition & intentional communication
P19 (sham/active)	MCS	7	nTBI	6 (1-1-2-1-0-1)	9 (1-**3**-2-1-0-2)	5 (1-0-2-1-0-1)	5 (1-0-2-1-0-1)	Gained visual pursuit
P24 (active/sham)	MCS	72	TBI	9 (2-0-5-1-0-1)	13 (2-**4**-5-1-0-1)	3 (0-0-2-1-0-0)	3 (0-0-2-1-0-0)	Gained object localization
P30 (active/sham)	EMCS	12	TBI	16 (3-3-5-1-2-2)	18 (**4**-**4**-**6**-1-1-2)	19 (3-5-6-1-2-2)	19 (3-5-6-1-2-2)	Gained systematic response to command, object localization & functional object use

P42 (sham/active)	EMCS	6	TBI	16 (3-4-5-2-0-2)	18 (3-**5**-5-1-2-2)	14 (4-3-5-1-0-1)	17 (4-4-5-1-2-1)	Gained object recognition
	tDCS- (n=7)							
ID (allocation)	**Diag.**	**TSO (months)**	**Etiol.**	**Active**		**Sham**		**Behavioral changes (appearing for the first time after active)**
				Before	**After**	**Before**	**After**	
P20 (active/sham)	MCS	6	nTBI	17 (3-3-5-3-1-2)	15 (3-3-5-3-**0**-1)	14 (2-1-5-3-1-2)	13 (2-0-5-3-1-2)	Lost intentional communication
P22 (sham/active)	MCS	8	nTBI	6 (0-3-1-1-0-1)	6 (1-**2**-1-1-0-1)	5 (1-1-1-1-0-1)	3 (0-0-1-1-0-1)	Lost visual pursuit
P26 (active/sham)	MCS	40	nTBI	10 (3-4-0-1-0-2)	6 (3-**0**-0-1-0-2)	5 (1-1-0-1-0-2)	6 (1-1-1-1-0-2)	Lost object localization
P27 (active/sham)	EMCS	4	TBI	18 (4-5-5-1-2-1)	16 (**3**-5-4-1-2-1)	16 (4-5-4-1-0-2)	16 (4-4-5-1-0-2)	Lost systematic response to command
P38 (sham/active)	MCS	60	nTBI	16 (4-4-5-2-0-1)	12 (**0**-4-5-2-0-1)	8 (0-0-5-2-0-1)	14 (2-3-5-2-0-2)	Lost systematic response to command
P40 (active/sham)	MCS	16	TBI	11 (3-3-1-	9 (**1**-3-1-	9 (1-3-2-	9 (1-3-1-	Lost reproducible response to command

				2-0-2)	2-0-2)	2-0-1)	2-0-2)	
P43 (active/sham)	MCS	69	TBI	14 (4-3-2-2-1-2)	11 (**3**-3-2-1-**0**-2)	12 (3-3-2-2-0-2)	18 (4-3-6-2-1-2)	Lost systematic response to command & intentional communication

Four CRS-R assessments and the allocation order are considered for the identification of individual behavioral changes: CRS-R before active, CRS-R after active, CRS-R before sham and CRS-R after sham. Subscores in bold depict gained or lost conscious behaviors. CRS-R scores are depicted as follows: Total Score (Auditory subscore – Visual subscore – Motor subscore – Oromotor/Verbal subscore – Communication subscore – Arousal subscore). Diag.= diagnosis; TSO= time since onset; Etiol.= etiology; MCS= Minimally Conscious State; EMCS= Emergence from the MCS; CRS-R= Coma Recovery Scale-Revised. TBI= traumatic brain injury; nTBI= non TBI (e.g., anoxia, stroke or mixed etiologies).

The group-level behavioral effects of frontoparietal tDCS in patients with DOC were not consistent with previous studies that stimulated the left DLPFC in single (Thibaut et al., 2014) or repeated sessions (Angelakis et al., 2014; Estraneo et al., 2017; Thibaut et al., 2017b). Given the fact that this was a new type of montage, we included both UWS, MCS and EMCS patients, in order to check if the level of consciousness affects the tDCS response the way it does for prefrontal tDCS. Therefore, we also analyzed treatment effects in subsamples of subjects, because this finding could have been attributed to the diagnostic or etiological heterogeneity of our sample. Regarding diagnosis, several studies have revealed that patients in MCS are more responsive to tDCS than those in UWS (Angelakis et al., 2014; Thibaut et al., 2014; Cavinato et al., 2019). However, when analyzing the treatment effect in MCS patients only, we did not find any behavioral changes. When looking at the etiology, there were no significant differences in CRS-R total scores changes for TBI and non-TBI patients separately. These findings suggest that the mechanism of injury may not determine clinical improvements following frontoparietal tDCS and tDCS-related improvements most likely depend on the localization of the lesions rather than on the mechanisms of injury, as tDCS responders in prior studies showed greater structural and metabolic preservation in the stimulated areas compared to non-responders (Thibaut et al., 2015c).

In addition to changes in CRS-R total scores, we also looked for clinically relevant behavioral changes at the single-subject level. Seven tDCS-responders were identified, all recovering visual abilities (e.g., visual pursuit, object localization), which suggests a selective effect of frontoparietal tDCS on visual-related behaviors. The fact that we also identified seven patients who lost a conscious behavior

after a-tDCS that was present before the stimulation (i.e., tDCS-group) raises concerns regarding the therapeutic efficacy of the chosen multichannel bihemispheric tDCS montage. The present study is indeed the first one to report this type of response and it would be interesting to better characterize these patients using neuroimaging, and to compare the structural and metabolic cerebral profile of patients who improved versus patients who worsened. This could help identify potential exclusion criteria for future studies that will need to pre-identify appropriate candidates for tDCS. Regarding individual behavioral responses observed after sham stimulation, some notable changes were observed too. Two patients showed indeed new signs of consciousness observed for the first time after sham tDCS (object localization and functional object use, respectively), considering the four CRS-R sessions conducted over the study period. Even though there were less "sham-responders" than "tDCS-responders", this raises the question of whether patients with DOC could present a kind of placebo response due to the sole intervention of placing a cap and electrodes over their scalp. This possible placebo response in DOC has not been discussed in the literature yet while some isolated cases of behavioral changes following sham interventions have been reported (Estraneo et al., 2017; Martens et al., 2019d). A placebo response would indeed need some conscious processing, which is – by definition – challenged in these patients. As a matter of fact, a more likely hypothesis is that these changes are due to spontaneous behavioral fluctuation. It is difficult to isolate tDCS-related effects from these spontaneous behavioral changes and further studies could mitigate this bias and select patients with particularly stable behaviors over time, as

assessed by repeated consecutive CRS-R assessments before assessing the effects of an external intervention.

The absence of a tDCS treatment effect may be related to the multifocal montage used in this study. Indeed, stimulating the frontoparietal network in a bilateral fashion may have paradoxically reduced the benefits of tDCS as a result of inter-hemispheric competition. The principle of inter-hemispheric competition is widely leveraged in rehabilitation (especially for stroke patients (Murase et al., 2004; Bütefisch et al., 2008) and many montages target the affected hemisphere with the anode while decreasing the excitability of the unaffected side with the cathode, leading to significant improvements in therapy (Schlaug et al., 2008) and reduced inter-hemispheric imbalance (Di Lazzaro et al., 2014). In our population, we stimulated both hemispheres with anodes, meaning that, even though our population typically sustains damage to both hemispheres (Guldenmund et al., 2016), the montage may have played a role in inhibiting rather than potentiating the inter-hemispheric balance. This unanticipated mechanistic effect may have led to decreased treatment effects and the emergence of 'paradoxical responders' who are unable to show some conscious behaviors such as response to command or intentional communication after bilateral tDCS. Future studies should investigate the effects of network-based unihemispheric tDCS montages to confirm this hypothesis. Additionally, the location of the cathodes over frontal and occipital areas might also have interfered with network activation by potentially decreasing the excitability of these areas. Another hypothesis for this lower clinical efficacy is related to our aim and not to the montage itself. It might be that increasing the level of consciousness using only a single session of tDCS is

insufficient to actively recruit the whole frontoparietal network and that it requires longer or more complex external interventions. The fact that prefrontal stimulation seems more efficient (as shown by the previously introduced studies) also suggests that tDCS is useful to improve the **behavioral responsiveness** of patients with DOC, that is more closely related to the prefrontal cortex functions (i.e., motor control, working memory, attention, decision-making (Heekeren et al., 2006; Collette et al., 2007; Barbey et al., 2012)). tDCS might therefore be a better option to stimulate patients' responsiveness (through the prefrontal cortex) than to increase patients' consciousness itself (through the frontoparietal network), for which other options targeting deeper subcortical structures could be more optimal.

Electrophysiological changes

Four EEGs could not be recorded due to too bad signal quality (impedances were too high and could not be reduced). Therefore, the EEG analyses were performed on 42 patients (14 UWS, 22 MCS, 6 EMCS, 22 TBI, 20 non-TBI, median [IQR] age: 46 [35 – 59] years; median [IQR] time-post injury: 13 [5 – 54] months). This sample did not significantly differ from the initial one (n=46) in terms of age (p=0.95), gender (p=0.76), time since injury (p=0.97), etiology (p=0.52) or diagnosis (p=0.80), as evaluated by Wilcoxon Rank Sum test for continuous variables, Fisher test for dichotomous variables and Chi-square test for categorical variables.

For the **relative power**, the POC was significantly different between a-tDCS and s-tDCS in the beta2 band only (W=177; p=0.008). Indeed, the median [IQR] POC_{beta2} was significantly greater

for s-tDCS (12.08 [-26.55 – 71.23] %) than for a-tDCS (-5.08 [-36.03 – 47.23] %), as shown in Figure 21. When separating by level of consciousness, there was still a significant difference in POC_{beta2} power, but only in the conscious patients (W=51; p=0.002). Median [IQR] POC_{beta2} power for the conscious patients was -7.10 [-34.19 – 35.77] % after a-tDCS and 12.08 [-21.30 – 88.04] % after s-tDCS. No other significant differences were found in the other bands. The median POC values by level of consciousness are presented in Figure 21. The data for pre, post and POC for relative power and LZW complexity can be found in Appendix 3.

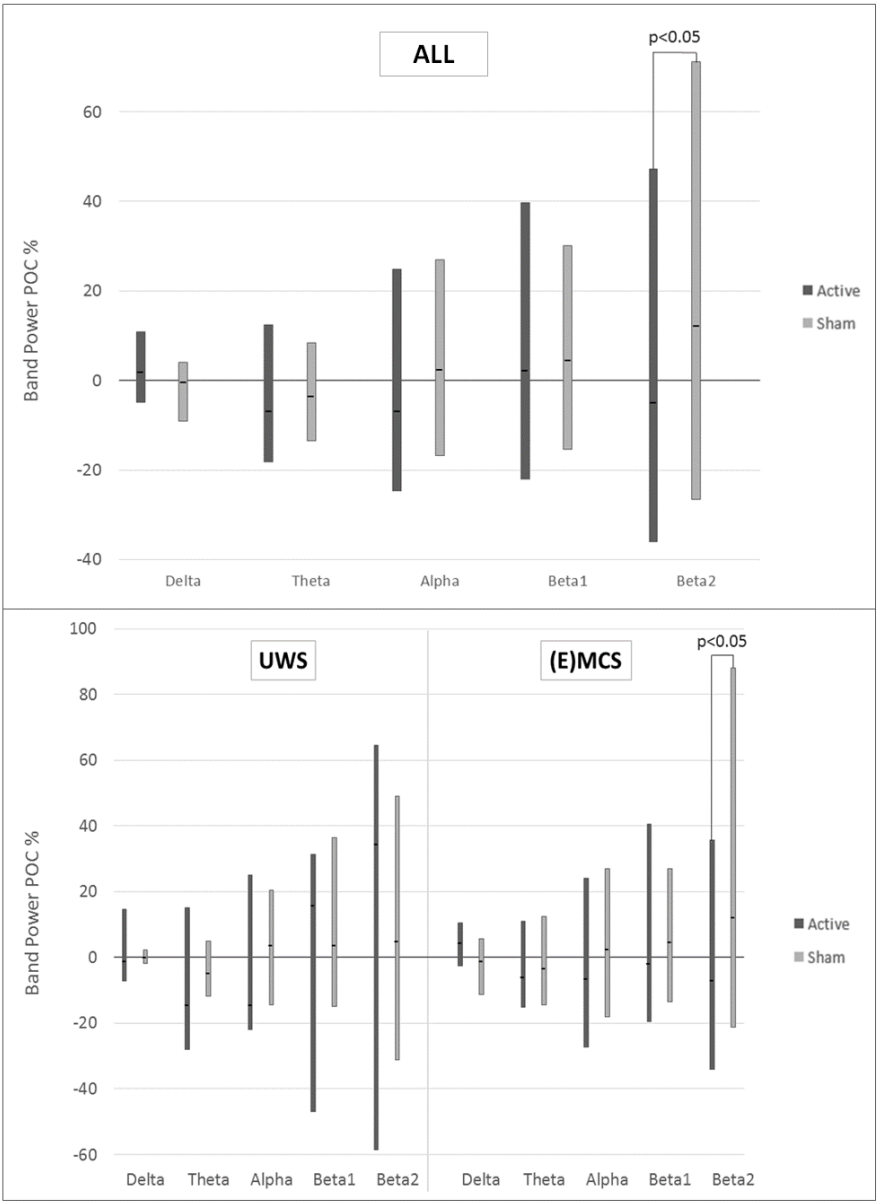

ALL
p<0.05
Band Power POC %
60
40
20
0
-20
-40
Delta
Theta
Alpha
Beta1
Beta2
Active
Sham
UWS
(E)MCS
p<0.05
Band Power POC %
100
80
60
40
20
0
-20
-40
-60
Delta
Theta
Alpha
Beta1
Beta2
Delta
Theta
Alpha
Beta1
Beta2
Active
Sham

Figure 21 – Median percentage of change (POC) between baseline and active/sham tDCS in relative power for each band for the whole sample (n=42; upper part) and for the subsamples of unconscious (i.e., UWS; n=14; lower left part) and conscious (i.e., (E)MCS=MCS and EMCS; n=28; lower right part) patients. The horizontal black lines represent the medians of the baseline (before active stimulation) complexity values and boxes represent the interquartile range. $p<0.05$= significant difference between active and sham with Wilcoxon rank sum test. UWS=Unresponsive Wakefulness Syndrome; MCS=Minimally Conscious State; EMCS= Emergence from the MCS.

For **LZW complexity**, the POC was significantly different between the active and sham conditions in the beta1 band only (W=69; p=0.006), for the whole sample (n=42). The median POC_{beta1} complexity decreased significantly more after a-tDCS (-0.23 [-0.69 – 0.002] %) than after s-tDCS (0.05 [-0.32 – 0.31] %) – see Figure 22. When separating by level of consciousness, there was still a significant difference between a-tDCS and s-tDCS (W=21; p=0.002; -0.18 [-0.72 – 0.001] % after a-tDCS and 0.08 [-0.18 – 0.34] % after s-tDCS) in POC_{beta1} in the conscious patients (MCS and EMCS) but not in the UWS group (median POC_{beta1} active: -0.25 [-0.63 – 0.07] %; median POC_{beta1} sham: -0.11 [-0.65 – 0.30]; W=10; p:0.578), as presented in Figure 22. No other significant differences were found in the other bands.

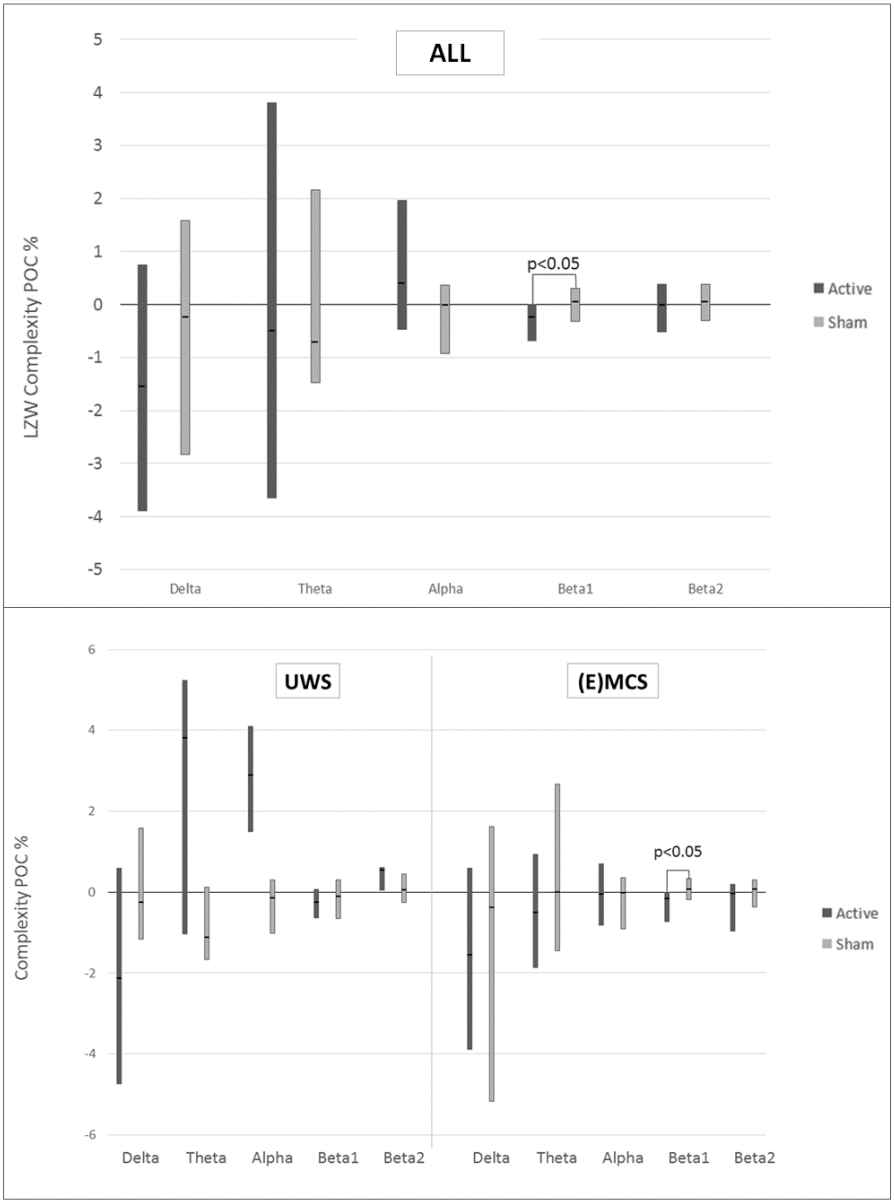

ALL
LZW Complexity POC %
5
4
3
2
1
0
-1
-2
-3
-4
-5
p<0.05
Active
Sham
Delta
Theta
Alpha
Beta1
Beta2
UWS
(E)MCS
Complexity POC %
6
4
2
0
-2
-4
-6
p<0.05
Active
Sham
Delta
Theta
Alpha
Beta1
Beta2
Delta
Theta
Alpha
Beta1
Beta2

Figure 22 – Median percentage of change (POC) between baseline and active/sham tDCS in LZW complexity for each band for the whole sample (n=42; upper part) and for the subsamples of unconscious (i.e., UWS; n=14; lower left part) and conscious (i.e., (E)MCS=MCS and EMCS; n=28; lower right part) patients. The horizontal black lines represent the medians of the baseline (before active stimulation) complexity values and boxes represent the interquartile range. $p<0.05$= significant difference between active and sham with Wilcoxon rank sum test. UWS=Unresponsive Wakefulness Syndrome; MCS=Minimally Conscious State; EMCS= Emergence from the MCS.

The only significantly measurable electrophysiological direct effect of tDCS were thus the decrease in beta1 complexity and the decrease in beta2 relative power. Up to now, only a few studies have used complexity metrics to evaluate the effects of tDCS and none used it for patients with DOC. A previous study compared left/right tDCS and sham while measuring CRS-R and EEG changes. The authors mentioned increased EEG functional connectivity in the beta band activity in the right frontal lobe following right DLPFC tDCS, while in the lower frequency bands (delta and theta), the increased connectivity was widely distributed across the cortex, with no notable behavioral changes for this montage (Wu et al., 2019). Even though this study was limited by a low sample size (i.e., 5 patients in each stimulation group), it contradicts our present findings. The decrease in beta2 could be due to a spectral shift to the delta band, that is the only one showing a power increase in conscious patients. The greater change in power in the beta2 band for the sham stimulation also implies the variability in this band is extremely high.

Relationship between behavioral and electrophysiological metrics

Both band power POC values and LZW complexity POC values following a-tDCS were not significantly correlated with the difference in the CRS-R total score following a-tDCS (ΔCRS-R active), as shown in Table 10.

Table 10 – Statistics of the Spearman's correlation tests

	POC Band Power					**POC LZW Complexity**				
	Delta	Theta	Alpha	Beta1	Beta2	Delta	Theta	Alpha	Beta1	Beta2
	All (n=42)									
rho	0.18	-0.05	-0.11	-0.14	-0.13	-0.07	-0.15	-0.13	-0.02	0.28
p	0.29	0.76	0.51	0.42	0.46	0.71	0.43	0.50	0.92	0.12
	UWS only (n=14)									
rho	0.32	-0.23	-0.15	-0.23	-0.44	-0.66	-0.61	-0.61	0.20	0.58
p	0.28	0.45	0.64	0.46	0.13	0.16	0.14	0.14	0.66	0.13
	MCS & EMCS (n=28)									
rho	0.17	-0.05	-0.18	-0.12	-0.07	-0.01	-0.08	-0.07	-0.03	0.34
p	0.39	0.83	0.39	0.56	0.74	0.97	0.72	0.76	0.89	0.11

Tests performed between the POC values following active stimulation and the difference in CRS-R total score (ΔCRS-R) following active stimulation, for both power and complexity in the sample who had complete behavioral and electrophysiological outcomes. POC= Percentage of Change; LZW= Lempel-Ziv-Welch; UWS= Unresponsive Wakefulness Syndrome; MCS= Minimally Conscious State; EMCS= Emergence from the MCS

There was no significant difference either between the three responders' groups in POC for band power and complexity, as shown in Table 11. However, when further investigating the relationships between baseline EEG metrics and behavioral changes, we found a significant difference between responders' groups ("tDCS+", "tDCS="

and “tDCS-”) in baseline complexity values for the theta (H=6.62; p=0.04) and the beta2 (H=6.29; p=0.04) bands – see Table 11. The median baseline complexity in the theta band was indeed higher for tDCS- (0.270 [0.256 – 0.280]) than for tDCS= (0.251 [0.247 – 0.258]) and tDCS+ (0.246 [0.243 – 0.264]). Post-hoc Bonferroni corrected (p<0.016) pairwise comparisons showed a significant difference between the tDCS= and the tDCS- groups (W=34; p=0.008; tDCS- being higher). This is presented in Figure 23.

Table 11 – Statistics of the Kruskal-Wallis tests performed to compare the three different responders groups (“tDCS+”, “tDCS=” and “tDCS-”)

	POC Band Power					**POC LZW Complexity**				
	Delta	**Theta**	**Alpha**	**Beta1**	**Beta2**	**Delta**	**Theta**	**Alpha**	**Beta1**	**Beta2**
H	1.35	0.76	0.59	0.25	1.19	0.88	0.63	2.37	0.27	1.21
p	0.51	0.68	0.75	0.88	0.55	0.64	0.73	0.31	0.87	0.55
	Baseline Band Power					**Baseline LZW Complexity**				
H	3.40	3.57	2.92	2.88	4.80	4.05	6.62	1.40	2.33	6.29
p	0.18	0.17	0.23	0.24	0.09	0.13	**0.04**	0.50	0.31	**0.04**

Comparison of percentage of change (POC) and baseline values for the active stimulation, for both power and complexity in the sample who had complete behavioral and electrophysiological outcomes (n=42). Values in bold depict a significant difference (p<0.05).

For the baseline complexity in the beta2 band, it was higher in tDCS= (0.606 [0.603 – 0.609]) than in tDCS- (0.605 [0.603 – 0.606]) and tDCS+ (0.602 [0.601 – 0.605]). Post-hoc Bonferroni corrected (p<0.016) pairwise comparisons showed a significant difference between the tDCS+ and the tDCS= groups (W=37; p=0.013; higher in tDCS=).

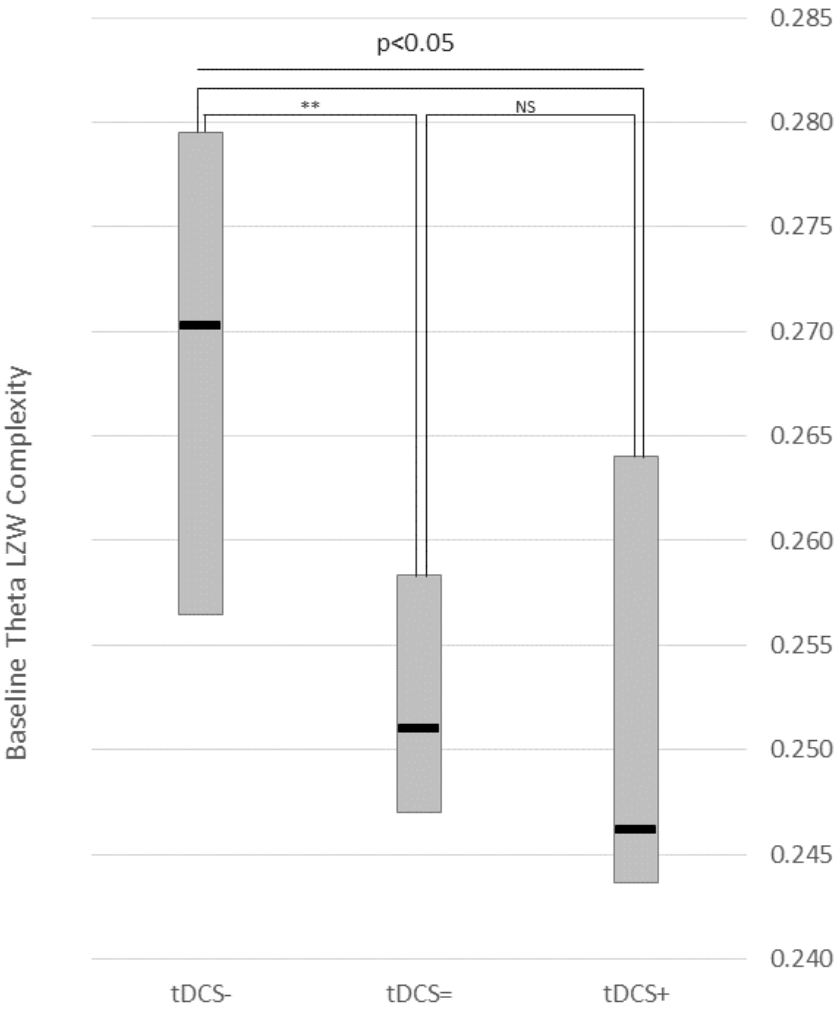


Figure 23 – Baseline LZW complexity values in the theta band for the three responders' groups: tDCS- (i.e., loss of a sign of consciousness following active stimulation); tDCS= (i.e., no loss nor gain of conscious behavior following active

stimulation) and; tDCS+ (i.e., gain of a sign of consciousness following active stimulation). The black lines represent the medians of the baseline (before active stimulation) complexity values and the boxes represent the interquartile range. $p<0.05$= Kruskal Wallis test comparing the 3 responders' groups; **= significant difference for Bonferroni corrected ($p<0.016$) Wilcoxon rank sum test pairwise comparison; NS=non-significant.

When looking at the ΔCRS-R active (i.e., for a-tDCS), we did not find a significant correlation with the baseline values for power or for complexity in the whole sample. When subgrouping by level of consciousness, we found a significant negative correlation between the baseline complexity in theta and the ΔCRS-R for the conscious patients ($r= -0.429$; $p=0.02$), as presented in Figure 24.

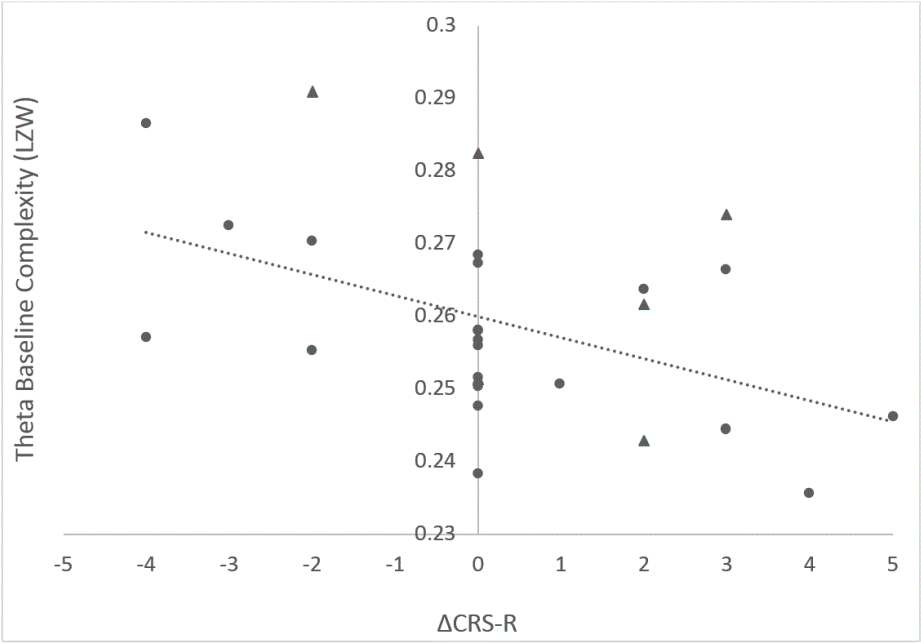


Figure 24 – Correlation between the baseline theta complexity values and the ΔCRS-R (i.e., CRS-R total score post active stimulation minus before active stimulation) in the MCS (n=23; dots) and the EMCS (n=6; triangles) patients. Spearman's rho= -0.429; p=0.02.

The electrophysiological changes were accordingly not correlated to behavioral changes, indicating that some subtle changes in EEG were not translated into behavioral changes. This inconsistency had been reported in the past by studies combining tDCS with electrophysiological measurements that showed interesting effects on functional connectivity. A modulation of cortical global excitability, that was different within UWS and MCS, was measured by TMS-EEG following left DLPFC stimulation (Bai et

al., 2017a). The same authors showed increased frontoparietal coherence in the EEG theta band after the same type of stimulation in MCS only (Bai et al., 2018). These effects were however not paralleled by any relevant clinical improvement but suggest that tDCS can alter connectivity in functional networks. In our study, a unique session of tDCS may have been not sufficient to promote the recovery of new conscious behaviors while still influencing neural activity measured with EEG (e.g., decrease for power and complexity in the beta bands). This absence of significant relationship between behavioral and electrophysiological outcomes does however not apply to our baseline EEG metrics.

Baseline neurophysiological values accurately discriminated between different behavioral response groups. This applies especially for the theta band, for which baseline low complexity appeared as a biomarker for responsiveness. A previous high density EEG-tDCS study with DOC patients also showed increased theta band spatial connectivity (using graph-theory analyses) and higher network centrality in the theta band (indicating a large density of richly connected hub regions) in DLPFC tDCS-responders as compared to non-responders (Thibaut et al., 2018b). In the present findings, patients with higher theta complexity would be more likely to show a paradoxical response to tDCS (i.e., losing conscious behaviors) whereas patients with lower baseline complexity are more likely to show a positive response (i.e., gaining conscious behaviors). This would mean there is a limit to the benefits of having rich theta activity to respond to tDCS; a high complexity (around 0.24) would indicate a high probability to positively respond to tDCS but a too high complexity (around 0.27) would be deleterious, in an inverted complexity-response U-shape. Another hypothesis would be that low

theta complexity at baseline can potentiate a spectral shift from theta to alpha, which would induce a clinical improvement, as suggested by previous works (Williams et al., 2013; Thibaut et al., 2018b). The shift to alpha was not observed here, which can be attributed to the low dose of tDCS and the small number of responders.

Some limitations in this study should be considered before generalizing the results. First, we applied a single session of tDCS while it is now known that behavioral effects of tDCS are enhanced with repeated stimulations (Boggio et al., 2007; Marangolo et al., 2013; Thibaut et al., 2017b). The heavy setup inherent to EEG recording and multifocal stimulation required to be performed at the University Hospital, as opposed to home-based tDCS studies using user-friendly stimulators (Charvet et al., 2015; Martens et al., 2018b; Garcia-Larrea et al., 2019). Second, the fluctuations in vigilance that are characteristic of this population (Candelieri et al., 2011; Piarulli et al., 2016) might have impacted the results, as suggested by changes happening after s-tDCS too. Indeed, it is impossible to exclude the impact of behavioral fluctuation over the results as some patients seem to lose and gain conscious behaviors repeatedly. This concern is nevertheless partly mitigated by the fact that no significant differences were identified between the baseline conditions (i.e., before a-tDCS and before s-tDCS) regarding CRS-R total score, baseline EEG power and baseline EEG complexity.

In conclusion, at the group level, a single session of multifocal frontoparietal tDCS does not induce clinically relevant effects in patients with DOC. The fact that some of the patients improved and other worsened following frontoparietal tDCS underlines the inter-individual variability in response to tDCS that was already observed in

previous studies. These results highlight the need to promote the use of individualized montages that are chosen based on prior structural and neuroimaging findings. To this end, we showed that baseline EEG activity in the theta band could be used to characterize tDCS responders and non-responders' profiles. Optimizing therapeutic approaches for patients with DOC is still a challenge and further efforts should be made toward individualized care and treatments combination (e.g., tDCS during rehabilitation intervention).

3.4. Closing the loop, a brain-state dependent approach

Another common feature concerning all of the above-presented tDCS studies, in DOC patients but in other populations as well, is that the application of the stimulation was performed at an arbitrary moment, in a so-called open-loop fashion, treating the brain as a "black box". As a matter of fact, the brain acts as a complex generator of behavior in an environment composed of inputs and outputs (Zrenner et al., 2016). The important variability in individual response to tDCS might partly be explained by this limiting approach (Wiethoff et al., 2014; López-Alonso et al., 2015). Using the brain's own output to trigger a future input (i.e., closed-loop model) represents a way to overcome this limitation. It is particularly relevant for tDCS since the **brain state** can condition its efficacy. For instance, during a choice reaction task, tDCS applied over the frontal gyrus produces increased salience network activation whereas during resting state, only activity in the default mode network is observed with deactivation of the salience network, as measured with fMRI in healthy subjects. Task-dependent effects of tDCS have been confirmed in others trials with cognitive testing on working memory in healthy subjects (Wu et al., 2014; Gill et al., 2015). The challenge of brain-state dependent tDCS efficacy is of particular importance for patients with DOC, who typically present **fluctuations in vigilance** impacting their responsiveness to external stimuli (Schiff, 2005; Schnakers et al., 2009, 2014; Cruse et al., 2013; Giacino et al., 2014; Piarulli et al., 2016). One of the surrogate markers of this level of vigilance is the spectral entropy of the EEG, which measures the

disorder characteristic of the irregularity, the complexity and the unpredictability of the signals (Palanca et al., 2009). It is known to be greater when individuals are in completely alert states, as compared to sleep (Mateos et al., 2018) or anesthesia (Bein, 2006). In DOC patients, this index correlates with the CRS-R total score, as measured in 56 patients with DOC for both chronic and acute states and both traumatic and non-traumatic etiologies (Gosseries et al., 2011). Piarulli and colleagues used the spectral entropy measured with resting EEG in six UWS and six MCS patients to highlight the periodicity in its fluctuation. They suggested that the EEG spectral entropy variability in MCS could mirror the fluctuation of vigilance previously described in this population. In this study, the authors showed that patients in MCS present a periodicity of 70 minutes in these fluctuations (range 57-80 minutes), comparable to the fluctuations in attention observed in healthy controls, while patient in UWS do not present this type of periodicity (Piarulli et al., 2016). A key component to tDCS responsiveness might therefore be the timing of the stimulations, that could also explain the inconsistent rate of responders reported in previous trials. Administering tDCS during specific time windows (i.e., periods of low or high arousal) could therefore influence its clinical efficacy in patients in MCS since it is known that the positive effects of tDCS are dependent on the brain state (Zrenner et al. 2016). To this end, recent advances in tDCS software and hardware enable the implementation of a closed-loop set-up by complex computations being performed in real-time. Proof-of-concept studies showed the efficiency of such approaches using EEG patterns to trigger tDCS in both animal models of epilepsy (Berényi et al., 2012) and healthy subjects (Leite et al., 2017). This brain state-dependent use of tDCS has never been investigated in

DOC patients, yet. Using this technology to target specific levels of vigilance when applying tDCS could give insight into patterns of responsiveness and optimize future applications. Based on these hypotheses, we wanted to test a closed-loop system using EEG-arousal measures (spectral entropy) to define the best moment of the day for the application of tDCS in patients in MCS. We therefore designed a protocol for a new randomized controlled trial aiming at investigating whether tDCS applied during high vigilance states is more effective in increasing the level of conscious awareness than during low vigilance states and/or sham stimulation in patients in MCS, as measured by behavioral and electrophysiological metrics.

Our primary outcome will be the changes in power spectra in all relevant frequency bands (1 – 35 Hz). We hypothesize to observe a greater shift from lower to higher frequencies
following active tDCS applied at high vigilance, as compared to tDCS applied at low vigilance and sham tDCS. Our secondary outcome will be the behavioral improvement measured with the CRS-R after stimulation. We hypothesize a greater increase in the CRS-R total score as well a larger number of responders in patients receiving tDCS during high vigilance states, than for the two other conditions.

Inclusion criteria will be as follows: centrally-active medication stable for at least a week; stable diagnosis of MCS (no diagnosis change based on two CRS-Rs performed within one week); adult (16 years old - 65 years old); at least three months post-injury. Exclusion criteria will be: open craniotomies; ventriculo-peritoneal shunt under the stimulated area (prefrontal cortex); pacemaker; metallic cerebral implant; severe medical condition(s) that might influence clinical diagnosis and EEG activity (e.g., severe hepatic

insufficiency or renal failure, or sub-continuous or abundant epileptiform discharges on standard EEG recordings).

We conducted an a priori sample size estimation based on the individual CRS-R data relative to the chronic (i.e., >3 months post-injury) MCS patients included in our previously published randomized clinical trial testing the effect of a single prefrontal tDCS session (Thibaut et al., 2014). The effect size in favor of the active tDCS treatment for this subsample of 21 patients was 1.03 (mean ± SD of the CRS-R total score difference for the active group: 1.048 ± 1.244; for the sham group: -0.095 ± 0.889). Based on this effect size and a power of 0.90 with an alpha error probability of 0.05, the sample size was estimated at 13 patients. To compensate for the potential amount of dropouts (20% based on our previous experience), we will include 16 patients.

The tDCS closed-loop system that will be used is a customized version of the Starstim 20 (Neuroelectrics, Barcelona) that enables generation of complex tDCS patterns driven by real-time analysis of EEG dynamics. As presented in Figure 25, the Closed-Loop Manager (CLM) receives the EEG streaming, analyzes vigilance levels in real-time, and remotely commands tDCS stimulation. CLM connects to Starstim's software suite (NIC) and receives via Lab Streaming Layer (LSL) the EEG measured in real time. LSL provides accurate synchronization and time-stamping of received EEG samples. Samples are filtered, buffered, cleaned and split into short-time epochs. Replicating the study conducted by Piarulli and co-workers (Piarulli et al., 2016), the spectral entropy time-courses are analyzed at midline electrodes Fz, Cz and Pz.

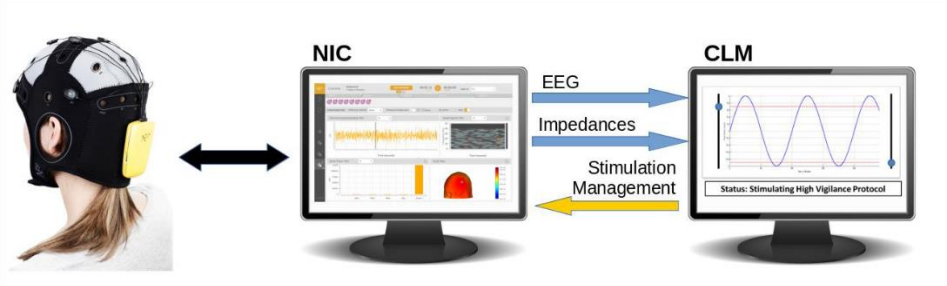


Figure 25 – Closed-loop system hardware and software setup, provided by Starlab Barcelona.

CLM monitors vigilance levels at a 1-minute rate remotely commanding NIC to launch two different stimulations protocols when low or high pre-set vigilance thresholds are reached. In order to ensure patient's safety, CLM limits the total stimulation dose and continuously monitors optimal impedance levels at stimulation channels.

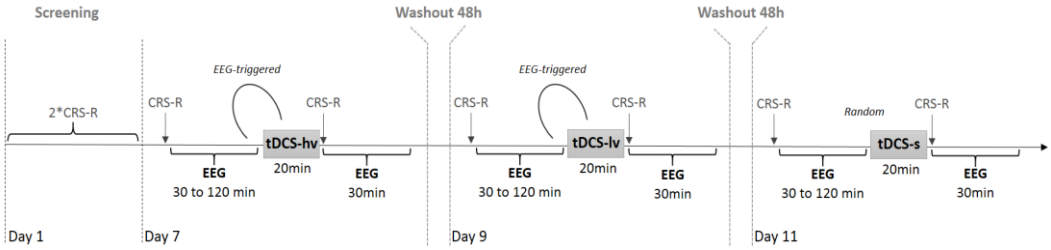


Figure 26 – Study Protocol consisting in a screening phase with two CRS-Rs to confirm the MCS diagnosis and a study phase with three different sessions applied in a randomized order: tDCS-hv = tDCS applied at high vigilance state using the closed-loop system computing the EEG spectral entropy in real time; tDCS-lv= tDCS applied at low vigilance state with the same system and; tDCS-s = sham tDCS applied at a random moment.

This study will be a double-blind sham-controlled study, using a crossover design with three sessions performed on three different days spaced by at least 48 hours: 1. tDCS applied at high vigilance levels; 2. tDCS applied at low vigilance levels and; 3. Sham tDCS applied at random vigilance levels. Each session will consist of an initial behavioral assessment using the CRS-R, a continuous EEG recording to detect changes in spectral entropy, a stimulation session, and a behavioral assessment with the CRS-R and an EEG recording post intervention. The protocol is presented in Figure 26.

Regarding the intervention, 20-channels EEG will be recorded using the Starstim 20 and the stimulation will be applied using a customized version of the device, designed in collaboration with the company. For the stimulation, anodes are placed over F3, Fz and F4 while the cathodes will be placed over P7, Cz, P8, to target the prefrontal cortex bilaterally and thereby executive functions. We decided to target the whole **executive functional network** to further increase patients' behavioral responsiveness. The seeds for highest

current density were therefore located in the DLPFC bilaterally. The optimized current modelling using Stimweaver (Ruffini et al., 2014) is presented in Figure 27.

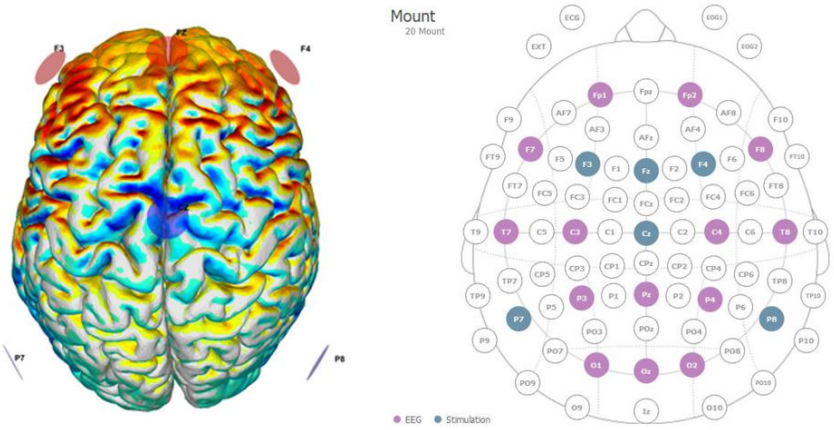


Figure 27 – Optimized stimulation montage based on current modelling (left) and montage that will be used for both EEG recordings and stimulation (right).

Stimulation will be applied for 20 minutes using six 3.14 cm2 Ag/AgCl gelled electrodes, each one delivering an intensity of 1 mA with 15 seconds ramp up/down period. Sham tDCS will consist of applying the same parameters as for active conditions, but the corresponding device will be turned off after 30 seconds, as to mimic the initial sensation of the active current.

Neurobehavioral assessments will be conducted using the Coma Recovery Scale Revised (CRS-R). Demographic and clinical data relating to the past and current medical history will be collected via review of the medical record or discussion with family members and

clinicians familiar with the case to supplement the data acquired from the medical chart.

Statistical analyses on the behavioral data will be performed using Kruskal Wallis rank sum tests to calculate the differences in delta CRS-R between the three conditions (high vigilance, low vigilance and sham). As secondary analyses, we will identify potential responders at the individual level and compute the rates of responders in the three subgroups (i.e., high vigilance, low vigilance and sham). Responders will be defined as patients showing a new sign of consciousness (based on the CRS-R) after active tDCS that was not observed before stimulation or during the baseline screening. We will also investigate the impact of etiology (i.e., traumatic or non-traumatic) and of time since injury using Fisher's test and Spearman correlation, respectively.

Before starting the trial above-presented, we conducted a pilot phase consisting in recording 6-hour spontaneous EEG in patients meeting inclusion and exclusion criteria. The aim was to improve the algorithms and the usability of the software. We conducted successful recordings in seven patients. As a sanity check, we first wanted to see if we could find the same fluctuations in vigilance described by Piarulli and colleagues (Piarulli et al., 2016) using our Starstim closed-loop EEG setup. As shown in Figure 28 as an example, there is a periodicity in the spectral entropy fluctuation, that is similar to the ones previously described. The results further showed that the changes in the spectral entropy of the arousal level in these patients were predictable from the software.

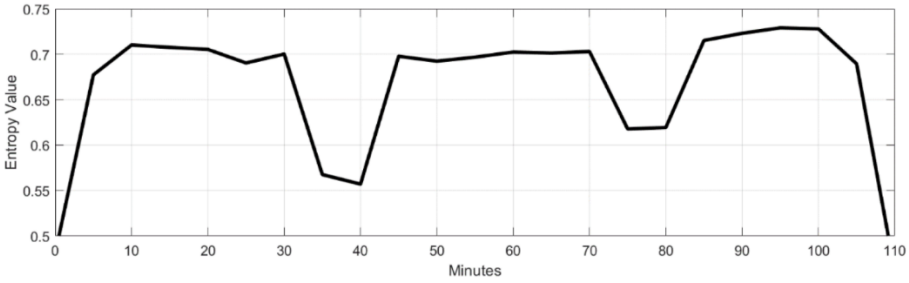


Figure 28 – Evolution of spectral entropy over time, measured in one pilot patient

After these encouraging results regarding usability of the software, the study could move on to the next phase of including patients in the randomized controlled trial. This represents the first step toward a new way to administer tDCS in patients with DOC.

Patients in DOC need targeted individual interventions in order to optimize their responsiveness to external interventions. This study will be the first of its kind to use a closed-loop EEG-tDCS approach to determine the optimal time window to apply stimulation, based on the patient's own vigilance level. Fluctuations in vigilance represent a well-known challenge for clinicians and researchers working with this population. They may prevent optimal diagnostic assessments and therapeutic interventions. Online EEG provides a relatively affordable tool to tackle this issue and to sharpen the use of non-invasive brain stimulation techniques. Several teams already used EEG as an outcome measure to evaluate the effects of tDCS. Combined with transcranial magnetic stimulation to assess cortical excitability, it could underlie differences in tDCS-response between UWS and MCS patients, with a premature decrease in global cerebral excitability in UWS (Bai et al., 2017a).

Differences between these two diagnostic groups have been confirmed by Cavinato et al., who showed that tDCS increases power and coherence on alpha and beta bands in MCS patients, that are correlated with behavioral improvements, while only slower frequencies are affected in UWS (Cavinato et al., 2019). Neuroimaging approaches have therefore a relevant role in identifying different response patterns. Thibaut and colleagues further retrospectively compared structural, metabolic and electrophysiological profiles of known tDCS-responders and non-responders. They showed a greater atrophy in non-responders in regions including the left dorsolateral prefrontal cortex, the medial prefrontal cortex and the left thalamus as compared to responders. The same areas as well as the thalamus were hypometabolic in non-responders as compared to responders (Thibaut et al., 2015c). Regarding EEG brain connectivity, they showed higher theta centrality in responders (as compared to non-responders) meaning this new biomarker can be used as well in order to predict tDCS response in patients with DOC (Thibaut et al. 2018). Metabolic, structural and electrophysiological patterns of responsiveness have therefore been investigated in DOC patients, but the literature is scarce regarding the brain-state dependent application of tDCS, although the effects of tDCS are reliant on the ongoing cortical activity (Ohn et al., 2008). Selectively administering tDCS during specific EEG state patterns reflecting brain states, in a closed-loop fashion, is therefore a promising approach. For patients in DOC, these patterns should reflect the level of vigilance, as these patients are prone to fluctuations, conditioning responsiveness at the bedside (Schnakers et al., 2009; Giacino et al., 2014). The spectral entropy of the frontal EEG signals has been shown to indicate reliably the level

of vigilance (Gosseries et al., 2011), while periodic fluctuations have only been observed in MCS patients (Piarulli et al., 2016). Applying tDCS when the spectral entropy is high (i.e., assuming when the level of vigilance is high) could improve the behavioral responsiveness as measured by the CRS-R, as compared to states of low vigilance.

From a feasibility perspective, recent works and the data presented here showed that scalp-recorded low-density EEG is able to detect patterns of interest based on a pre-defined algorithm and to trigger tDCS within milliseconds time frame (Leite et al., 2017). The implication of feasibility of brain state-triggered interventions hereby goes beyond application of tDCS. A couple of studies on transcranial alternating current stimulation (tACS) used a closed-loop system to modulate specific brain oscillations. During sleep for instance, tACS could be triggered during fast spindles to enhance motor memory consolidation (Lustenberger et al., 2016). The effects of closed-loop tACS in memory consolidation have been confirmed in other studies (Jones et al., 2018; Ketz et al., 2018) and this setup has shown therapeutic benefits in sleep quality (Robinson et al., 2018) and tremor suppression as well (Brittain et al., 2013).

The field of closed-loop application of non-invasive brain stimulation is still subject to important challenges such as development of accurate and optimized triggering algorithms, translation into clinical use and correct identification of inputs to feed the system with. Nevertheless, the clinical applications are multiple, and could be part of the therapeutic options for patients with DOC, which are still limited. The present study will be the first proof of concept toward this application. As patients with DOC cannot be actively engaged in a specific task to prompt their brain to be in an “active state”, using such closed-loop EEG-tDCS approach to

monitor patients' vigilance and determine the most appropriate moment to trigger the stimulation represents an important step forward in the management and care of this population.

4. Discussion and Perspectives

“Your assumptions are your windows on the world. Scrub them off every once in a while, or the light won't come in.”

Isaac Asimov

Patients with DOC following a severe acquired brain injury represent a challenging population to take care of, both from a clinical and a research perspective. The clinician working with them may feel ill-equipped facing the complexity of the clinical picture: typically lying in a bed, with no communicative behavior, requiring a lot of nursing, with a varying number of comorbidities or complications such as spasticity, aphasia or pain (Majerus et al., 2009; Schnakers et al., 2012; Nakase-Richardson et al., 2013) and a very limited amount of treatment options (Thibaut et al., 2019b). From a research perspective, the scientist apprehending this population will also be confronted with the heterogeneity of this group in terms of injury mechanisms, location of the lesions, potential for recovery, responsiveness to the treatments, etc. which makes conducting studies with a high level of evidence, such as randomized controlled trials, difficult. The present thesis had the ambitious aim to tackle both of these issues using a two-step approach: focusing on the diagnosis, and then focusing on the treatment.

While the first step in the care of such dramatic cases is indeed to pin down the diagnosis reflecting the level of consciousness of the patient, even that initial approach is intricate. The high misdiagnosis rates, consistently reported around 40% (Andrews et al., 1996; Childs and Mercer, 1996; Schnakers et al., 2009), can make the clinician reasonably insecure, which in turn impacts the patient's family, the therapy staff and their counseling capacities. Bearing in mind these difficulties encountered on the field, we investigated in Part One how to provide relevant and helpful information to the clinician, using existing data from a specialized rehabilitation setting.

We first focused on a not-so-rare scenario of a patient being admitted to a rehabilitation facility several weeks after a serious injury to the head, with no observable signs of consciousness. The main rehabilitation objectives for this comatose or UWS patient will be to increase his level of consciousness and thereafter improve his functional status. The transition from his state of unconsciousness to a state of consciousness (MCS or EMCS) will therefore be a pivotal point in his management. The earlier this transition will be identified, the better, especially in specific contexts where insurance policies limit the length of stay in specialized facilities. More importantly, the therapeutic management for conscious or unconscious patients will differ, in terms of pain management for instance, responsiveness to treatments and rehabilitation interventions. Our first retrospective study therefore better characterized which behaviors mark this transition and when (Martens et al., 2019b). Thanks to bi-weekly CRS-R assessments collected on a sample of 79 patients, we showed that we can expect to observe the first sign(s) of consciousness within six weeks after injury and after two weeks of rehabilitation. We further showed that visual pursuit was the most prevalent by far, and that in 72% of the cases, only a single subscale of the CRS-R marked this transition. This confirms the key role of visual pursuit as early indicator of consciousness (Dolce et al., 2011). Visual pathways seem therefore to be part of the areas with the most potential for early recovery, through neuroplastic processes and long-range connectivity between the cortex and the brainstem, which is often impacted in the UWS (Laureys et al., 1999; Silva et al., 2010). While interestingly, the traumatic or non-traumatic nature of the injury did not significantly impact these results, there was a notable exception for behaviors with an important motor contribution such as

localization to pain or functional object use, which were significantly more observed in TBI patients. Automatic movements (e.g., scratching nose, grabbing sheets) largely contributed to this difference, possibly due to the fact that they can be triggered either by internal or external stimuli, whereas the other motor behaviors have a stronger reliance on external stimuli only. The pathophysiological differences between TBI and non-TBI in terms of preferential damage of highly oxygen-demanding areas could also drive the greater motor impairment in non-traumatic cases. With these results, we narrowed down the previously reported time window of about three months for consciousness recovery during rehabilitation (Bagnato et al., 2016) to six weeks. We also emphasized the importance of conducting thorough and repeated bedside assessments, as the risk of missing a sign of consciousness can be high since only one of them tends to appear first. This has been underlined by recently updated DOC care guidelines as well (Giacino et al., 2018b).

We then attempted to embrace the perspective of the relatives and the caregivers by investigating, with a similar study design, one of the most anticipated milestones in recovery that is undoubtedly the recovery of communication (Krishnan et al., 2017; Lugo et al., 2017). We showed in a larger sample of 175 patients that it takes between two to four months post-injury to recover functional communication, and that nearly two thirds of the DOC patients analyzed in this study recover it, either during the two first months of rehabilitation or later on. Patients with shorter acute stays and with older age have greater chance to regain this ability. This important step also marks the next transition to EMCS, a clinical state often associated with a confusional state and that is still in need for better

characterization and appropriate care (Nakase-Richardson et al., 2009; Bodien et al., 2019).

Based on these behavioral findings combined with modern neuroimaging tools, clinicians and researchers can now rely on objective data to complement the path to recovery in the subacute phase of a DOC. These two studies are of course limited by their retrospective design and the inherent absence of intervention-based outcomes. It would have been interesting, for instance, to investigate the effects of specific rehabilitation interventions, such as environmental management or the addition of communicative devices and strategies, on communication recovery. Regarding the identification of the most prevalent behaviors denoting consciousness, the next step would be to investigate if any patterns in recovery can be properly identified and clustered (e.g., concurrent recovery of visual pursuit and command-following, recovery of automatic movement only) and whether any of these clusters are significantly associated with a better outcome. Long-term outcome data are difficult to collect, partly because it is easy to lose track of the patients once they are discharged home or to nursing facilities and the repositories are rare. On top of that, collecting data such as the CRS-R requires expertise and time commitment and the burden in terms of travels and human resources is therefore too high. A potential solution is the “tele-diagnosis” using phone-based questionnaires extracted from the CRS-R that are currently being validated in large multi-center trials. Another option is the use of abbreviated scales providing the same diagnostic accuracy than the CRS-R but requiring shorter completion time. Again, validation studies built on the most frequently observed behaviors depicting consciousness are ongoing (Wannez et al., 2017a). This type of tools

could then also be used in constraining settings such as intensive care units. However, these options still require some training and experienced clinicians. An approach addressing these issues has been investigated by Hermann and colleagues (Hermann et al., 2019). Taking advantage of the time spent by the caregivers at the patient's bedside, they used the principle of wisdom of the crowds to pool a large amount of subjective reports regarding the level of consciousness (about 700 ratings from 80 nurses). Based on a visual analog scale quantifying the own feeling about the patient's level of consciousness and compared to the CRS-R, they used receiver operating characteristics curve to assess the diagnostic accuracy of the "DoC-feeling" and report an area under the curve of 0.92. This excellent diagnostic performance encourages further use of this tool to complement the clinician's diagnosis and to multiply the assessments as it is easily implementable in any setting and is way less time-demanding than behavioral assessment at the bedside. Finally, another limitation pertaining to both of these studies is the fact that they were conducted in US healthcare facilities, with the specific healthcare context described above. The population of patients with DOC can therefore not plainly be compared with European ones and the results presented in these two studies may not completely translate to public healthcare settings.

Bearing these limitations in mind, Part One thus contributed to characterizing clinical signs of consciousness, thereby improving the behavioral diagnosis at the bedside in a rehabilitation setting, while also anticipating relatives' expectations and providing objective answers. Moving forward to offering curative treatment solutions, Part Two widely investigated the use of tDCS within the therapeutic options available for these patients.

We first explored different types of environments to perform tDCS. In a randomized controlled trial investigating long-term prefrontal tDCS in chronic MCS patients, we showed that when delivered at home or in a rehabilitation or nursing facility by trained caregivers or relatives, long-term prefrontal tDCS can safely and significantly improve CRS-R scores (Martens et al., 2018b). This has tremendous implications regarding affordability and clinical efficacy of the technique, as we are expanding the environment of tDCS application beyond research and medical facilities. As long as the compliance was satisfying (i.e., patients received at least 80% of the planned sessions), the treatment effect of active 4-week tDCS was significant, suggesting the dose of applied tDCS is another important parameter of responsiveness. This has been confirmed by a previous randomized controlled trial performed with the same type of population (16 chronic MCS patients), where some patients started to show new signs of consciousness after one, two or three days of consecutive stimulations (Thibaut et al., 2017b). Repeating the sessions therefore unsurprisingly appears as a valuable option to increase the amount of responders. Home-based application of tDCS opens the door for larger application reaching more patients and for expanded results that will guide the effective and appropriate clinical use of tDCS (Charvet et al., 2015).

Given the imbalance between trials investigating prefrontal stimulation and the ones targeting other areas, in favor of the DLPFC, we decided to explore the potential benefits of stimulating zones that have not been investigated yet. Our pilot trial in 10 DOC patients (MCS and UWS) focusing on the motor cortex showed no behavioral treatment effect at the group level (Martens et al., 2019d), which contradicts a more recent open label study reporting 80% of

responders in a MCS TBI population (Straudi et al., 2019). This inconsistency encourages further investigation in properly designed randomized controlled trials with a priori sample size estimation based on this available data. Repeated sessions should also be investigated given the known cumulative effects of tDCS that could increase the patient's behavioral responsiveness if directed to the motor cortex (Boggio et al., 2007).

We also investigated, in a large randomized controlled trial on 46 patients, the use of multifocal tDCS targeting a whole network: the external awareness network located in the frontoparietal areas. Again, no behavioral treatment was identified at the group level while seven tDCS-responders were identified at the individual level. Noteworthy, we also identified for the first time patients who behaviorally worsened following stimulation by losing a sign of consciousness after the active tDCS session. These "paradoxical" responders shared a common baseline EEG pattern that significantly differed from the other patients: they had a higher initial complexity in the theta band. This is the first known report of a biomarker for negative treatment response in the DOC population. It is unsurprising that this finding concerned the theta band, as it appears as a key frequency band for patients with DOC. Indeed, as a diagnostic marker, patients in UWS have significantly lower connectivity as compared to MCS in this band (Lehembre et al., 2012). In active EEG paradigms (e.g., counting own name *versus* other names), it is also in that band that changes in power and synchronization are observed following the stimulus condition in both MCS and UWS (Fellinger et al., 2011). Following application of prefrontal tDCS, changes in coherence have also been reported in the theta band exclusively (Bai et al., 2018). Furthermore, higher initial spatial connectivity and

network centrality in the theta band are known features of positive response to tDCS, as revealed when comparing known tDCS-responders and non-responders using high-density EEG (Thibaut et al., 2018b). It would be interesting to conduct complexity analyses on the same dataset and check if the level of complexity is higher in these non-responders as compared to responders.

Overall, when investigating alternative targets such as the motor cortex or the frontoparietal network and comparing them with the existing literature, it appeared that the behavioral effects were way less remarkable than for the DLPFC. Therefore, as highlighted by Figure 29, the left DLPFC still appears for now as the optimal target for tDCS, especially in MCS patients.

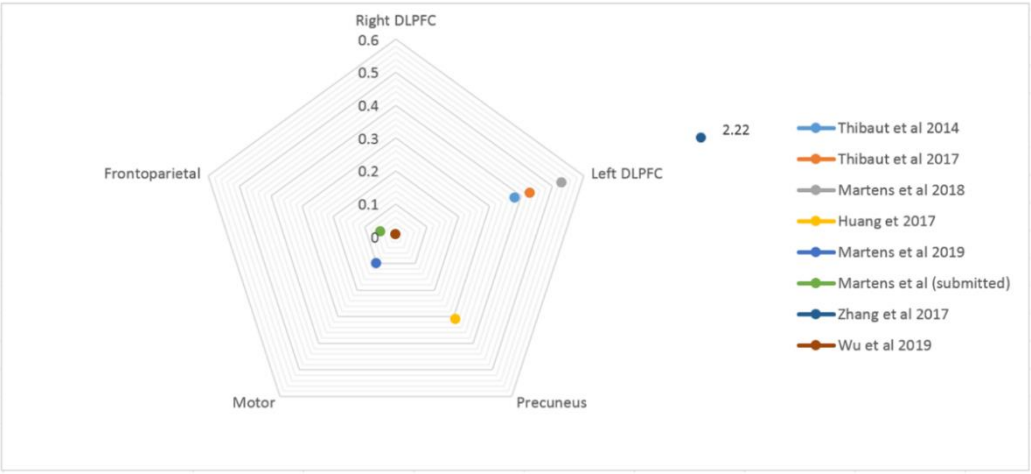


Figure 29 – Pooled effect sizes for different targets extracted from all available data in randomized controlled trials investigating tDCS in patients with DOC.

This might be explained by the mesocircuit hypothesis stating that the strong connectivity between the prefrontal cortex and the

striatum might be potentiated by stimulating the prefrontal area and thereby could down regulate the inhibition on the thalamus from the striatum, enhancing thalamo-cortical connectivity, critical for consciousness recovery (Schiff, 2010; Fridman et al., 2014). It could also be explained by another hypothesis introduced above: that non-invasive neuromodulation methods can act on the patient's behavioral responsiveness, through the prefrontal cortex, but not on his/her consciousness *per se*. This would explain why stimulating the external awareness network (i.e., frontoparietal areas) with tDCS did not lead to drastic clinical improvements. Eliciting increased levels of consciousness may indeed need stronger activation of subcortical areas located in the thalamus or the precuneus for instance, as opposed to elective intervention on the cortical areas, as enabled by tDCS. Given its inherent physiological functioning, tDCS cannot reach deep subcortical structures, as about half of the current is already lost when crossing the scalp and the skull (Miranda et al., 2006; Stagg and Nitsche, 2011). Reaching subcortical structures underlying consciousness could be achieved using other techniques. Invasive approaches, with DBS for instance, could be an option to directly activate the thalamic nuclei, with the known associated challenges regarding surgical risks and eligibility criteria however. Invasive VNS could also be a way to activate the thalamus and hypothalamus through the nucleus of the solitary tract located in the brainstem. Feasibility of the surgical implantation has been proven in an uncontrolled case-report performed with a patient who was UWS for 15 years and transitioned to MCS following one month of stimulation, accompanied by metabolic (higher activity in cortical and basal ganglia regions) and electrophysiological (increase in theta power) changes (Corazzol et al., 2017). This encouraging first step

needs to be followed-up by randomized controlled trials. The non-invasive alternative, transauricular VNS has also been investigated in an uncontrolled cohort study of 14 patients in UWS or MCS and induced significant improvement in total CRS-R scores at the group level after one month on bi-daily 30-minute stimulation (Noé et al., 2019). Another completely innovative approach that can target deeper subcortical structures is the use of ultrasonic stimulation. Using sound waves focused on the thalamus, a case-report showed recovery of spatio-temporal orientation and language comprehension in an acute young TBI patient (Monti et al., 2016). Again, further investigation is warranted. Other theories also suggest that critical hubs in consciousness processes are posterior and not depending on the frontal areas. A temporo-parietal-occipital hot zone located in the posterior cerebral cortex would support conscious experiences in general, or in a particular context (e.g., recognizing faces), based on fMRI and EEG findings (Koch et al., 2016). The only trial targeting the posterior parietal cortex with tDCS in MCS showed significant effects yet to a lesser extent than for the DLPFC but again, tDCS might not be the most suitable tool to increase the excitability of these hubs. Invasive options could be investigated to corroborate these hypotheses.

Taken together, our findings show that the best target to increase the behavioral responsiveness of patients with DOC, as measured by the CRS-R, is the left DLPFC (see Figure 29). However, we did not conduct a priori sample size estimation before conducting our studies and there might therefore have been under-powered, from a statistical perspective. As a post-hoc analysis, we calculated the achieved power of the three tDCS trials above-presented and based on the effect sizes we found, we recalculated the sample size

needed to obtain a statistical power of 90%. These results are presented in Table 12.

Table 12 – Power and sample size estimation for the three tDCS studies presented

tDCS study	N	p value [a]	Cohen's d effect size	Achieved power	Recalculated sample size
Left DLPFC (home-based, 20 sessions), (Martens et al., 2018b)	27	0.053	0.47	0.63	52
Motor cortex (single session), (Martens et al., 2019d)	10	0.55	0.1	0.06	1103
Frontoparietal network (single session), *submitted*	46	0.92	0.05	0.06	4404

Power calculated using effect size, N and α =0.05; sample size estimated using effect size, power (1-β) =0.90 and α =0.05. [a] Wilcoxon Rank-Sum Test

An overarching observation is that all of the studies are underpowered with too small sample sizes but it is most striking for the two studies using single sessions of tDCS on other targets than the DLPFC, corroborating our previous hypotheses. For the motor and frontoparietal targets, the effect is indeed way too small which makes recruiting enough patients an impossible task. The optimal sample size for the left DLPFC target is more achievable but still underlines the important difficulty of achieving enough statistical power with patients with DOC. This condition is indeed very rare and study recruitment is a major challenge. From a clinical perspective, it is also relevant to not focus on the group level treatment effect only

but on the individual changes observed as well and how to improve single-level responsiveness.

Moving a step beyond in that direction, shifting from an arbitrary timing of stimulation toward a brain-state dependent application represents a promising option to further increase the response rate. This closed-loop model could fit in research, clinical and home-based settings. As stated above, EEG can be used to identify patterns of individual responsiveness, notably in the theta band. It could further be used to monitor ongoing cerebral activity and trigger interventions, in a brain state-dependent fashion. We hypothesize, based on previous works (Gosseries et al., 2011; Piarulli et al., 2016), that spectral entropy can be an appropriate marker for vigilance, and that vigilance-dependent application of tDCS could represent an additional therapeutic benefit for patients in MCS. We here chose to stimulate the DLPFC bilaterally, focusing this time on improving executive functions, directly involved in the patient's behavioral responsiveness. Shifting our aims from improving the level of consciousness toward improving the behavioral responsiveness, as discussed above, may represent a more optimal and realistic way to use tDCS, as it can only reach cortical structures, whereas consciousness lies in both cortical and subcortical areas. If the upcoming results confirm our hypotheses, this would imply that accounting for the timing of the stimulations has to be featured in future stimulation parameters.

In the future, thanks to fast evolving technological advancements, we could imagine a combination of home-based and closed-loop approaches, with long-term monitoring of brain EEG signals and optimal delivering of tDCS. This requires of course portable user-friendly closed-loop devices, along with an extensive

training of all the participants. It could offer new ways to treat patients with DOC using non-invasive brain stimulation in a way never observed before. Remotely-supervised tDCS is already efficiently used in other conditions (Palm et al., 2018) such as multiple sclerosis (Charvet et al., 2017), chronic tinnitus (Hyvärinen et al., 2016) or vascular dementia (André et al., 2016).

While the present work aimed at improving both diagnostic and therapeutic aspects of the management of patients with DOC, a series of challenges still need to be addressed. The first one being to change the overly-pessimistic misconception that failure to recover consciousness within the first weeks post-injury portends an unfavorable outcome. This widespread belief probably originates from the first consensus statement of the Multi-Society Task Force on the "persistent vegetative state" (stating that recovery from a non-TBI UWS is exceedingly rare after three months) (The Multi-Society Task Force on PVS, 1994a), but it also largely relies on individual clinical judgment. Premature end-of-life decisions have been reflected by a large retrospective Canadian study including 720 patients admitted to intensive care following a TBI (Turgeon et al., 2011). They highlighted that 70% of deaths in the intensive care units were due to life-sustaining therapy withdrawal and that in 65% of these cases, it happened within the first three days following admission. Our investigations however show that patients admitted to rehabilitation later on, with still major deficits, are able to recover complex cortically mediated behaviors such as response to command or communication (Martens et al., 2019b). These findings have been confirmed by an observational cohort study conducted on 95 TBI patients in MCS or UWS in the subacute phase of injury (four to 16 weeks) and focusing on the recovery of the most complex behavior

of each CRS-R subscale (Giacino et al., 2019). The authors showed that 20% of the sample recovered all six of the target behaviors within the 6-week observation period and further suggest to consider aggressive rehabilitation and medical interventions for the patients in the subacute phase. Clinicians working in intensive care settings should therefore keep this data in view when discussing prognosis with families or caregivers, especially since even long-term outcome studies demonstrate that about 20% of patients with DOC are able to live independently within two to five years post-injury (Katz et al., 2009).

The second challenge pertains to the widely discussed important variations in individual response to interventions such as tDCS. The fluctuations in vigilance, characteristics of patients with DOC, are a well-known yet under investigated phenomenon, as no study has focused on properly characterizing them. The approach currently used to reduce the related diagnostic errors (with potentially dramatic consequences) is to repeat the behavioral assessments in order to decrease the chances of misdiagnosis (Wannez et al., 2017b). Accurate monitoring of vigilance could however help the clinician identify the best moment to perform a bedside assessment. To this end, EEG and pupillometry appear as valuable potential markers (Schleicher et al., 2008; Landsness et al., 2011; Piarulli et al., 2016). If such markers are validated, they could also be integrated into closed-loop systems to complement the inputs feeding the algorithm. Another part of the variations in responsiveness is the heterogeneity of the lesions in DOC patients, that also affects the clinical presentation and leads to various phenotypes of treatment response. Neuroimaging is undoubtedly a valuable tool to explore why some patients respond to tDCS and

others do not. Grey matter integrity and relative metabolic preservation of the stimulated brain area clearly appear to play a role in the observed behavioral improvements in DOC patients. Indeed, when retrospectively investigating the T1 MRI and FDG PET data of known tDCS responders and non-responders, significant differences in structural integrity and metabolic activity were observed between the two subgroups in the stimulated cortical area (i.e., the DLPFC) but also in distant connected areas (i.e., precuneus and thalamus) with a greater preservation for the responders group, suggesting clinical responsiveness appears to rely on, at least partial, structural and functional preservation of the stimulated area (Thibaut et al., 2015c). Another characteristic of tDCS responders is that they present higher connectivity in the executive control network as measured by fMRI (Cavaliere et al., 2016). However, MRI and PET scanner machines are expensive and not available in every facility. It also requires skilled nursing teams and expertise for signal analysis and cannot be used at the bedside. To tackle these issues, EEG can be used at the bedside, is more affordable and requires less training. It has been shown for instance that in MCS patients, responders show higher cortical connectivity in the theta band as compared to non-responders (Thibaut et al., 2018b). As a matter of fact, additional work needs to be done in identifying patterns of responsiveness in patients with DOC. To this end, developing biomarkers of responsiveness using machine learning approaches to categorize EEG signals according to clinical responsiveness to the treatment could represent a valuable support. Treatment for patients with DOC using non-invasive neuromodulation methods such as tDCS needs to further evolve toward individualized approaches. Clearly, the varying nature and

extend of brain lesions in DOC patients make “one size fits all” stimulation montages challenging if not inappropriate.

On the other hand, there is emerging evidence that tDCS is more efficient when applied in combination with other rehabilitation interventions such as physical and occupational therapies (Nair et al., 2011; Allman et al., 2016; Lefebvre et al., 2017; Dehem et al., 2018). Simultaneous stimulation of the central and the peripheral nervous systems (i.e., neuromuscular facilitation or sensorimotor techniques) could better enhance synaptic plasticity and skill relearning (Schlaug et al., 2008). It would therefore also be of interest to apply tDCS in patients with DOC during rehabilitation interventions, to induce stronger effects on neural plasticity. In the same vein, even passively engaging the patient through external sensorial stimuli such as music, flavors and fragrances, could also potentiate the effects of tDCS. As a matter of fact, tDCS responsiveness is multifactorial, as it also includes the repetition of tDCS sessions, as previously stated (Boggio et al., 2007; Ulam et al., 2014).

Finally, the aim of moving toward home-based application to enable better clinical translation, larger samples and more powerful results is ambitious but comes of course with its own challenges, including safety and efficacy monitoring. To this end, some new tools could have the overarching goal to assist in both diagnostic assessments and treatment efficacy outcome measurements and deserve some attention. The DOC-feeling for instance (Hermann et al., 2019), offers this opportunity in addition to giving an important role to families and caregivers, who sometimes feel helpless when taking care of this kind of patients.

5. Conclusion

"*The important thing is not to stop questioning. Curiosity has its own reason for existence.*"

Albert Einstein

DOC represent a rare and dramatic condition, with the unfortunate consequence of few available treatment options, partly because they are under investigated. Patients affected by this alteration in consciousness demonstrate various clinical presentations, in terms of level of awareness, behavioral output, motor complications, etc. which adds on the challenge for optimized care. Beyond better characterizing the potential for recovery of these patients, we explored further a specific treatment option; tDCS, and its modes of application: where to stimulate? In which environment? When?

In view of our findings, the left DLPFC is the target with the most potential behavioral benefits, as compared to the motor or the frontoparietal cortices. We should bear in mind however that individual response has a key and often neglected role to play and that assumptions on the group level effects should be taken cautiously. Regarding clinical translation, tDCS can be safely and efficiently applied by non-experts in the patient's daily environment. This open several doors for home-based and long-term use, as well as better involvement of the caregivers and relatives, who are often eager to take an active part in the therapeutic management. The optimal moment of application could be identified using EEG entropic patterns, that could in turn trigger tDCS and thereby potentiate its neuroplastic effects. This approach is still in the early stages but represents a promising therapeutic option.

Considering the fact that one of the aims of the present work was to help clinicians facing the challenges surrounding the management of DOC, we would like to conclude with some clinical recommendations offered by this thesis:

- Clinicians working in rehabilitation facilities should put a specific emphasis on tracking visual pursuit, reproducible response to command and automatic motor movements when diagnosing comatose or UWS patients at the bedside
- Clinicians working in rehabilitation facilities with a specialized DOC program may expect patients admitted as comatose or UWS to change their bedside diagnosis to MCS or EMCS within six weeks of injury
- Clinicians working in intensive care settings should be aware that non-communicative patients discharged to rehabilitation may recover functional communication in 61% of cases
- Clinicians working in rehabilitation facilities may expect non-communicative admitted patients to recover IC within 40 days of injury and FC within 49 days of injury in 70% of cases. Cases of late communication recovery (i.e., past 8 weeks of rehabilitation) can occur in 33% of cases
- Clinicians treating patients with DOC should consider tDCS in their therapeutic options to improve behavioral responsiveness without having safety concerns
- One session of tDCS should be applied as follows: 20 minutes of anodal stimulation (2 mA) over the left DLPFC (F3) using sponge 35 cm^2 electrodes
- Clinicians can anticipate a greater rate of behavioral improvement for MCS patients than for UWS patients, regardless of etiology and chronicity of the injury
- The behavioral effects of one tDCS session are transient (max. 1 hour) and are enhanced when repeating the tDCS

sessions on a daily basis. Repeated tDCS sessions can be safely applied for five consecutive days, up to 20 days of stimulation

- tDCS sessions can be applied by trained relatives and caregivers, upon regular professional supervision and use of tDCS devices designed to this application
- Clinicians treating patients with DOC should not prioritize right prefrontal, motor, posterior parietal nor bilateral frontoparietal tDCS

These recommendations finely complement the most recent ones based on systematic reviews of the literature (Giacino et al., 2018b; Kondziella et al., 2020) and build on the foundations of comprehensive evidence-based guidelines for the management of patients with DOC following severe brain injury.

As stated above, other treatment options are available and deserve more investigations too: VNS, ultrasound or repeated TMS to name a few. The findings presented here, notably in terms of remote supervision, can be easily translated to these techniques and contribute to enrich the still too scattered panel of treatment options for patients with DOC.

6. References

Allman, C., Amadi, U., Winkler, A. M., Wilkins, L., Filippini, N., Kischka, U., et al. (2016). Ipsilesional anodal tDCS enhances the functional benefits of rehabilitation in patients after stroke. *Sci. Transl. Med.* 8, 330re1-330re1. doi:10.1126/scitranslmed.aad5651.

André, S., Heinrich, S., Kayser, F., Menzler, K., Kesselring, J., Khader, P. H., et al. (2016). At-home tDCS of the left dorsolateral prefrontal cortex improves visual short-term memory in mild vascular dementia. *J. Neurol. Sci.* 369, 185–190. doi:10.1016/j.jns.2016.07.065.

Andrews, K. (1993). Recovery of patients after four months or more in the persistent vegetative state. *BMJ* 306, 1597–600. doi:10.1136/bmj.306.6892.1597.

Andrews, K., Murphy, L., Munday, R., and Littlewood, C. (1996). Misdiagnosis of the vegetative state: retrospective study in a rehabilitation unit. *BMJ* 313, 13–16.

Angelakis, E., Liouta, E., Andreadis, N., Korfias, S., Ktonas, P., Stranjalis, G., et al. (2014). Transcranial direct current stimulation effects in disorders of consciousness. *Arch Phys Med Rehabil* 95, 283–289. doi:10.1016/j.apmr.2013.09.002.

Anokhin, A. P., Birbaumer, N., Lutzenberger, W., Nikolaev, A., and Vogel, F. (1996). Age increases brain complexity. *Electroencephalogr. Clin. Neurophysiol.* 99, 63–68.

Aubinet, C., Larroque, S. K., Heine, L., Martial, C., Majerus, S., Laureys, S., et al. (2018). Clinical subcategorization of minimally conscious state according to resting functional connectivity. *Hum. Brain Mapp.* 39. doi:10.1002/hbm.24303.

Bagnato, S., Boccagni, C., Sant'Angelo, A., Fingelkurts, A. A., Fingelkurts, A. A., and Galardi, G. (2016). Longitudinal assessment of clinical signs of recovery in patients with unresponsive wakefulness syndrome after traumatic or nontraumatic brain injury. *J. Neurotrauma* 33, 1–5. doi:10.1089/neu.2016.4418.

Bagnato, S., Boccagni, C., Sant'Angelo, A., Prestandrea, C., Mazzilli, R., and Galardi, G. (2015). EEG predictors of outcome in patients with disorders of consciousness admitted for intensive rehabilitation. *Clin. Neurophysiol.* 126, 959–966. doi:10.1016/J.CLINPH.2014.08.005.

Bai, Y., Xia, X., Kang, J., Yang, Y., He, J., and Li, X. (2017a). TDCS modulates cortical excitability in patients with disorders of consciousness. *NeuroImage Clin.* 15, 702–709. doi:10.1016/j.nicl.2017.01.025.

Bai, Y., Xia, X., Li, X., Wang, Y., Yang, Y., Liu, Y., et al. (2017b). Spinal cord stimulation modulates frontal delta and gamma in patients of minimally consciousness state. *Neuroscience* 346, 247–254. doi:10.1016/j.neuroscience.2017.01.036.

Bai, Y., Xia, X., Liang, Z., Wang, Y., Yang, Y., He, J., et al. (2017c). Frontal Connectivity in EEG Gamma (30–45 Hz) Respond to Spinal Cord Stimulation in Minimally Conscious State Patients. *Front. Cell. Neurosci.* 11, 177. doi:10.3389/fncel.2017.00177.

Bai, Y., Xia, X., Wang, Y., Guo, Y., Yang, Y., He, J., et al. (2018). Fronto-parietal coherence response to tDCS modulation in patients with disorders of consciousness. *Int. J. Neurosci.* 128, 587–594. doi:10.1080/00207454.2017.1403440.

Balakrishnan, S., and Ward, A. B. (2013). "The diagnosis and management of adults with spasticity," in *Handbook of Clinical Neurology* (Elsevier B.V.), 145–160. doi:10.1016/B978-0-444-52901-5.00013-7.

Barbey, A. K., Koenigs, M., and Grafman, J. (2012). Dorsolateral prefrontal contributions to human working memory. *Cortex*. doi:10.1016/j.cortex.2012.05.022.

Barclay, W. R. (1981). Guidelines for the Determination of Death. *JAMA J. Am. Med. Assoc.* 246, 2194. doi:10.1001/jama.1981.03320190052031.

Bein, B. (2006). Entropy. *Best Pract. Res. Clin. Anaesthesiol.* 20, 101–109. doi:10.1016/j.bpa.2005.07.009.

Berényi, A., Belluscio, M., Mao, D., and Buzsáki, G. (2012). Closed-loop control of epilepsy by transcranial electrical stimulation. *Science* 337, 735–7. doi:10.1126/science.1223154.

Bernat, J. L. (1998). A defense of the whole-brain concept of death. *Hast. Cent Rep* 28, 14–23.

Bernat, J. L. (2002). Questions remaining about the minimally conscious state. *Neurology* 58, 337–338. doi:10.1212/WNL.58.3.337.

Bikson, M., Grossman, P., Thomas, C., Zannou, A. L., Jiang, J., Adnan, T., et al. (2016). Safety of Transcranial Direct Current Stimulation: Evidence Based Update 2016. *Brain Stimul.* doi:10.1016/j.brs.2016.06.004.

Bikson, M., Inoue, M., Akiyama, H., Deans, J. K., Fox, J. E., Miyakawa, H., et al. (2004). Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices *in vitro*. *J. Physiol.* 557, 175–190. doi:10.1113/jphysiol.2003.055772.

Bindman, L. J., Lippold, O. C. J., and Redfearn, J. W. T. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *J. Physiol.* 172, 369–382. doi:10.1113/jphysiol.1964.sp007425.

Bodien, Y., Chatelle, C., and Edlow, B. (2017). Functional Networks in Disorders of Consciousness. *Semin. Neurol.* 37, 485–502. doi:10.1055/s-0037-1607310.

Bodien, Y. G., Martens, G., Ostrow, J., Sheau, K., and Giacino, J. T. (2019). Cognitive Impairment, Clinical Symptoms and Functional Disability in Patients Emerging from the Minimally Conscious State. *NeuroRehabilitation* In Press.

Boggio, P. S., Ferrucci, R., Rigonatti, S. P., Covre, P., Nitsche, M., Pascual-Leone, A., et al. (2006). Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease. *J Neurol Sci* 249, 31–38. doi:10.1016/j.jns.2006.05.062.

Boggio, P. S., Nunes, A., Rigonatti, S. P., Nitsche, M. A., Pascual-Leone, A., and Fregni, F. (2007). Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients. *Restor Neurol Neurosci* 25, 123–129.

Boggio, P. S., Rigonatti, S. P., Ribeiro, R. B., Myczkowski, M. L., Nitsche, M. A., Pascual-Leone, A., et al. (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, 249–254. doi:10.1017/S1461145707007833.

Boly, M., Faymonville, M. E., Peigneux, P., Lambermont, B., Damas, F., Luxen, A., et al. (2005). Cerebral processing of auditory and noxious stimuli in severely brain injured patients: differences between VS and MCS. *Neuropsychol Rehabil* 15, 283–289. doi:10.1080/09602010443000371.

Born, J. D. (1988). The Glasgow-Liege Scale. *Acta Neurochir. (Wien).* 91, 1–11. doi:10.1007/BF01400520.

Bourdillon, P., Hermann, B., Sitt, J. D., and Naccache, L. (2019). Electromagnetic Brain Stimulation in Patients With Disorders of Consciousness. *Front. Neurosci.* 13. doi:10.3389/fnins.2019.00223.

Brittain, J. S., Probert-Smith, P., Aziz, T. Z., and Brown, P. (2013). Tremor suppression by rhythmic transcranial current stimulation. *Curr. Biol.* 23, 436–440. doi:10.1016/j.cub.2013.01.068.

Bruno, M. A., Majerus, S., Boly, M., Vanhaudenhuyse, A., Schnakers, C., Gosseries, O., et al. (2012). Functional neuroanatomy underlying the clinical subcategorization of minimally conscious state patients. *J Neurol* 259, 1087–1098. doi:10.1007/s00415-011-6303-7.

Bruno, M. A., Vanhaudenhuyse, A., Thibaut, A., Moonen, G., and Laureys, S. (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, 1373–1384. doi:10.1007/s00415-011-6114-x.

Bucki, B., Spitz, E., and Baumann, M. (2019). Emotional and social repercussions of stroke on patient-family caregiver dyads: Analysis of diverging attitudes and profiles of the differing dyads. *PLoS One* 14, e0215425. doi:10.1371/journal.pone.0215425.

Busl, K. M., and Greer, D. M. (2010). Hypoxic-ischemic brain injury: Pathophysiology, neuropathology and mechanisms. *NeuroRehabilitation* 26, 5–13. doi:10.3233/NRE-2010-0531.

Bütefisch, C. M., Weβling, M., Netz, J., Seitz, R. J., and Hömberg, V. (2008).

Relationship Between Interhemispheric Inhibition and Motor Cortex Excitability in Subacute Stroke Patients. *Neurorehabil. Neural Repair* 22, 4–21. doi:10.1177/1545968307301769.

Buzsáki, G. (2006). *Rhythms of the brain*. Oxford University Press

Candelieri, A., Cortese, M. D., Dolce, G., Riganello, F., and Sannita, W. G. (2011). Visual Pursuit: Within-Day Variability in the Severe Disorder of Consciousness. *J. Neurotrauma* 28, 2013–2017. doi:10.1089/neu.2011.1885.

Carboncini, M. C., Piarulli, A., Virgillito, A., Arrighi, P., Andre, P., Tomaiuolo, F., et al. (2014). A case of post-traumatic minimally conscious state reversed by midazolam: Clinical aspects and neurophysiological correlates. *Restor. Neurol. Neurosci.* 32, 767–787. doi:10.3233/RNN-140426.

Casali, A. G., Gosseries, O., Rosanova, M., Boly, M., Sarasso, S., Casali, K. R., et al. (2013). A theoretically based index of consciousness independent of sensory processing and behavior. *Sci. Transl. Med.* 5, 198ra105. doi:10.1126/scitranslmed.3006294.

Casarotto, S., Comanducci, A., Rosanova, M., Sarasso, S., Fecchio, M., Napolitani, M., et al. (2016). Stratification of unresponsive patients by an independently validated index of brain complexity. *Ann. Neurol.* 80, 718–729. doi:10.1002/ana.24779.

Cavaliere, C., Aiello, M., Di Perri, C., Amico, E., Martial, C., Thibaut, A., et al. (2016). Functional Connectivity Substrates for tDCS Response in Minimally Conscious State Patients. *Front. Cell. Neurosci.* 10, 257. doi:10.3389/fncel.2016.00257.

Cavinato, M., Genna, C., Formaggio, E., Gregorio, C., Storti, S. F., Manganotti, P., et al. (2019). Behavioural and electrophysiological effects of tDCS to prefrontal cortex in patients with disorders of consciousness. *Clin. Neurophysiol.* 130, 231–238. doi:10.1016/j.clinph.2018.10.018.

Cervós-Navarro, J., and Diemer, N. H. (1991). Selective vulnerability in brain hypoxia. *Crit. Rev. Neurobiol.* 6, 149–82.

Charvet, L. E., Kasschau, M., Datta, A., Knotkova, H., Michael, C., Alonzo, A., et al. (2015). Remotely-supervised transcranial direct current stimulation (tDCS) for clinical trials : guidelines for technology and protocols. 9, 1–13. doi:10.3389/fnsys.2015.00026.

Charvet, L., Shaw, M., Dobbs, B., Frontario, A., Sherman, K., Bikson, M., et al. (2017). Remotely Supervised Transcranial Direct Current Stimulation Increases the Benefit of At-Home Cognitive Training in Multiple Sclerosis. *Neuromodulation Technol. Neural Interface* 2017. doi:10.1111/ner.12583.

Chatelle, C., and Laureys, S. (2011). “Understanding disorders of consciousness,” in *The Oxford Handbook of Neuroethics*, eds. J. Illes and B. Sahakian (New York: Oxford University Press), 119–133.

Chatelle, C., Majerus, S., Whyte, J., Laureys, S., and Schnakers, C. (2012). A sensitive scale to assess nociceptive pain in patients with disorders of consciousness. *J*

Neurol Neurosurg Psychiatry 83, 1233–1237. doi:10.1136/jnnp-2012-302987.

Cheng, L., Gosseries, O., Ying, L., Hu, X., Yu, D., Gao, H., et al. (2013). Assessment of localisation to auditory stimulation in post-comatose states: use the patient's own name. *BMC Neurol* 13, 27. doi:10.1186/1471-2377-13-27.

Chennu, S., Annen, J., Wannez, S., Thibaut, A., Chatelle, C., Cassol, H., et al. (2017). Brain networks predict metabolism, diagnosis and prognosis at the bedside in disorders of consciousness. *Brain* 140, 2120–2132. doi:10.1093/brain/awx163.

Childs, N. L., and Mercer, W. N. (1996). Misdiagnosing the persistent vegetative state. Misdiagnosis certainly occurs. *BMJ* 313, 944.

Childs, N. L., Mercer, W. N., and Childs, H. W. (1993). Accuracy of diagnosis of persistent vegetative state. *Neurology* 43, 1465–7. doi:10.1212/WNL.43.8.1465.

Chudy, D., Deletis, V., Almahariq, F., Marčinković, P., Škrlin, J., and Paradžik, V. (2018). Deep brain stimulation for the early treatment of the minimally conscious state and vegetative state: experience in 14 patients. *J. Neurosurg.* 128, 1189–1198. doi:10.3171/2016.10.JNS161071.

Cincotta, M., Giovannelli, F., Chiaramonti, R., Bianco, G., Godone, M., Battista, D., et al. (2015). No effects of 20 Hz-rTMS of the primary motor cortex in vegetative state: A randomised, sham-controlled study. *Cortex.* 71, 368–376. doi:10.1016/j.cortex.2015.07.027.

Coben, R., and Evans, J. R. (2011). *Neurofeedback and Neuromodulation Techniques and Applications*. Elsevier Inc. doi:10.1016/C2009-0-64101-5.

Collette, F., Van der Linden, M., Laureys, S., Arigoni, F., Delfiore, G., Degueldre, C., et al. (2007). Mapping the updating process: common and specific brain activations across different versions of the running span task. *Cortex* 43, 146–158.

Corazzol, M., Lio, G., Lefevre, A., Deiana, G., Tell, L., André-Obadia, N., et al. (2017). Restoring consciousness with vagus nerve stimulation. *Curr. Biol.* 27, R994–R996. doi:10.1016/j.cub.2017.07.060.

Cruse, D., Thibaut, A., Demertzi, A., Nantes, J. C., Bruno, M.-A., Gosseries, O., et al. (2013). Actigraphy assessments of circadian sleep-wake cycles in the Vegetative and Minimally Conscious States. *BMC Med.* 11, 11.

D'Esposito, M., Aguirre, G. K., Zarahn, E., Ballard, D., Shin, R. K., and Lease, J. (1998). Functional MRI studies of spatial and nonspatial working memory. *Brain Res Cogn Brain Res* 7, 1–13.

D'Esposito, M., Detre, J. A., Alsop, D. C., Shin, R. K., Atlas, S., and Grossman, M. (1995). The neural basis of the central executive system of working memory. *Nature* 378, 279–281. doi:10.1038/378279a0.

Dehaene, S., and Naccache, L. (2001). Towards a cognitive neuroscience of consciousness: basic evidence and a workspace framework. *Cognition* 79, 1–37.

Dehem, S., Gilliaux, M., Lejeune, T., Delaunois, E., Mbonda, P., Vandermeeren, Y., et al. (2018). Effectiveness of a single session of dual-transcranial direct current stimulation in combination with upper limb robotic-assisted rehabilitation in chronic stroke patients: A randomized, double-blind, cross-over study. *Int. J. Rehabil. Res.* 41, 138–145. doi:10.1097/MRR.0000000000000274.

Demertzi, A., Antonopoulos, G., Heine, L., Voss, H. U., Crone, J. S., De Los Angeles, C., et al. (2015). Intrinsic functional connectivity differentiates minimally conscious from unresponsive patients. *Brain* 138, 2619–2631. doi:10.1093/brain/awv169.

Demertzi, A., Ledoux, D., Bruno, M. A., Vanhaudenhuyse, A., Gosseries, O., Soddu, A., et al. (2011). Attitudes towards end-of-life issues in disorders of consciousness: a European survey. *J Neurol* 258, 1058–1065. doi:10.1007/s00415-010-5882-z.

Devinsky, O., and D'Esposito, M. (2004). *Neurology of cognitive and behavioural disorders*. Oxford, New York: Oxord University Press.

Di, H. B., Yu, S. M., Weng, X. C., Laureys, S., Yu, D., Li, J. Q., et al. (2007). Cerebral response to patient's own name in the vegetative and minimally conscious states. *Neurology* 68, 895–899. doi:10.1212/01.wnl.0000258544.79024.d0.

Di, H., Boly, M., Weng, X., Ledoux, D., and Laureys, S. (2008). Neuroimaging activation studies in the vegetative state: predictors of recovery? *Clin Med* 8, 502–507.

Di, H., Nie, Y., Hu, X., Tong, Y., Heine, L., Wannez, S., et al. (2014). Assessment of visual fixation in vegetative and minimally conscious states. *BMC Neurol.* 14, 147.

Di Lazzaro, V., Dileone, M., Capone, F., Pellegrino, G., Ranieri, F., Musumeci, G., et al. (2014). Immediate and late modulation of interhemipheric imbalance with bilateral transcranial direct current stimulation in acute stroke. *Brain Stimul.* 7, 841–848. doi:10.1016/j.brs.2014.10.001.

Di Perri, C., Bahri, M. A., Amico, E., Thibaut, A., Heine, L., Antonopoulos, G., et al. (2016). Neural correlates of consciousness in patients who have emerged from a minimally conscious state: a cross-sectional multimodal imaging study. *Lancet Neurol.* 15, 830–842. doi:10.1016/S1474-4422(16)00111-3.

Dolce, G., Lucca, L. F., Candelieri, A., Rogano, S., Pignolo, L., and Sannita, W. G. (2011). Visual pursuit in the severe disorder of consciousness. *J. Neurotrauma* 28, 1149–1154. doi:10.1089/neu.2010.1405.

Dolce, G., Quintieri, M., Serra, S., Lagani, V., and Pignolo, L. (2008). Clinical signs and early prognosis in vegetative state: A decisional tree, data-mining study. *Brain Inj.* 22, 617–623. doi:10.1080/02699050802132503.

Estraneo, A., Loreto, V., Guarino, I., Boemia, V., Paone, G., Moretta, P., et al. (2016). Standard EEG in diagnostic process of prolonged disorders of consciousness. *Clin. Neurophysiol.* 127, 2379–2385. doi:10.1016/j.clinph.2016.03.021.

Estraneo, A., Moretta, P., Cardinale, V., De Tanti, A., Gatta, G., Giacino, J. T., et al. (2015). A multicentre study of intentional behavioural responses measured using the Coma Recovery Scale–Revised in patients with minimally conscious state. *Clin. Rehabil.* 29, 803–808. doi:10.1177/0269215514556002.

Estraneo, A., Moretta, P., Loreto, V., Lanzillo, B., Santoro, L., and Trojano, L. (2010). Late recovery after traumatic, anoxic, or hemorrhagic long-lasting vegetative state. *Neurology* 75, 239–245. doi:10.1212/WNL.0b013e3181e8e8cc.

Estraneo, A., Pascarella, A., Moretta, P., Masotta, O., Fiorenza, S., Chirico, G., et al. (2017). Repeated transcranial direct current stimulation in prolonged disorders of consciousness: A double-blind cross-over study. *J. Neurol. Sci.* 375, 464–470. doi:10.1016/j.jns.2017.02.036.

Fellinger, R., Klimesch, W., Schnakers, C., Perrin, F., Freunberger, R., Gruber, W., et al. (2011). Cognitive processes in disorders of consciousness as revealed by EEG time-frequency analyses. *Clin Neurophysiol* 122, 2177–2184. doi:10.1016/j.clinph.2011.03.004.

Fernández-Espejo, D., Rossit, S., and Owen, A. M. (2015). A Thalamocortical Mechanism for the Absence of Overt Motor Behavior in Covertly Aware Patients. *JAMA Neurol.* 72, 1442. doi:10.1001/jamaneurol.2015.2614.

Fernández, A., López-Ibor, M.-I., Turrero, A., Santos, J.-M., Morón, M.-D., Hornero, R., et al. (2011). Lempel–Ziv complexity in schizophrenia: a MEG study. *Clin. Neurophysiol.* 122, 2227–2235.

Fernández, A., Zuluaga, P., Abásolo, D., Gómez, C., Serra, A., Méndez, M. A., et al. (2012). Brain oscillatory complexity across the life span. *Clin. Neurophysiol.* 123, 2154–2162.

Ferrucci, R., Bortolomasi, M., Vergari, M., Tadini, L., Salvoro, B., Giacopuzzi, M., et al. (2009). Transcranial direct current stimulation in severe, drug-resistant major depression. *J. Affect. Disord.* 118.

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., and Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc. Natl. Acad. Sci. U. S. A.* 102, 9673–9678. doi:10.1073/pnas.0504136102.

Francois, B., Vacher, P., Roustan, J., Salle, J. Y., Vidal, J., Moreau, J. J., et al. (2001). Intrathecal baclofen after traumatic brain injury: Early treatment using a new technique to prevent spasticity. *J. Trauma - Inj. Infect. Crit. Care* 50, 158–161.

Fregni, F., Boggio, P. S., Lima, M. C., Ferreira, M. J., Wagner, T., Rigonatti, S. P., et al. (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, 197–209. doi:10.1016/j.pain.2006.02.023.

Fregni, F., and Pascual-Leone, A. (2006). Hand motor recovery after stroke: tuning the orchestra to improve hand motor function. *Cogn Behav Neurol* 19, 21–33.

Fridman, E. A., Beattie, B. J., Broft, A., Laureys, S., and Schiff, N. D. (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, 6473–6478. doi:10.1073/pnas.1320969111.

Fridman, E. A., Calvar, J., Bonetto, M., Gamzu, E., Krimchansky, B. Z., Meli, F., et al. (2009). Fast awakening from minimally conscious state with apomorphine. *Brain Inj* 23, 172–177. doi:10.1080/02699050802649662.

Fridman, E. A., Krimchansky, B. Z., Bonetto, M., Galperin, T., Gamzu, E. R., Leiguarda, R. C., et al. (2010). Continuous subcutaneous apomorphine for severe disorders of consciousness after traumatic brain injury. *Brain Inj.* 24, 636–641. doi:10.3109/02699051003610433.

Gallucci, A., Lucena, P. H., Martens, G., Thibaut, A., and Fregni, F. (2019). Transcranial direct current stimulation to prevent and treat surgery-induced opioid dependence: a systematic review. *Pain Manag.* 9, 93–106. doi:10.2217/pmt-2018-0053.

Ganesh, S., Guernon, A., Chalcraft, L., Harton, B., Smith, B., and Louise-Bender Pape, T. (2013). Medical comorbidities in disorders of consciousness patients and their association with functional outcomes. *Arch Phys Med Rehabil* 94, 1899–1907. doi:10.1016/j.apmr.2012.12.026.

Garcia-Larrea, L., Perchet, C., Hagiwara, K., and André-Obadia, N. (2019). At-Home Cortical Stimulation for Neuropathic Pain: a Feasibility Study with Initial Clinical Results. *Neurotherapeutics*. doi:10.1007/s13311-019-00734-3.

Giacino, J. T., Ashwal, S., Childs, N., Cranford, R., Jennett, B., Katz, D. I., et al. (2002). The minimally conscious state: definition and diagnostic criteria. *Neurology* 58, 349–353.

Giacino, J. T., Fins, J. J., Laureys, S., and Schiff, N. D. (2014). Disorders of consciousness after acquired brain injury: the state of the science. *Nat Rev Neurol* 10, 99–114. doi:10.1038/nrneurol.2013.279.

Giacino, J. T., Kalmar, K., and Whyte, J. (2004). The JFK Coma Recovery Scale-Revised: measurement characteristics and diagnostic utility. *Arch Phys Med Rehabil* 85, 2020–2029.

Giacino, J. T., Katz, D. I., Schiff, N. D., Whyte, J., Ashman, E. J., Ashwal, S., et al. (2018a). Comprehensive systematic review update summary: Disorders of consciousness: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology; The American Congress of Rehabilitation Medicine; And the National Institute on Disability, Independent Living, and Rehabilitati. *Neurology* 91, 461–470. doi:10.1212/WNL.0000000000005928.

Giacino, J. T., Katz, D. I., Schiff, N. D., Whyte, J., Ashman, E. J., Ashwal, S., et al. (2018b). Practice guideline update recommendations summary: Disorders of consciousness: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology; the

American Congress of Rehabilitation Medicine; and the National Institute on Disability, Independent Living, and Rehabilitation Research. *Neurology* 91, 450–460. doi:10.1212/WNL.0000000000005926.

Giacino, J. T., Schnakers, C., Rodriguez-Moreno, D., Kalmar, K., Schiff, N., and Hirsch, J. (2009). Behavioral assessment in patients with disorders of consciousness: gold standard or fool's gold? *Prog. Brain Res.* 177, 33–48. doi:10.1016/S0079-6123(09)17704-X.

Giacino, J. T., Sherer, M., Christoforou, A., Maurer-Karattup, P., Hammond, F. M., Long, D., et al. (2019). Behavioral Recovery and Early Decision Making in Patients with Prolonged Disturbance in Consciousness after Traumatic Brain Injury. *J. Neurotrauma*, neu.2019.6429. doi:10.1089/neu.2019.6429.

Giacino, J. T., Whyte, J., Bagiella, E., Kalmar, K., Childs, N., Khademi, A., et al. (2012). Placebo-controlled trial of amantadine for severe traumatic brain injury. *N. Engl. J. Med.* 366, 819–826. doi:10.1056/NEJMoa1102609.

Giacino, J. T., Zasler, N. D., Katz, D. I., Kelly, J. P., Rosenberg, J. H., and Filley, C. M. (1997). Development of practice guidelines for assessment and management of the vegetative and minimally conscious states. *J. Head Trauma Rehabil.* 12, 79–89.

Gill-Thwaites, H., and Munday, R. (1999). The Sensory Modality Assessment and Rehabilitation Technique (SMART): a comprehensive and integrated assessment and treatment protocol for the vegetative state and minimally responsive patient. *Neuropsychol. Rehabil.* 9, 305–320. doi:10.1080/096020199389392.

Gill, J., Shah-Basak, P. P., and Hamilton, R. (2015). It's the thought that counts: Examining the task-dependent effects of transcranial direct current stimulation on executive function. *Brain Stimul.* 8, 253–259. doi:10.1016/j.brs.2014.10.018.

Giovannella, M., Ibañez, D., Gregori-Pla, C., Kacprzak, M., Mitjà, G., Ruffini, G., et al. (2018). Concurrent measurement of cerebral hemodynamics and electroencephalography during transcranial direct current stimulation. *Neurophotonics* 5, 1. doi:10.1117/1.NPh.5.1.015001.

Gladson, B. (2010). *Pharmacology for Rehabilitation Professionals*. Elsevier Health Sciences.

Golland, Y., Bentin, S., Gelbard, H., Benjamini, Y., Heller, R., Nir, Y., et al. (2007). Extrinsic and intrinsic systems in the posterior cortex of the human brain revealed during natural sensory stimulation. *Cereb. Cortex* 17, 766–77. doi:10.1093/cercor/bhk030.

Gomez-Tames, J., Asai, A., and Hirata, A. (2020). Significant group-level hotspots found in deep brain regions during transcranial direct current stimulation (tDCS): A computational analysis of electric fields. *Clin. Neurophysiol.* 131, 755–765. doi:10.1016/j.clinph.2019.11.018.

Gosseries, O., Demertzi, A., Ledoux, D., Bruno, M.-A. M.-A., Vanhaudenhuyse, A., Thibaut, A., et al. (2012). Burnout in healthcare workers managing chronic patients with disorders of consciousness. *Brain Inj.* 26, 1493–1499. doi:10.3109/02699052.2012.695426.

Gosseries, O., Di, H., Laureys, S., and Boly, M. (2014). Measuring Consciousness in Severely Damaged Brains. *Annu. Rev. Neurosci.* 37, 457–478. doi:10.1146/annurev-neuro-062012-170339.

Gosseries, O., Schnakers, C., Ledoux, D., Vanhaudenhuyse, A., Bruno, M. A., Demertzi, A., et al. (2011). Automated EEG entropy measurements in coma, vegetative state/unresponsive wakefulness syndrome and minimally conscious state. *Funct Neurol* 26, 25–30.

Gracies, J. M. (2005). Pathophysiology of spastic paresis. II: Emergence of muscle overactivity. *Muscle Nerve* 31, 552–571. doi:10.1002/mus.20285.

Grillner, S., Hellgren, J., Ménard, A., Saitoh, K., and Wikström, M. A. (2005). Mechanisms for selection of basic motor programs – roles for the striatum and pallidum. *Trends Neurosci.* 28, 364–370. doi:10.1016/J.TINS.2005.05.004.

Guldenmund, P., Soddu, A., Baquero, K., Vanhaudenhuyse, A., Bruno, M.-A., Gosseries, O., et al. (2016). Structural brain injury in patients with disorders of consciousness: A voxel-based morphometry study. *Brain Inj.* 30, 343–352. doi:10.3109/02699052.2015.1118765.

Guo, Y., Bai, Y., Xia, X., Li, J., Wang, X., Dai, Y., et al. (2019). Effects of Long-Lasting High-Definition Transcranial Direct Current Stimulation in Chronic Disorders of Consciousness: A Pilot Study. *Front. Neurosci.* 13, 412. doi:10.3389/fnins.2019.00412.

Harris, P. A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., and Conde, J. G. (2009). Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. *J. Biomed. Inform.* 42, 377–381. doi:10.1016/J.JBI.2008.08.010.

Heekeren, H. R., Marrett, S., Ruff, D. A., Bandettini, P. A., and Ungerleider, L. G. (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, 10023–10028. doi:10.1073/pnas.0603949103.

Hermann, B., Goudard, G., Courcoux, K., Valente, M., Labat, S., Despois, L., et al. (2019). Wisdom of the caregivers: Pooling individual subjective reports to diagnose states of consciousness in brain-injured patients, a monocentric prospective study. *BMJ Open* 9. doi:10.1136/bmjopen-2018-026211.

Herrold, A. A., Pape, T. L. B., Guernon, A., Mallinson, T., Collins, E., and Jordan, N. (2014). Prescribing multiple neurostimulants during rehabilitation for severe brain injury. *Sci. World J.* 2014. doi:10.1155/2014/964578.

Herwig, U., Satrapi, P., and Schonfeldt-Lecuona, C. (2003). Using the international 10-20 EEG system for positioning of transcranial magnetic stimulation. *Brain*

Topogr 16, 95–99.

Huang, W., Wannez, S., Fregni, F., Hu, X., Jing, S., Martens, G., et al. (2017). Repeated stimulation of the posterior parietal cortex in patients in minimally conscious state: A sham-controlled randomized clinical trial. *Brain Stimul.* 10, 718–720. doi:10.1016/j.brs.2017.02.001.

Huang, Y., and Parra, L. C. (2019). Can transcranial electric stimulation with multiple electrodes reach deep targets? *Brain Stimul.* 12, 30–40. doi:10.1016/j.brs.2018.09.010.

Hyvärinen, P., Mäkitie, A., and Aarnisalo, A. A. (2016). Self-Administered Domiciliary tDCS Treatment for Tinnitus: A Double-Blind Sham-Controlled Study. *PLoS One* 11, e0154286. doi:10.1371/journal.pone.0154286.

Jennett, B., and Plum, F. (1972). Persistent vegetative state after brain damage. A syndrome in search of a name. *Lancet* 1, 734–737.

Jones, A. P., Choe, J., Bryant, N. B., Robinson, C. S. H., Ketz, N. A., Skorheim, S. W., et al. (2018). Dose-dependent effects of closed-loop tACS delivered during slow-wave oscillations on memory consolidation. *Front. Neurosci.* 12. doi:10.3389/fnins.2018.00867.

Kampfl, A., Schmutzhard, E., Franz, G., Pfausler, B., Haring, H.-P., Ulmer, H., et al. (1998). Prediction of recovery from post-traumatic vegetative state with cerebral magnetic-resonance imaging. *Lancet* 351, 1763–1767. doi:10.1016/S0140-6736(97)10301-4.

Katz, D. I., Polyak, M., Coughlan, D., Nichols, M., and Roche, A. (2009). Natural history of recovery from brain injury after prolonged disorders of consciousness: outcome of patients admitted to inpatient rehabilitation with 1–4 year follow-up. *Prog. Brain Res.* 177, 73–88.

Katz, R. T., Rovai, G. P., Brait, C., and Rymer, W. Z. (1992). Objective quantification of spastic hypertonia: correlation with clinical findings. *Arch Phys Med Rehabil* 73, 339–347.

Katzenschlager, R., Hughes, A., Evans, A., Manson, A. J., Hoffman, M., Swinn, L., et al. (2005). Continuous subcutaneous apomorphine therapy improves dyskinesias in Parkinson's disease: A prospective study using single-dose challenges. *Mov. Disord.* 20, 151–157. doi:10.1002/mds.20276.

Ketz, N., Jones, A. P., Bryant, N. B., Clark, V. P., and Pilly, P. K. (2018). Closed-loop slow-wave tACS improves sleep-dependent long-term memory generalization by modulating endogenous oscillations. *J. Neurosci.* 38, 7314–7326. doi:10.1523/JNEUROSCI.0273-18.2018.

Khedr, E. M., Gamal, N. F., El-Fetoh, N. A., Khalifa, H., Ahmed, E. M., Ali, A. M., et al. (2014). A double-blind randomized clinical trial on the efficacy of cortical direct current stimulation for the treatment of Alzheimer's disease. *Front Aging Neurosci* 6, 275. doi:10.3389/fnagi.2014.00275.

Koch, C., Massimini, M., Boly, M., and Tononi, G. (2016). Neural correlates of

consciousness: progress and problems. *Nat. Rev. Neurosci.* 17. doi:10.1038/nrn.2016.22.

Kolls, B. J., and Stacy, M. (2006). Apomorphine: A Rapid Rescue Agent for the Management of Motor Fluctuations in Advanced Parkinson Disease. *Clin. Neuropharmacol.* 29, 292–301. doi:10.1097/01.WNF.0000220824.57769.E5.

Kondziella, D., Bender, A., Diserens, K., van Erp, W., Estraneo, A., Formisano, R., et al. (2020). European Academy of Neurology guideline on the diagnosis of coma and other disorders of consciousness. *Eur. J. Neurol.*, ene.14151. doi:10.1111/ene.14151.

Krewer, C., Luther, M., Koenig, E., and Müller, F. (2015). Tilt Table Therapies for Patients with Severe Disorders of Consciousness: A Randomized, Controlled Trial. *PLoS One* 10, e0143180. doi:10.1371/journal.pone.0143180.

Krishnan, S., Pappadis, M. R., Weller, S. C., Stearnes, M., Kumar, A., Ottenbacher, K. J., et al. (2017). Needs of Stroke Survivors as Perceived by Their Caregivers: A Scoping Review. *Am. J. Phys. Med. Rehabil.* 96, 487–505. doi:10.1097/PHM.0000000000000717.

Landsness, E., Bruno, M. A., Noirhomme, Q., Riedner, B., Gosseries, O., Schnakers, C., et al. (2011). Electrophysiological correlates of behavioural changes in vigilance in vegetative state and minimally conscious state. *Brain* 134, 2222–2232. doi:10.1093/brain/awr152.

Laureys, S. (2005). The neural correlate of (un)awareness: lessons from the vegetative state. *Trends Cogn Sci* 9, 556–559. doi:10.1016/j.tics.2005.10.010.

Laureys, S., Celesia, G. G., Cohadon, F., Lavrijsen, J., Leon-Carrion, J., Sannita, W. G., et al. (2010). Unresponsive wakefulness syndrome: a new name for the vegetative state or apallic syndrome. *BMC Med* 8, 68. doi:10.1186/1741-7015-8-68.

Laureys, S., Faymonville, M. E., Luxen, A., Lamy, M., Franck, G., and Maquet, P. (2000). Restoration of thalamocortical connectivity after recovery from persistent vegetative state. *Lancet* 355, 1790–1791.

Laureys, S., Faymonville, M. E., Peigneux, P., Damas, P., Lambermont, B., Del Fiore, G., et al. (2002). Cortical processing of noxious somatosensory stimuli in the persistent vegetative state. *Neuroimage* 17, 732–741.

Laureys, S., Goldman, S., Phillips, C., Van Bogaert, P., Aerts, J., Luxen, A., et al. (1999). Impaired effective cortical connectivity in vegetative state: preliminary investigation using PET. *Neuroimage* 9, 377–382. doi:10.1006/nimg.1998.0414.

Laureys, S., Owen, A. M., and Schiff, N. D. (2004). Brain function in coma, vegetative state, and related disorders. *Lancet Neurol* 3, 537–546. doi:10.1016/S1474-4422(04)00852-X.

Laureys, S., and Schiff, N. D. (2012). Coma and consciousness: Paradigms (re)framed by neuroimaging. *Neuroimage* 61, 478–491.

doi:10.1016/j.neuroimage.2011.12.041.
Lefaucheur, J. P. (2016). A comprehensive database of published tDCS clinical trials (2005–2016). *Neurophysiol. Clin.* 46, 319–398. doi:10.1016/j.neucli.2016.10.002.
Lefebvre, S., Dricot, L., Laloux, P., Desfontaines, P., Evrard, F., Peeters, A., et al. (2017). Increased functional connectivity one week after motor learning and tDCS in stroke patients. *Neuroscience* 340, 424–435. doi:10.1016/j.neuroscience.2016.10.066.
Lehembre, R., Bruno, M. A., Vanhaudenhuyse, A., Chatelle, C., Cologan, V., Leclercq, Y., et al. (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, 41–47.
Leite, J., Morales-Quezada, L., Carvalho, S., Thibaut, A., Doruk, D., Chen, C.-F., et al. (2017). Surface EEG-Transcranial Direct Current Stimulation (tDCS) Closed-Loop System. *Int. J. Neural Syst.*, 1750026. doi:10.1142/S0129065717500265.
Lempel, A., and Ziv, J. (1976). On the complexity of finite sequences. *IEEE Trans. Inf. theory* 22, 75–81.
Leuchter, A. F., Cook, I. A., Feifel, D., Goethe, J. W., Husain, M., Carpenter, L. L., et al. (2015). Efficacy and safety of low-field synchronized transcranial magnetic stimulation (sTMS) for treatment of major depression. *Brain Stimul.* 8, 787–794. doi:10.1016/j.brs.2015.05.005.
Li, D., and Mashour, G. A. (2019). Cortical dynamics during psychedelic and anesthetized states induced by ketamine. *Neuroimage* 196, 32–40. doi:10.1016/j.neuroimage.2019.03.076.
Li, Y., Tong, S., Liu, D., Gai, Y., Wang, X., Wang, J., et al. (2008). Abnormal EEG complexity in patients with schizophrenia and depression. *Clin. Neurophysiol.* 119, 1232–1241.
Liebetanz, D., Nitsche, M. A., Tergau, F., and Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain* 125, 2238–2247.
Lisman, J. E. (2001). Three Ca^{2+} levels affect plasticity differently: the LTP zone, the LTD zone and no man's land. *J. Physiol.* 532, 285–285. doi:10.1111/j.1469-7793.2001.0285f.x.
Liu, P., Gao, J., Pan, S., Meng, F., Pan, G., Li, J., et al. (2016a). Effects of High-Frequency Repetitive Transcranial Magnetic Stimulation on Cerebral Hemodynamics in Patients with Disorders of Consciousness: A Sham-Controlled Study. *Eur. Neurol.* 76, 1–7. doi:10.1159/000447325.
Liu, P., Gao, J., Pan, S., Meng, F., Pan, G., Li, J., et al. (2016b). Effects of High-Frequency Repetitive Transcranial Magnetic Stimulation on Cerebral Hemodynamics in Patients with Disorders of Consciousness: A Sham-Controlled Study. *Eur. Neurol.* 76, 1–7. doi:10.1159/000447325.

Locke, J. (1841). *An essay concerning human understanding*.

López-Alonso, V., Fernández-Del-Olmo, M., Costantini, A., Gonzalez-Henriquez, J. J., and Cheeran, B. (2015). Intra-individual variability in the response to anodal transcranial direct current stimulation. *Clin. Neurophysiol.* 126, 2342–7. doi:10.1016/j.clinph.2015.03.022.

Løvstad, M., Andelic, N., Knoph, R., Jerstad, T., Anke, A., Skandsen, T., et al. (2014). Rate of Disorders of Consciousness in a Prospective Population-Based Study of Adults With Traumatic Brain Injury. *J. Head Trauma Rehabil.* 29, E31–E43. doi:10.1097/HTR.0000000000000017.

Luaute, J., Maucort-Boulch, D., Tell, L., Quelard, F., Sarraf, T., Iwaz, J., et al. (2010). Long-term outcomes of chronic minimally conscious and vegetative states. *Neurology* 75, 246–252. doi:10.1212/WNL.0b013e3181e8e8df.

Lugo, Z., Pellas, F., Blandin, V., Laureys, S., and Gosseries, O. (2017). Assessment of needs, psychological impact and quality of life in families of patients with locked-in syndrome. *Brain Inj.* 31, 1590–1596. doi:10.1080/02699052.2017.1347277.

Lustenberger, C., Boyle, M. R., Alagapan, S., Mellin, J. M., Vaughn, B. V., and Fröhlich, F. (2016). Feedback-Controlled Transcranial Alternating Current Stimulation Reveals a Functional Role of Sleep Spindles in Motor Memory Consolidation. *Curr. Biol.* 26, 2127–2136. doi:10.1016/j.cub.2016.06.044.

Magrassi, L., Maggioni, G., Pistarini, C., Di Perri, C., Bastianello, S., Zippo, A. G., et al. (2016). Results of a prospective study (CATS) on the effects of thalamic stimulation in minimally conscious and vegetative state patients. *J. Neurosurg.* 125, 972–981. doi:10.3171/2015.7.JNS15700.

Majerus, S., Bruno, M. A., Schnakers, C., Giacino, J. T., and Laureys, S. (2009). The problem of aphasia in the assessment of consciousness in brain-damaged patients. *Prog Brain Res* 177, 49–61. doi:10.1016/S0079-6123(09)17705-1.

Marangolo, P., Fiori, V., Calpagnano, M. A., Campana, S., Razzano, C., Caltagirone, C., et al. (2013). tDCS over the left inferior frontal cortex improves speech production in aphasia. *Front Hum Neurosci* 7, 539. doi:10.3389/fnhum.2013.00539.

Margetis, K., Korfias, S. I., Gatzonis, S., Boutos, N., Stranjalis, G., Boviatsis, E., et al. (2014). Intrathecal baclofen associated with improvement of consciousness disorders in spasticity patients. *Neuromodulation* 17, 699–704. doi:10.1111/ner.12147.

Martens, G., Barra, A., and Thibaut, A. (2019a). Neuromodulation for patients with disorders of consciousness following severe brain injury: a systematic review. *PROSPERO CRD42019122420*.

Martens, G., Bodien, Y., Sheau, K., Christoforou, A., and Giacino, J. T. (2019b). Which behaviours are first to emerge during recovery of consciousness after severe brain injury? *Ann. Phys. Rehabil. Med.* doi:10.1016/j.rehab.2019.10.004.

Martens, G., Deltombe, T., Foidart-Dessalle, M., Laureys, S., and Thibaut, A. (2019c). Clinical and electrophysiological investigation of spastic muscle overactivity in patients with disorders of consciousness following severe brain injury. *Clin. Neurophysiol.* 130, 207–213. doi:10.1016/J.CLINPH.2018.10.021.

Martens, G., Foidart-Dessalle, M., Laureys, S., and Thibaut, A. (2018a). "How Does Spasticity Affect Patients with Disorders of Consciousness?," in *Coma and Disorders of Consciousness*, eds. C. Schnakers and S. Laureys (Cham: Springer International Publishing), 119–135. doi:10.1007/978-3-319-55964-3_7.

Martens, G., Fregni, F., Carrière, M., Barra, A., Laureys, S., and Thibaut, A. (2019d). Single tDCS session of motor cortex in patients with disorders of consciousness: a pilot study. *Brain Inj.*, 1–5. doi:10.1080/02699052.2019.1667537.

Martens, G., Laureys, S., and Thibaut, A. (2017). Spasticity management in disorders of consciousness. *Brain Sci.* 7, 162. doi:10.3390/brainsci7120162.

Martens, G., Lejeune, N., O'Brien, A. T., Fregni, F., Martial, C., Wannez, S., et al. (2018b). Randomized controlled trial of home-based 4-week tDCS in chronic minimally conscious state. *Brain Stimul.* 11, 982–990. doi:10.1016/J.BRS.2018.04.021.

Mateos, D. M., Guevara Erra, R., Wennberg, R., and Perez Velazquez, J. L. (2018). Measures of entropy and complexity in altered states of consciousness. *Cogn. Neurodyn.* 12, 73–84. doi:10.1007/s11571-017-9459-8.

Matsumoto-Miyazaki, J., Asano, Y., Ikegame, Y., Kawasaki, T., Nomura, Y., and Shinoda, J. (2016). Acupuncture Reduces Excitability of Spinal Motor Neurons in Patients with Spastic Muscle Overactivity and Chronic Disorder of Consciousness Following Traumatic Brain Injury. *J. Altern. Complement. Med.* 22, 895–902. doi:10.1089/acm.2016.0180.

Méndez, M. A., Zuluaga, P., Hornero, R., Gómez, C., Escudero, J., Rodríguez-Palancas, A., et al. (2012). Complexity analysis of spontaneous brain activity: effects of depression and antidepressant treatment. *J. Psychopharmacol.* 26, 636–643.

Miranda, P. C., Lomarev, M., and Hallett, M. (2006). Modeling the current distribution during transcranial direct current stimulation. *Clin Neurophysiol* 117, 1623–1629. doi:10.1016/j.clinph.2006.04.009.

Monai, H., and Hirase, H. (2018). Astrocytes as a target of transcranial direct current stimulation (tDCS) to treat depression. *Neurosci. Res.* 126, 15–21. doi:10.1016/j.neures.2017.08.012.

Monai, H., Ohkura, M., Tanaka, M., Oe, Y., Konno, A., Hirai, H., et al. (2016). Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain. *Nat. Commun.* 7, 11100. doi:10.1038/ncomms11100.

Monti, M. M., Schnakers, C., Korb, A. S., Bystritsky, A., and Vespa, P. M. (2016). Non-Invasive Ultrasonic Thalamic Stimulation in Disorders of Consciousness after

Severe Brain Injury: A First-in-Man Report. *Brain Stimul.* 9, 940–941. doi:10.1016/j.brs.2016.07.008.

Monti, M. M., Vanhaudenhuyse, A., Coleman, M. R., Boly, M., Pickard, J. D., Tshibanda, L., et al. (2010). Willful modulation of brain activity in disorders of consciousness. *N Engl J Med* 362, 579–589. doi:10.1056/NEJMoa0905370.

Murase, N., Duque, J., Mazzocchio, R., and Cohen, L. G. (2004). Influence of interhemispheric interactions on motor function in chronic stroke. *Ann. Neurol.* 55, 400–409. doi:10.1002/ana.10848.

Naccache, L. (2018). Minimally conscious state or cortically mediated state? *Brain* 141, 949–960. doi:10.1093/brain/awx324.

Nair, D. G., Renga, V., Lindenberg, R., Zhu, L., and Schlaug, G. (2011). Optimizing recovery potential through simultaneous occupational therapy and non-invasive brain-stimulation using tDCS. *Restor Neurol Neurosci* 29, 411–420. doi:10.3233/RNN-2011-0612.

Najenson, T., Sazbon, L., Fiselzon, J., Becker, E., and Schechter, I. (1978). Recovery of communicative functions after prolonged traumatic coma. *Scand. J. Rehabil. Med.* 10, 15–21.

Nakase-Richardson, R., McNamee, S., Howe, L. L., Massengale, J., Peterson, M., Barnett, S. D., et al. (2013). Descriptive characteristics and rehabilitation outcomes in active duty military personnel and veterans with disorders of consciousness with combat- and noncombat-related brain injury. *Arch. Phys. Med. Rehabil.* 94, 1861–1869. doi:10.1016/j.apmr.2013.05.027.

Nakase-Richardson, R., Yablon, S. A., Sherer, M., Nick, T. G., and Evans, C. C. (2009). Emergence from minimally conscious state: Insights from evaluation of posttraumatic confusion. *Neurology* 73, 1120–1126. doi:10.1212/WNL.0b013e3181bacf34.

Nakayama, N., Okumura, A., Shinoda, J., Nakashima, T., and Iwama, T. (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, 856–862. doi:10.1136/jnnp.2005.080523.

Naro, A., Calabro, R. S., Russo, M., Leo, A., Pollicino, P., Quartarone, A., et al. (2015). Can transcranial direct current stimulation be useful in differentiating unresponsive wakefulness syndrome from minimally conscious state patients? - PubMed - NCBI. *Restor Neurol Neurosci* 33, 159–176.

Newcombe, V., Chatfield, D., Outtrim, J., Vowler, S., Manktelow, A., Cross, J., et al. (2011). Mapping traumatic axonal injury using diffusion tensor imaging: Correlations with functional outcome. *PLoS One* 6. doi:10.1371/journal.pone.0019214.

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., et al. (2003a). Pharmacological modulation of cortical excitability shifts induced by

transcranial direct current stimulation in humans. *J Physiol* 553, 293–301. doi:10.1113/jphysiol.2003.049916.

Nitsche, M. A., Jaussi, W., Liebetanz, D., Lang, N., Tergau, F., and Paulus, W. (2004). Consolidation of human motor cortical neuroplasticity by D-cycloserine. *Neuropsychopharmacology* 29, 1573–1578. doi:10.1038/sj.npp.1300517.

Nitsche, M. A., Liebetanz, D., Lang, N., Antal, A., Tergau, F., and Paulus, W. (2003b). Safety criteria for transcranial direct current stimulation (tDCS) in humans. *Clin Neurophysiol* 114, 2220–2223.

Nitsche, M. A., and Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology* 57, 1899–901. doi:10.1212/WNL.57.10.1899.

Noé, E., Ferri, J., Colomer, C., Moliner, B., O'Valle, M., Ugart, P., et al. (2019). Feasibility, safety and efficacy of transauricular vagus nerve stimulation in a cohort of patients with disorders of consciousness. *Brain Stimul.* doi:10.1016/j.brs.2019.12.005.

Noé, E., Olaya, J., Navarro, M. D., Noguera, P., Colomer, C., García-Panach, J., et al. (2012). Behavioral recovery in disorders of consciousness: A prospective study with the Spanish version of the coma recovery scale-revised. *Arch. Phys. Med. Rehabil.* 93, 428-433.e12. doi:10.1016/j.apmr.2011.08.048.

Ohn, S. H., Park, C. I., Yoo, W. K., Ko, M. H., Choi, K. P., Kim, G. M., et al. (2008). Time-dependent effect of transcranial direct current stimulation on the enhancement of working memory. *Neuroreport* 19, 43–47. doi:10.1097/WNR.0b013e3282f2adfd.

Palanca, B. J. A., Mashour, G. A., and Avidan, M. S. (2009). Processed electroencephalogram in depth of anesthesia monitoring. *Curr. Opin. Anaesthesiol.* 22, 553–559. doi:10.1097/ACO.0b013e3283304032.

Palm, U., Kumpf, U., Behler, N., Wulf, L., Kirsch, B., Wörsching, J., et al. (2018). Home Use, Remotely Supervised, and Remotely Controlled Transcranial Direct Current Stimulation: A Systematic Review of the Available Evidence. *Neuromodulation Technol. Neural Interface* 21, 323–333. doi:10.1111/ner.12686.

Palm, U., Reisinger, E., Keeser, D., Kuo, M.-F., Pogarell, O., Leicht, G., et al. (2013). Evaluation of Sham Transcranial Direct Current Stimulation for Randomized, Placebo-Controlled Clinical Trials. *Brain Stimul.* 6, 690–695. doi:10.1016/J.BRS.2013.01.005.

Pandyan, A. D., Gregoric, M., and Barnes, M. P. (2005). Spasticity: clinical perceptions, neurological realities and meaningful measurement. *Disabil Rehabil* 27, 2–6.

Pape, T. L.-B., Heinemann, A. W., Kelly, J. P., Hurder, A. G., and Lundgren, S. (2005). A measure of neurobehavioral functioning after coma. Part I: Theory, reliability, and validity of the Disorders of Consciousness Scale. *J. Rehabil. Res. Dev.* 42, 1.

doi:10.1682/JRRD.2004.03.0032.

Peeters, M., Page, G., Maloteaux, J.-M., and Hermans, E. (2002). Hypersensitivity of dopamine transmission in the rat striatum after treatment with the NMDA receptor antagonist amantadine. *Brain Res.* 949, 32–41. doi:10.1016/S0006-8993(02)02961-X.

Piarulli, A., Bergamasco, M., Thibaut, A., Cologan, V., Gosseries, O., and Laureys, S. (2016). EEG ultradian rhythmicity differences in disorders of consciousness during wakefulness. *J. Neurol.* 263, 1746–1760. doi:10.1007/s00415-016-8196-y.

Pisani, L. R., Naro, A., Leo, A., Aricò, I., Pisani, F., Silvestri, R., et al. (2015a). Repetitive transcranial magnetic stimulation induced slow wave activity modification: A possible role in disorder of consciousness differential diagnosis? *Conscious. Cogn.* 38, 1–8. doi:10.1016/J.CONCOG.2015.09.012.

Pisani, L. R., Naro, A., Leo, A., Aricò, I., Pisani, F., Silvestri, R., et al. (2015b). Repetitive transcranial magnetic stimulation induced slow wave activity modification: A possible role in disorder of consciousness differential diagnosis? *Conscious. Cogn.* 38, 1–8. doi:10.1016/J.CONCOG.2015.09.012.

Pistoia, F., Sacco, S., Sarà, M., Franceschini, M., and Carolei, A. (2015). Intrathecal Baclofen: Effects on Spasticity, Pain, and Consciousness in Disorders of Consciousness and Locked-in Syndrome. *Curr. Pain Headache Rep.* 19. doi:10.1007/s11916-014-0466-8.

Plum, F., and Posner, J. B. (1972). The diagnosis of stupor and coma. *Contemp Neurol Ser* 10, 1–286.

Polanía, R., Paulus, W., Antal, A., and Nitsche, M. A. (2011). Introducing graph theory to track for neuroplastic alterations in the resting human brain: A transcranial direct current stimulation study. *Neuroimage* 54, 2287–2296. doi:10.1016/j.neuroimage.2010.09.085.

Purpura, D. P., and McMurtry, J. G. (1965). Intracellular activities and evoked potential changes during polarization of motor cortex. *J. Neurophysiol.* 28, 166–185. doi:10.1152/jn.1965.28.1.166.

R Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raine, T. R., Harper, C. C., Rocca, C. H., Fischer, R., Padian, N., Klausner, J. D., et al. (2005). Direct access to emergency contraception through pharmacies and effect on unintended pregnancy and STIs: A randomized controlled trial. *J. Am. Med. Assoc.* 293, 54–62. doi:10.1001/jama.293.1.54.

Rémi, J., Pfefferkorn, T., Owens, R. L., Schankin, C., Dehning, S., Birnbaum, T., et al. (2011). The crossed leg sign indicates a favorable outcome after severe stroke. *Neurology* 77, 1453–6. doi:10.1212/WNL.0b013e318232abe4.

Richard, I., and Menei, P. (2007). Intrathecal baclofen in the treatment of adult spasticity. *Neurosurg. Focus* 21, e5. doi:10.3171/foc.2006.21.2.6.

Robinson, C. S. H., Bryant, N. B., Maxwell, J. W., Jones, A. P., Robert, B., Lamphere, M., et al. (2018). The benefits of closed-loop transcranial alternating current stimulation on subjective sleep quality. *Brain Sci.* 8. doi:10.3390/brainsci8120204.

Rosanova, M., Gosseries, O., Casarotto, S., Boly, M., Casali, A. G., Bruno, M. A., et al. (2012). Recovery of cortical effective connectivity and recovery of consciousness in vegetative patients. *Brain* 135, 1308–1320. doi:10.1093/brain/awr340.

Ruffini, G. (2017). An algorithmic information theory of consciousness. *Neurosci. Conscious.* 3.

Ruffini, G., Fox, M. D., Ripolles, O., Miranda, P. C., and Pascual-Leone, A. (2014). Optimization of multifocal transcranial current stimulation for weighted cortical pattern targeting from realistic modeling of electric fields. *Neuroimage* 89, 216–225. doi:10.1016/j.neuroimage.2013.12.002.

Russo, C., Souza Carneiro, M., Bolognini, N., and Fregni, F. (2017). Safety Review of Transcranial Direct Current Stimulation in Stroke. *Neuromodulation* 20, 21–222.

Sacco, K., Galetto, V., Dimitri, D., Geda, E., Perotti, F., Zettin, M., et al. (2016). Concomitant Use of Transcranial Direct Current Stimulation and Computer-Assisted Training for the Rehabilitation of Attention in Traumatic Brain Injured Patients: Behavioral and Neuroimaging Results. *Front. Behav. Neurosci.* 10, 57. doi:10.3389/fnbeh.2016.00057.

Sanz, L., Lejeune, N., Thibaut, A., Blandiaux, S., Stender, J., Farber, N., et al. (2018). Treating severely brain-injured patients with apomorphine: study protocol for a double blind randomized placebo-controlled trial using behavioral and neuroimaging assessments. *Front. Neurosci.* 12. doi:10.3389/conf.fnins.2018.95.00065.

Sara, M., Sacco, S., Cipolla, F., Onorati, P., Scoppetta, C., Albertini, G., et al. (2007). An unexpected recovery from permanent vegetative state. *Brain Inj* 21, 101–103. doi:10.1080/02699050601151761.

Schartner, M. M., Pigorini, A., Gibbs, S. A., Arnulfo, G., Sarasso, S., Barnett, L., et al. (2017). Global and local complexity of intracranial EEG decreases during NREM sleep. *Neurosci. Conscious.*, niw022. doi:10.1093/nc/niw022.

Schartner, M., Seth, A., Noirhomme, Q., Boly, M., Bruno, M.-A., Laureys, S., et al. (2015). Complexity of multi-dimensional spontaneous EEG decreases during propofol induced general anaesthesia. *PLoS One* 10, e0133532.

Schiff, N. D. (2005). Modeling the minimally conscious state: measurements of brain function and therapeutic possibilities. *Prog Brain Res* 150, 473–493. doi:10.1016/S0079-6123(05)50033-5.

Schiff, N. D. (2010). Recovery of consciousness after brain injury: a mesocircuit hypothesis. *Trends Neurosci.* 33, 1–9. doi:10.1016/j.tins.2009.11.002.

Schiff, N. D., Giacino, J. T., Kalmar, K., Victor, J. D., Baker, K., Gerber, M., et al. (2007). Behavioural improvements with thalamic stimulation after severe traumatic brain injury. *Nature* 448, 600–603. doi:10.1038/nature06041.

Schlaug, G., Renga, V., and Nair, D. (2008). Transcranial Direct Current Stimulation in Stroke Recovery. *Arch. Neurol.* 65, 1571–1576. doi:10.1001/archneur.65.12.1571.

Schleicher, R., Galley, N., Briest, S., and Galley, L. (2008). Blinks and saccades as indicators of fatigue in sleepiness warnings: looking tired? *Ergonomics* 51, 982–1010. doi:10.1080/00140130701817062.

Schnakers, C., Chatelle, C., Demertzi, A., Majerus, S., and Laureys, S. (2012). What about Pain in Disorders of Consciousness? *AAPS J* 14, 437–444. doi:10.1208/s12248-012-9346-5.

Schnakers, C., Edlow, B. L., Chatelle, C., and Giacino, J. T. (2015). "Minimally Conscious State," in *The Neurology of Consciousness*, eds. S. Laureys, O. Gosseries, and G. Tononi (Academic Press), 488.

Schnakers, C., Giacino, J. T., Lovstad, M., Habbal, D., Boly, M., Di, H., et al. (2014). Preserved Covert Cognition in Noncommunicative Patients With Severe Brain Injury? *Neurorehabil Neural Repair*. doi:10.1177/1545968314547767.

Schnakers, C., Hustinx, R., Vandewalle, G., Majerus, S., Moonen, G., Boly, M., et al. (2008). Measuring the effect of amantadine in chronic anoxic minimally conscious state. *J Neurol Neurosurg Psychiatry* 79, 225–227. doi:10.1136/jnnp.2007.124099.

Schnakers, C., Vanhaudenhuyse, A., Giacino, J., Ventura, M., Boly, M., Majerus, S., et al. (2009). Diagnostic accuracy of the vegetative and minimally conscious state: clinical consensus versus standardized neurobehavioral assessment. *BMC Neurol* 9, 35. doi:10.1186/1471-2377-9-35.

Seel, R. T., Sherer, M., Whyte, J., Katz, D. I., Giacino, J. T., Rosenbaum, A. M., et al. (2010). Assessment Scales for Disorders of Consciousness: Evidence-Based Recommendations for Clinical Practice and Research. *Arch. Phys. Med. Rehabil.* 91, 1795–1813. doi:10.1016/j.apmr.2010.07.218.

Sheean, G. (2002). The pathophysiology of spasticity. *Eur J Neurol* 9 Suppl 1, 3–61.

Sherer, M., Nakase-Thompson, R., Yablon, S. A., and Gontkovsky, S. T. (2005). Multidimensional Assessment of Acute Confusion After Traumatic Brain Injury. *Arch. Phys. Med. Rehabil.* 86, 896–904. doi:10.1016/j.apmr.2004.09.029.

Shiel, A., Horn, S. A., Wilson, B. A., Watson, M. J., Campbell, M. J., and McLellan, D. L. (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, 408–416.

Shrestha, P., Malla, H., Pant, B., and Taira, T. (2011). Intrathecal baclofen therapy in severe head injury, first time in Nepal, a technique suitable for underdeveloped countries. *Asian J. Neurosurg.* 6, 49–51. doi:10.4103/1793-

5482.85638.

Silva, S., Alacoque, X., Fourcade, O., Samii, K., Marque, P., Woods, R., et al. (2010). Wakefulness and loss of awareness: brain and brainstem interaction in the vegetative state. *Neurology* 74, 313–320. doi:10.1212/WNL.0b013e3181cbcd96.

Simis, M., Bravo, G. L., Boggio, P. S., Devido, M., Gagliardi, R. J., and Fregni, F. (2014). Transcranial Direct Current Stimulation in de novo Artistic Ability After Stroke. *Neuromodulation Technol. Neural Interface* 17, 497–501. doi:10.1111/ner.12140.

Stagg, C. J., Antal, A., and Nitsche, M. A. (2018). Physiology of Transcranial Direct Current Stimulation. *J. ECT* 34, 144–152. doi:10.1097/YCT.0000000000000510.

Stagg, C. J., and Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. *Neuroscientist* 17, 37–53. doi:10.1177/1073858410386614.

Stender, J., Gosseries, O., Bruno, M.-A., Charland-Verville, V., Vanhaudenhuyse, A., Demertzi, A., et al. (2014). Diagnostic precision of PET imaging and functional MRI in disorders of consciousness: a clinical validation study. *Lancet* 6736, 8–16. doi:10.1016/S0140-6736(14)60042-8.

Straudi, S., Bonsangue, V., Mele, S., Craighero, L., Montis, A., Fregni, F., et al. (2019). Bilateral M1 anodal transcranial direct current stimulation in post traumatic chronic minimally conscious state: a pilot EEG-tDCS study. *Brain Inj.*, 1–6. doi:10.1080/02699052.2019.1565894.

Sturm, W., and Willmes, K. (2001). On the functional neuroanatomy of intrinsic and phasic alertness. in *NeuroImage* (Academic Press Inc.), S76–S84. doi:10.1006/nimg.2001.0839.

Sun, Y., Wang, J., Heine, L., Huang, W., Wang, J., Hu, N., et al. (2018). Personalized objects can optimize the diagnosis of EMCS in the assessment of functional object use in the CRS-R: a double blind, randomized clinical trial. *BMC Neurol.* 18, 38. doi:10.1186/s12883-018-1040-5.

Teasdale, G., and Jennett, B. (1974). Assessment of Coma and Impaired Consciousness. *Lancet* 304, 81–84. doi:10.1016/S0140-6736(74)91639-0.

The Multi-Society Task Force on PVS (1994a). Medical aspects of the persistent vegetative state (1). *N Engl J Med* 330, 1499–1508. doi:10.1056/NEJM199405263302107.

The Multi-Society Task Force on PVS (1994b). Medical aspects of the persistent vegetative state (2). *N Engl J Med* 330, 1572–1579. doi:10.1056/NEJM199406023302206.

Thibaut, A., Bruno, M.-A., Ledoux, D., Demertzi, A., and Laureys, S. (2014). tDCS in patients with disorders of consciousness Sham-controlled randomized double-blind study. *Neurology* 82, 1112–1118. doi:10.1212/WNL.0000000000000260.

Thibaut, A., Chatelle, C., Vanhaudenhuyse, A., Martens, G., Cassol, H., Martial, C., et

al. (2018a). Transcranial direct current stimulation unveils covert consciousness. *Brain Stimul.* 11, 642–644. doi:10.1016/j.brs.2018.02.002.

Thibaut, A., Chatelle, C., Wannez, S., Deltombe, T., Stender, J., Schnakers, C., et al. (2015a). Spasticity in disorders of consciousness: A behavioral study. *Eur J Phys Rehabil Med* 51, 289–397.

Thibaut, A., Chatelle, C., Ziegler, E., Bruno, M.-A., Laureys, S., and Gosseries, O. (2013). Spasticity after stroke: physiology, assessment and treatment. *Brain Inj.* 27, 1093–1105. doi:10.3109/02699052.2013.804202.

Thibaut, A., Chennu, S., Chatelle, C., Martens, G., Annen, J., Cassol, H., et al. (2018b). Theta network centrality correlates with tDCS response in disorders of consciousness. *Brain Stimul.* 11, 1407–1409. doi:10.1016/j.brs.2018.09.002.

Thibaut, A., Deltombe, T., Wannez, S., Gosseries, O., Ziegler, E., Dieni, C., et al. (2015b). Impact of soft splints on upper limb spasticity in chronic patients with disorders of consciousness: A randomized, single-blind, controlled trial. *Brain Inj.* 29, 830–836. doi:10.3109/02699052.2015.1005132.

Thibaut, A., Di Perri, C., Chatelle, C., Bruno, M.-A., Bahri, M. A., Wannez, S., et al. (2015c). Clinical response to tDCS depends on residual brain metabolism and grey matter integrity in patients with minimally conscious state. *Brain Stimul.* 8, 1116–1123. doi:10.1016/j.brs.2015.07.024.

Thibaut, A., O'Brien, A. T., and Fregni, F. (2017a). Strategies for replacing non-invasive brain stimulation sessions: recommendations for designing neurostimulation clinical trials. *Expert Rev. Med. Devices* 14, 633–649. doi:10.1080/17434440.2017.1352470.

Thibaut, A., Piarulli, A., Martens, G., Chatelle, C., and Laureys, S. (2019a). Effect of multichannel transcranial direct current stimulation to reduce hypertonia in individuals with prolonged disorders of consciousness: A randomized controlled pilot study. *Ann. Phys. Rehabil. Med.* doi:10.1016/J.REHAB.2019.05.009.

Thibaut, A., Schiff, N., Giacino, J., Laureys, S., and Gosseries, O. (2019b). Therapeutic interventions in patients with prolonged disorders of consciousness. *Lancet Neurol.* 18, 600–614. doi:10.1016/S1474-4422(19)30031-6.

Thibaut, A., Wannez, S., Donneau, A.-F., Chatelle, C., Gosseries, O., Bruno, M.-A., et al. (2017b). Controlled clinical trial of repeated prefrontal tDCS in patients with chronic minimally conscious state. *Brain Inj.* 31, 1–9. doi:10.1080/02699052.2016.1274776.

Thonnard, M., Gosseries, O., Demertzi, A., Lugo, Z., Vanhaudenhuyse, A., Bruno, M.-A. A., et al. (2014). Effect of zolpidem in chronic disorders of consciousness: A prospective open-label study. *Funct Neurol* 28, 259–264.

Threlkeld, Z. D., Bodien, Y. G., Rosenthal, E. S., Giacino, J. T., Nieto-Castanon, A., Wu, O., et al. (2018). Functional networks reemerge during recovery of consciousness after acute severe traumatic brain injury. *Cortex* 106, 299–

308. doi:10.1016/j.cortex.2018.05.004.

Tononi, G., and Edelman, G. M. (1998). Consciousness and complexity. *Science (80-.).* 282, 1846–1851.

Turgeon, A. F., Lauzier, F., Simard, J.-F., Scales, D. C., Burns, K. E. A., Moore, L., et al. (2011). Mortality associated with withdrawal of life-sustaining therapy for patients with severe traumatic brain injury: a Canadian multicentre cohort study. *CMAJ* 183, 1581–8. doi:10.1503/cmaj.101786.

Ulam, F., Shelton, C., Richards, L., Davis, L., Hunter, B., Fregni, F., et al. (2014). Cumulative effects of transcranial direct current stimulation on EEG oscillations and attention/working memory during subacute neurorehabilitation of traumatic brain injury. *Clin Neurophysiol* 126, 486–496. doi:10.1016/j.clinph.2014.05.015.

Valle, A., Roizenblatt, S., Botte, S., Zaghi, S., Riberto, M., Tufik, M., et al. (2009). Efficacy of anodal transcranial direct current stimulation (tDCS) for the treatment of fibromyalgia: results of a randomized, sham controlled longitudinal clinical trial. *J Pain Manag* 2, 353–361.

van Erp, W. S., Lavrijsen, J. C. M., van de Laar, F. A., Vos, P. E., Laureys, S., and Koopmans, R. T. C. M. (2014). The vegetative state/unresponsive wakefulness syndrome: a systematic review of prevalence studies. *Eur. J. Neurol.* 21, 1361–1368. doi:10.1111/ene.12483.

Vanhaudenhuyse, A., Demertzi, A., Schabus, M., Noirhomme, Q., Bredart, S., Boly, M., et al. (2011). Two distinct neuronal networks mediate the awareness of environment and of self. *J Cogn Neurosci* 23, 570–578. doi:10.1162/jocn.2010.21488.

Vanhaudenhuyse, A., Noirhomme, Q., Tshibanda, L. J., Bruno, M. A., Boveroux, P., Schnakers, C., et al. (2010). Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients. *Brain* 133, 161–171. doi:10.1093/brain/awp313.

Vanhaudenhuyse, A., Schnakers, C., Bredart, S., and Laureys, S. (2008). Assessment of visual pursuit in post-comatose states: use a mirror. *J Neurol Neurosurg Psychiatry* 79, 223. doi:10.1136/jnnp.2007.121624.

Voss, H. U., Uluc, A. M., Dyke, J. P., Watts, R., Kobylarz, E. J., McCandliss, B. D., et al. (2006). Possible axonal regrowth in late recovery from the minimally conscious state. *J Clin Invest* 116, 2005–2011. doi:10.1172/JCI27021.

Wallace, T., and Bradshaw, A. (2011). Technologies and strategies for people with communication problems following brain injury or stroke. *NeuroRehabilitation* 28, 199–209. doi:10.3233/NRE-2011-0649.

Wannez, S., Gosseries, O., Azzolini, D., Martial, C., Cassol, H., Aubinet, C., et al. (2017a). Prevalence of coma-recovery scale-revised signs of consciousness in patients in minimally conscious state. *Neuropsychol. Rehabil.* doi:10.1080/09602011.2017.1310656.

Wannez, S., Heine, L., Thonnard, M., Gosseries, O., and Laureys, S. (2017b). The repetition of behavioral assessments in disorders of consciousness. *Ann. Neurol.* 81, 883–889.

Wannez, S., Vanhaudenhuyse, A., Laureys, S., and Brédart, S. (2017c). Mirror efficiency in the assessment of visual pursuit in patients in minimally conscious state. *Brain Inj.* 31, 1429–1435. doi:10.1080/02699052.2017.1376755.

Whyte, J., DiPasquale, M. C., and Vaccaro, M. (1999). Assessment of command-following in minimally conscious brain injured patients. *Arch. Phys. Med. Rehabil.* 80, 653–660. doi:10.1016/S0003-9993(99)90168-5.

Whyte, J., Rajan, R., Rosenbaum, A., Katz, D., Kalmar, K., Seel, R., et al. (2014). Zolpidem and restoration of consciousness. *Am J Phys Med Rehabil* 93, 101–113. doi:10.1097/PHM.0000000000000069.

Wiethoff, S., Hamada, M., and Rothwell, J. C. (2014). Variability in response to transcranial direct current stimulation of the motor cortex. *Brain Stimul* 7, 468–475. doi:10.1016/j.brs.2014.02.003.

Wijdicks, E. F., Bamlet, W. R., Maramattom, B. V, Manno, E. M., and McClelland, R. L. (2005). Validation of a new coma scale: The FOUR score. *Ann Neurol* 58, 585–593. doi:10.1002/ana.20611.

Williams, S. T., Conte, M. M., Goldfine, A. M., Noirhomme, Q., Gosseries, O., Thonnard, M., et al. (2013). Common resting brain dynamics indicate a possible mechanism underlying zolpidem response in severe brain injury. *Elife* 2, e01157.

Wu, M., Yu, Y., Luo, L., Wu, Y., Gao, J., Ye, X., et al. (2019). Efficiency of Repetitive Transcranial Direct Current Stimulation of the Dorsolateral Prefrontal Cortex in Disorders of Consciousness: A Randomized Sham-Controlled Study. *Neural Plast.* 2019, 1–11. doi:10.1155/2019/7089543.

Wu, Y. J., Tseng, P., Chang, C. F., Pai, M. C., Hsu, K. Sen, Lin, C. C., et al. (2014). Modulating the interference effect on spatial working memory by applying transcranial direct current stimulation over the right dorsolateral prefrontal cortex. *Brain Cogn.* 91, 87–94. doi:10.1016/j.bandc.2014.09.002.

Yampolsky, C., Hem, S., and Bendersky, D. (2012). Dorsal column stimulator applications. *Surg. Neurol. Int.* 3, 275. doi:10.4103/2152-7806.103019.

Yu, Y., Yang, Y., Wang, L., Fang, J., Chen, Y., He, J., et al. (2017). Transcutaneous auricular vagus nerve stimulation in disorders of consciousness monitored by fMRI: The first case report. *Brain Stimul.* 10, 328–330. doi:10.1016/j.brs.2016.12.004.

Zhang, Y., Song, W., Du, J., Huo, S., Shan, G., and Li, R. (2017). Transcranial Direct Current Stimulation in Patients with Prolonged Disorders of Consciousness: Combined Behavioral and Event-Related Potential Evidence. *Front. Neurol.* 8, 620. doi:10.3389/fneur.2017.00620.

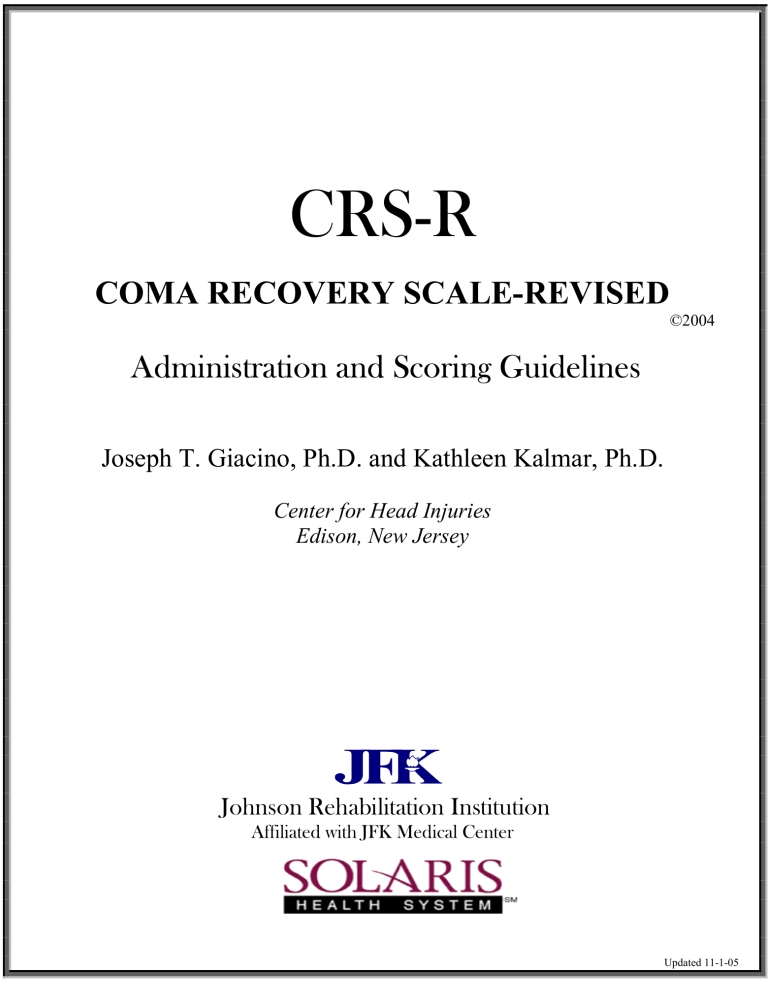
Zheng, Z. S., Reggente, N., Lutkenhoff, E., Owen, A. M., and Monti, M. M. (2017). Disentangling disorders of consciousness: Insights from diffusion tensor imaging and machine learning. *Hum. Brain Mapp.* 38, 431–443. doi:10.1002/hbm.23370.

Ziv, J., and Lempel, A. (1978). Compression of individual sequences via variable-rate coding. *IEEE Trans. Inf. Theory* 24, 530–536. doi:10.1109/TIT.1978.1055934.

Zrenner, C., Belardinelli, P., Müller-Dahlhaus, F., and Ziemann, U. (2016). Closed-Loop Neuroscience and Non-Invasive Brain Stimulation: A Tale of Two Loops. *Front. Cell. Neurosci.* 10, 92. doi:10.3389/fncel.2016.00092.

7. Appendix

7.1. Appendix 1 – CRS-R administration and scoring guidelines



CRS-R

COMA RECOVERY SCALE-REVISED

©2004

Administration and Scoring Guidelines

Joseph T. Giacino, Ph.D. and Kathleen Kalmar, Ph.D.

Center for Head Injuries
Edison, New Jersey

JFK
Johnson Rehabilitation Institution
Affiliated with JFK Medical Center
SOLARIS HEALTH SYSTEM℠

Updated 11-1-05

JFK COMA RECOVERY SCALE - REVISED ©2004

Record Form

This form should only be used in association with the "CRS-R ADMINISTRATION AND SCORING GUIDELINES" which provide instructions for standardized administration of the scale.

Patient:	**Diagnosis:**	**Etiology:**
Date of Onset:	**Date of Admission:**	

Date																
Week	**ADM**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
AUDITORY FUNCTION SCALE																
4 - Consistent Movement to Command *																
3 - Reproducible Movement to Command *																
2 - Localization to Sound																
1 - Auditory Startle																
0 - None																
VISUAL FUNCTION SCALE																
5 - Object Recognition *																
4 - Object Localization: Reaching *																
3 - Visual Pursuit *																
2 - Fixation *																
1 - Visual Startle																
0 - None																
MOTOR FUNCTION SCALE																
6 - Functional Object Use †																
5 - Automatic Motor Response *																
4 - Object Manipulation *																
3 - Localization to Noxious Stimulation *																
2 - Flexion Withdrawal																
1 - Abnormal Posturing																
0 - None/Flaccid																
OROMOTOR/VERBAL FUNCTION SCALE																
3 - Intelligible Verbalization *																
2 - Vocalization/Oral Movement																
1 - Oral Reflexive Movement																
0 - None																
COMMUNICATION SCALE																
2 - Functional: Accurate †																
1 - Non-Functional: Intentional *																
0 - None																
AROUSAL SCALE																
3 - Attention																
2 - Eye Opening w/o Stimulation																
1 - Eye Opening with Stimulation																
0 - Unarousable																
TOTAL SCORE																

Denotes emergence from MCS†

Denotes MCS *

1

BRAIN STEM REFLEX GRID ©2004

Record Form

Patient:	**Date:**						
Pupillary Light	Reactive						
	Equal						
	Constricted						
	Dilated						
	Pinpoint						
	Accommodation						
Corneal Reflex	Absent						
	Present Unilateral						
	Present Bilateral						
Spontaneous Eye Movements	None						
	Skew Deviation						
	Conjugate Gaze Deviation						
	Roving						
	Dysconjugate						
Oculocephalic Reflex	None						
	Abnormal						
	Full						
	Normal						
Postural Responses (Indicate Limb)	Abnormal Extension						
	Abnormal Flexion						

NOTES

2

AROUSAL FACILITATION PROTOCOL ©2004

GUIDELINES

1) The goal of this intervention is to prolong the length of time the patient maintains arousal (i.e. eye opening)

2) The protocol is administered anytime the patient is observed to:

- Exhibit sustained eyelid closure ***AND/OR***
- Stops following commands for a period of at least one minute.

3) Readminister the arousal facilitation protocol when:

- Sustained eye closure re-occurs ***OR***
- Behavioral responsiveness ceases despite sustained eye opening.

INTERVENTIONS

Deep Pressure:

1) Present deep pressure stimulation unilaterally to the face, neck, shoulder, arm, hand, chest, back, leg, foot, and toes. The muscle should be firmly grasped at its base between the thumb and forefinger. While squeezing the muscle firmly, it should be "rolled" back and forth through the finger tips three to four times. This procedure should be repeated sequentially working from the facial musculature to the toes. The examiner should assure that there are no internal lines, local injuries (e.g., fractures, contusions, decubiti) or systemic complications (e.g., heterotopic ossification) before administering deep pressure.

2) Administer same on contralateral side.

AUDITORY FUNCTION SCALE ©2004

Score	Item	Method	Response
		1. Observe frequency of spontaneous movement for a one minute interval (See **Baseline Observation** and **Command Following Protocol** on page 5).	Clearly discernible and accurate responses occur within 10 seconds on all 4 trials administered.
		2. Choose at least 1 object-related and 1 non-object related command from the Command Following Protocol. The type of command chosen (eye, limb, oral) should be based on patient's physical capacity and should be of low spontaneous frequency. If time permits, more than one type of command from each category may be used. The command should be repeated once during the 10 second response interval.	This item is credited only when ***all 4 trials*** of ***2 different*** commands are passed.
4	***Consistent Movement to Command***	**a. Object-Related Eye Movement Commands:** Present 2 common objects simultaneously and approximately 16 inches apart within the patient's field of view. Ask the patient to look at the object named (i.e. "Look at the [name object]". Next, reverse the positions of the 2 objects and ask the patient to look at the ***same*** object again (i.e. "Look at the [name object]"). Administer two additional trials using the same 2 objects and repeat the above procedure with instruction to look at the ***other*** object on both trials. Two trials per object should be administered for a total of 4 trials.	
		b. Object-Related Limb Movement Command: Present 2 common objects simultaneously and approximately 16 inches apart within the patient's field of view and within arm's (or leg's) length and ask the patient to touch the object named with their hand (or foot). Next, reverse the positions of the 2 objects and ask the patient to touch the ***same*** object again. Administer two additional trials using the same two objects and repeat the above procedure with instruction to touch the ***other object on both trials***. Two trials per object should be administered for a total of 4 trials.	
		c. Non-Object Related Commands: Select at least 1 eye movement, limb movement or oral movement/vocalization command and present it over 4 trials at 15 second intervals. The same command should be used for all 4 trials. Movements that occur between commands (ie: after the response interval has elapsed) should be noted but not scored.	
3	***Reproducible Movement to Command***	Same as above	3 clearly discernible responses occur over the 4 trials on any one of the object ***or*** non-object related commands.

Continued

AUDITORY FUNCTION SCALE ©2004			
Score	**Item**	**Method**	**Response**
2	***Localization to Sound***	Standing behind the patient and out of view, present an auditory stimulus (eg. voice, noise) from the right side for 5 seconds. Perform a second trial presenting the auditory stimulus from the left side. Repeat above procedure for a total of 4 trials, 2 on each side.	Head and/or eyes orient toward the location of the stimulus on **both trials** in at least one direction. This item is scored when there is clear evidence of head and/or eye movement. It is not dependent on the degree or duration of movement.
1	***Auditory Startle***	Present a loud noise directly above the patient's head and out of view. Administer 4 trials.	Eyelid flutter or blink occurs immediately following the stimulus on at least 2 trials.
0	***None***	See above	No response to any of the above

BASELINE OBSERVATION AND COMMAND FOLLOWING PROTOCOL ©2004

Commands	Baseline	Trial 1	Trial 2	Trial 3	Trial 4
	1 minute frequency count				
I Object Related Commands					
A. Eye Movement Commands					
Look at the *(object #1)*					
Look at the *(object #2)*					
B. Limb Movement Commands					
Take the *(name object #1)*					
Take the *(name object #2)*					
Kick the *(name object #1)*					
Kick the *(name object #2)*					
II Non-Object Related Commands					
A. Eye Movement Commands					
Look away from me					
Look up *(at ceiling)*					
Look down *(at floor)*					
B. Limb Movement Commands					
Touch my hand					
Touch your nose					
Move your *(object/body part)*					
C. Oral Movement/ Vocalization Commands					
Stick out your tongue					
Open your mouth					
Close your mouth					
Say "ah"					

Spontaneous Eye Opening	**Yes:**		**No:**	
Spontaneous Visual Tracking	**Yes:**		**No:**	

Resting Posture	
RUE:	
RLE:	
LUE:	
LLE:	

VISUAL FUNCTION SCALE ©2004

Score	Item	Method	Response
5	***Object Recognition***	Same as Consistent Movement to Command on Auditory Function Scale, Section 2a and b (p. 3).	3 to 4 clearly discernible responses occur over the 4 trials administered.
4	***Object Localization: Reaching***	**1.** Identify the arm or leg with the greatest range of movement. **2.** For upper extremity reaching, select common ADL objects (e.g. comb, toothbrush, etc.). For lower extremity assessment, select a ball suitable for kicking. **3.** Present the object approximately 8 inches to the left or right of the limb's resting position. The object should be placed in a position that is not obstructed from view. The patient should be instructed to "Touch the ***(name object)*** " with the appropriate arm or leg. **4.** The command may be repeated once within the assessment interval. Do not provide any tactile cues, as these may stimulate random limb movement. **5.** Present an object twice to the left of the limb and twice to the right of the limb, in random order for a total of 4 trials.	Score the ***direction*** in which the limb ***first*** moves within a 10 second observation period, or score as no movement. The limb does not need to make contact with the object, only to move toward it; *and* Movement must occur in the correct direction on 3 of the 4 trials administered.
3	***Visual Pursuit***	Hold a hand mirror 4-6 inches directly in front of the patient's face and verbally encourage the patient to fixate on the mirror. Move mirror slowly 45 degrees to the right and left of the vertical midline and 45 degrees above and below the horizontal midline. Repeat the above procedure so that a total of 2 trials are administered in each plane.	Eyes must follow the mirror for 45 degrees ***without loss of fixation*** on 2 occasions in any direction. *If above criterion is not met, repeat the procedure assessing one eye at a time (using an eye patch).*
2	***Fixation***	Present a brightly colored or illuminated object 6 to 8 inches in front of the patient's face and then rapidly move to upper, lower, right and left visual fields for a total of 4 trials.	Eyes change from initial fixation poin and refixate on the new target location for more than 2 seconds. At least 2 episodes of fixation are required.
1	***Visual Startle***	Present visual threat by passing finger 1 inch in front of patient' eye. Be careful not to touch eyelashes or create a breeze (manually open eyes if necessary). Conduct 4 trials per eye.	Eyelid flutter or blink following presentation of visual threat on at least 2 trials with **either** eye.
0	***None***	See above	No response to any of the above.

MOTOR FUNCTION SCALE ©2004

Score	Item	Method	Response
6	***Functional Object Use***	Select 2 common objects (e.g. comb, cup). Place one of the objects in the patient's hand and instruct the patient to "Show me how to use a [name object]." Next, place the second object in the patient's hand and restate the same instruction. Repeat the above procedure using the same objects so that a total of 2 trials are administered with each object.	Movements executed are generally compatible with both object's specific function (e.g. comb is placed on or near the head) on all 4 trials administered. *If the patient is unable to hold the object because of neuromuscular involvement, this should be noted on the record form and the item should* ***not*** *be scored.*
5	***Automatic Motor Response***	Observe for automatic motor behaviors such as nose scratching, grasping bedrail that occur spontaneously during the examination. If spontaneous automatic motor behaviors are not observed, present a familiar gesture (e.g. wave) in association with the following series of alternating commands: **1)** "Show me how to wave" ***(demonstrate gesture).*** **2)** "I'm going to wave again. Do not move at all. Just hold still." ***(demonstrate gesture).*** **3)** "Show me how to wave" ***(demonstrate gesture)*** . **4)** "I'm going to wave again. Do not move at all. Just hold still." ***(demonstrate gesture)*** . For patients with limited ability to move the limbs, objects associated with oromotor activity may be used (e.g. spoon). Place the object in front of the patient's mouth ***without making contact.*** Administer the following series of alternating commands: **1)** "Show me how to use ***(name object)*** . **2)** "I'm going to show you ***(name object)*** again. Do not move at all. Just hold still." **3)** "Show me how to use ***(name object).*** " **4)** "I'm going to show you ***(name object)*** again. Do not move at all. Just hold still."	At least 2 episodes of automatic motor behavior are observed within the session and each episode can be clearly differentiated from a reflexive response. Patient performs the gesture (e.g. waves) *on trials 2 and 4* (regardless of performance on trials 1 and 3). Patient performs the oral movement pattern (e.g. mouth opening occurs when spoon is brought to mouth by examiner) *on trials 2 and 4* (regardless of performance on trials 1 and 3).

Continued

MOTOR FUNCTION SCALE ©2004

Score	Item	Method	Response
4	***Object Manipulation***	Place a baseball size ball on the ***dorsal*** surface of one of the patient's hands. Roll the ball across the index finger and thumb without touching the undersurface of the hand or fingers. While moving the ball, instruct the patient to, "Take the ball." Repeat the above for a total of 4 trials.	The following criteria must be met on 3 of the 4 trials administered: **1.** The wrist must rotate and the fingers should extend as the object is moved along the dorsal surface of the hand; *and* **2.** The object must be grasped and held for a minimum of 5 seconds. The object cannot be held by means of a grasp reflex or increased finger flexor tone.
3	***Localization to Noxious Stimulation***	Extend all four extremities. Apply pressure to the finger or toe of an extremity (use best extremity on each side of the body) for a minimum of 5 seconds (ie.squeeze the finger or toe between your thumb and index finger). Administer 2 trials on each side for a total of 4 trials.	The non-stimulated limb must locate and make contact with the stimulated body part at the point of stimulation on at least 2 of the four trials.
2	***Flexion Withdrawal***	Extend all 4 extremities. Apply deep pressure to nailbeds of each extremity (ie. press the ridge of a pencil into the cuticle). Administer1 trial per extremity.	There is ***isolated*** flexion withdrawal of at least one limb. The limb must move ***away*** from the point of stimulation. If quality of response is uncertain, the trial may be repeated.
1	***Abnormal Posturing***	Observe response to above method	Slow, stereotyped flexion ***or*** extension of the upper and/or lower extremities occurs immediately after the stimulus is applied.
0	***None/Flaccid***	Observe response to above method	There is no discernible movement following application of noxious stimulation, secondary to hypertonic ***or*** flaccid muscle tone.

OROMOTOR/VERBAL FUNCTION SCALE ©2004

Score	Item	Method	Response
3	***Intelligible Verbalization***	**1.** Tell patient "I would like to hear your voice." This should be followed by an attempt to directly elicit speech using the verbal prompts shown below. At least one prompt should be selected from the Aural Set and at least one from the Visual Set. **2.** A maximum of 3 trials should be administered for each prompt chosen from the Aural and Visual Sets. Prompts should be administered at 15 second intervals. **Aural Set:** **a)** "What is your name?' **b)** "How are you today?" **c)** "Where do you live?" **Visual Set:** **a)** "What do you call this thing?" (Hold up common object in front of the patient's right and then left visual field for 10 seconds). **b)** "How many fingers am I holding up right now?" (Hold up 1 finger in front of the right and then left visual field for 10 seconds). **c)** "What part of my body is this?" (Point to your nose while positioned at the patient's visual midline).	Each of the following criteria must be met: **1.** Each verbalization must consist of at least 1 consonant-vowel-consonant (C-V-C) triad. For example, "ma" would not be acceptable, but "mom" would. Make sure objects chosen have a C-V-C sequence; *and* **2.** Two different words must be documented by the examiner to ensure that a repetitive word-like sound is not mistaken for a word. Words need not be appropriate or accurate for the context, but must be fully intelligible; *and* **3.** Words produced by writing or alphabet board are acceptable. *Verbalizations that occur spontaneously or at other times during the assessment and meet the above criteria should also receive a score of 3.*
2	***Vocalization / Oral Movement***	Observe for non-reflexive oral movements, spontaneous vocalizations or vocalizations that occur during administration of vocalization commands (see page 5).	At least one episode of non-reflexive oral movement and/or vocalization occurs spontaneously or in response to application of sensory stimulation. *Yawning is scored as reflexive oral movement.*
1	***Oral Reflexive Movement***	Present tongue blade between patient's lips and/or teeth	There is clamping of jaws, tongue pumping, or chewing movement following introduction of tongue blade into mouth.
0	***None***	See above	No response to any of the above.

COMMUNICATION SCALE ©2004

(if there is no evidence of reproducible command following or spontaneous communicative behavior, the Communication subscale is not administered)

Score	Item	Method	Response
2	***Functional: Accurate***	Administer the 6 Situational Orientation questions from the Communication Assessment Protocol (page12). The examiner may use the Visual set, Auditory set or both sets, if appropriate.	Clearly discernible and accurate responses occur on all 6 of the Visual ***or*** Auditory Situational Orientation questions from the Communication Assessment Protocol (see page 12).
1	***Non-Functional: Intentional***	Same as above	A clearly discernible communicative response* (e.g. head nods/shakes, thumbs up) must occur within 10 seconds on at least 2 of the 6 Situational Orientation questions (irrespective of accuracy). **The examiner must determine that this response occurs more frequently following verbal prompting (e.g. questions) than when non-specific auditory stimulation (e.g. hand clapping) is administered.*
0	***None***	See above	No discernible verbal or non-verbal communication responses occur at any time.

COMMUNICATION ASSESSMENT PROTOCOL ©2004						
Situational Orientation						
Visually Based				**Aurally Based**		
Am I touching my ear right now? (do not touch ear) Am I touching my nose right now? (touch nose) Am I touching my nose right now? (touch nose) Am I touching my ear right now? (do not touch ear) Am I touching my nose right now? (do not touch nose) Am I touching my ear right now? (touch ear)				Am I clapping my hands right now? (do not clap) Am I clapping my hands right now? (clap) Am I clapping my hands right now? (clap) Am I clapping my hands right now? (do not clap) Am I clapping my hands right now? (clap) Am I clapping my hands right now? (do not clap)		
			Date			
Score						
of 6	of 6	of 6		of 6	of 6	of 6
			Date			
Score						
of 6	of 6	of 6		of 6	of 6	of 6

AROUSAL SCALE ©2004

Score	Item	Method	Response
3	***Attention***	Observe consistency of behavioral responses following verbal or gestural prompts.	There are no more than 3 occasions across the length of the evaluation in which the patient fails to respond to a verbal prompt.
2	***Eye Opening w/o Stimulation***	Observe status of the eyelids across length of assessment.	Eyes remain open across the length of the examination without the need for tactile, pressure or noxious stimulation.
1	***Eye Opening with Stimulation***	Same as above	Tactile, pressure or noxious stimulation must be applied at least once during the examination in order for the patient to sustain eye opening (the length of time the eyes remain open may vary and is not considered in the scoring).
0	***Unarousable***	See above	No eye opening noted.

ASSESSMENT OF CONTINGENT BEHAVIOR ©2004

(Supplementary Item)

Score	Item	Method	Response
Not Scored	***Contingent Vocalization / Gesture / Affective Response***	**1.** Vocalizations, gestures and affective responses are assessed through a combination of reports from family and clinicians, and direct observations from treating staff. Family and clinical staff should be questioned about any vocalizations, gestures or affective responses (i.e. smiling, laughing, frowning, crying) that are observed to occur spontaneously or in response to a specific stimulus. **2.** If above response is based on report, staff should attempt to directly elicit the behavior again with the assistance of the individual who reported it. **3.** If affective responses are observed during direct examination, the examiner should attempt to re-elicit the behavior using the same eliciting stimulus previously noted to produce the behavior. Examples of appropriate eliciting stimuli include verbal requests ("What's your name?"), limb gestures (wave), facial gestures (sticking out tongue) and pictures (family photos). **4.** The examiner should document: **a.** The nature of the eliciting stimulus (e.g. Verbal: "Are you feeling sad?"; Limb gesture: handshake); **b.** Specific characteristics of the behavioral response (e.g. facial grimace with tearing of the eyes; smiling, moaning); **c.** Number of times the behavior has been observed to occur within 10 seconds of the eliciting stimulus; **d.** Number of times the behavior has been observed to occur spontaneously; **e.** The time frame allowed for **"c"** and **"d"** should be specified and approximately the same.	A vocalization, gesture or affective response occurs significantly more often in response to a specific eliciting stimulus, than when the stimulus is absent. *Contingent responses do not include those that occur following administration of painful stimuli.*

RECORD DATE AND DESCRIPTION OF ABOVE STIMULI UTILIZED AND RESPONSES OBSERVED

DATE	ELICITING STIMULUS	TARGET BEHAVIOR	# SPONTANEOUS OCCURRENCES OF TARGET BEHAVIOR	# OCCURRENCES OF TARGET BEHAVIOR WITHIN 10 SEC OF ELICITING STIMULU

7.2. Appendix 2 – Operational definitions of CRS-R behaviors indicating conscious awareness.

Behavior	Operational Definition†
Auditory Subscale	
Consistent movement to command†	A clearly-discernible, accurate response is observed following administration of a one-step command (eye, limb, oral). Responses must be accurate on 4 consecutive trials of 2 different commands.
Reproducible movement to command†	A clearly-discernible, accurate response is observed following administration of a one-step command. Responses must be accurate on 3 of 4 trials of at least one command.
Visual Subscale	
Object recognition†	Two different familiar objects presented together are correctly identified by pointing or touch. Responses must be accurate on at least 3 of 4 trials administered.
Object localization†	Following presentation of an object to the right and left of an extremity, the extremity moves in the direction of the object. The limb does not need to make contact with the object, only to move toward it. Movement must occur in the correct direction on 3 of 4 trials administered.
Visual pursuit†	One or both eyes follow movement of a mirror without loss of fixation for at least 45 degrees from midline. Response must occur at least twice in any direction over 4 trials.
Visual fixation†	Following movement of a visual stimulus from midline to a new position within the

	visual field, one or both eyes move from the initial position to the new stimulus location and re-fixate for at least 3 seconds. Response must occur at least twice in any direction over 4 trials.
Motor Subscale	
Functional object use *	Following instruction to demonstrate use of a common object placed in the hand, a movement sequence is executed that is generally compatible with the object's specific function. Responses must be accurate on 2 of 2 trials with two different objects.
Automatic movement†	At least 2 episodes of non-reflexive motor behavior (e.g., scratching, wave) are observed during the examination.
Object manipulation†	Following placement of a ball on the dorsal surface of the hand, there is rotation of the wrist and sustained (≥5s) grasp of the object. Cannot be accomplished through grasp reflex.
Localization to noxious stimulation†	Following pressure applied for a minimum of 5 seconds on the finger or toe, the non-stimulated limb locates and makes contact with the stimulated body part on at least 2 of the 4 trials administered on each side.
Oromotor/Verbal Subscale	
Intelligible verbalization†	At least two different fully-intelligible words, consisting of a consonant-vowel-consonant blend, are verbalized during the course of the examination.
Communication Subscale	
Functional communication *	A clearly discernible, accurate verbal or gestural yes-no responses occurs following administration of 6 consecutive questions concerning situational orientation (e.g. "Am

	I touching my ear/nose?").
Intentional communication†	A clearly discernible verbal or gestural yes-no response occurs following administration of at least 2 of 6 questions concerning situational orientation (e.g. "Am I touching my ear/nose?").

† Denotes the minimally conscious state; * denotes emergence from the minimally conscious state. Operational definitions are extracted from the CRS-R administration and scoring guidelines.

7.3. Appendix 3 – Median EEG values

	Band Power					
	Active tDCS			**Sham tDCS**		
	Pre	**Post**	**POC** (%)	**Pre**	**Post**	**POC** (%)
	All (n=42)					
Delta (1-4 Hz)	0.6832	0.7119	1.8385	0.6373	0.6086	-0.5191
Theta (4-8 Hz)	0.1114	0.1001	-6.9476	0.1078	0.1035	-3.5746
Alpha (8-13 Hz)	0.0614	0.0702	-6.9252	0.0772	0.0720	2.3533
Beta1 (13-23 Hz)	0.0580	0.0752	2.1878	0.0773	0.0925	4.3483
Beta2 (23-35 Hz)	0.0258	0.0209	-5.0804	0.0342	0.0392	12.0768
	UWS (n=14)					
Delta (1-4 Hz)	0.7478	0.7378	-1.4414	0.7077	0.6485	-0.1335
Theta (4-8 Hz)	0.0816	0.0682	-14.5778	0.0924	0.0775	-5.0588
Alpha (8-13 Hz)	0.0355	0.0412	-14.7607	0.0299	0.0589	3.4494
Beta1 (13-23 Hz)	0.0379	0.0528	15.6503	0.0309	0.0701	3.6072
Beta2 (23-35 Hz)	0.0203	0.0209	34.2773	0.0187	0.0329	4.6304
	MCS & EMCS (n=28)					
Delta (1-4 Hz)	0.6822	0.6682	4.1525	0.6279	0.5978	-1.3138
Theta (4-8 Hz)	0.1169	0.1055	-6.224	0.1166	0.1087	-3.4368
Alpha (8-13 Hz)	0.0616	0.0769	-6.6933	0.0803	0.0888	2.3533
Beta1 (13-23 Hz)	0.0732	0.0862	-2.1058	0.0852	0.0925	4.3483
Beta2 (23-35 Hz)	0.0288	0.0226	-7.1006	0.0353	0.0392	12.0791
	LZW Complexity					
	All (n=42)					
Delta (1-4 Hz)	0.1477	0.1476	-1.5524	0.1512	0.1469	-0.2489
Theta (4-8 Hz)	0.2552	0.2575	-0.2245	0.2576	0.2589	-0.7161
Alpha (8-13 Hz)	0.4068	0.4113	0.3103	0.4145	0.4132	-0.0133
Beta1 (13-23 Hz)	0.5149	0.5137	-0.2398	0.5160	0.5165	0.0545
Beta2 (23-35 Hz)	0.6053	0.6050	-0.0149	0.6037	0.6048	0.0504
	UWS (n=14)					
Delta (1-4 Hz)	0.1456	0.1459	-2.1267	0.1436	0.1448	-0.2489
Theta (4-8 Hz)	0.2472	0.2509	3.8052	0.2472	0.2462	-1.1196
Alpha (8-13 Hz)	0.4047	0.4120	2.8911	0.4133	0.4106	-0.1517
Beta1 (13-23 Hz)	0.5148	0.5118	-0.2515	0.5170	0.5164	-0.1102

Beta2 (23-35 Hz)	0.6054	0.6056	0.5424	0.6034	0.6049	0.0504
	MCS & EMCS (n=28)					
Delta (1-4 Hz)	0.1478	0.1477	-1.5524	0.1519	0.1475	-0.3877
Theta (4-8 Hz)	0.2569	0.2591	-0.5019	0.2623	0.2600	-0.0113
Alpha (8-13 Hz)	0.4068	0.4105	-0.0537	0.4148	0.4132	-0.0133
Beta1 (13-23 Hz)	0.5151	0.5140	-0.1750	0.5155	0.5165	0.0769
Beta2 (23-35 Hz)	0.6051	0.6037	-0.0332	0.6039	0.6039	0.0692

Median values of the relative EEG band power and LZW complexity averaged across all electrodes per patient and per band. POC= median percentage of change; UWS= Unresponsive Wakefulness Syndrome; MCS= Minimally Conscious State; EMCS= Emergence from the MCS.

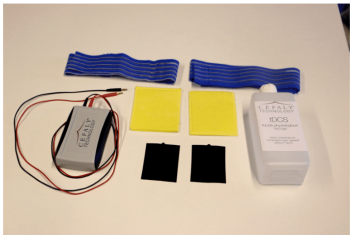
7.4. **Appendix 4 –** Instruction manual of the Cefaly tDCS

Utilisation du CEFALY tDCS

Matériel

La boîte contient :

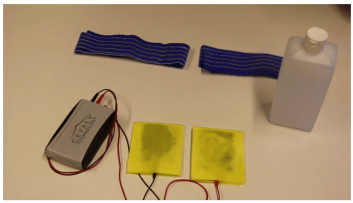
- Un appareil de stimulation tDCS avec 2 câbles et 2 piles
- 2 électrodes
- 2 éponges
- 2 bandeaux
- 1 bouteille de liquide physiologique



Matériel fourni

Préparation

1. Enficher les câbles dans les électrodes
2. Placer les électrodes dans les éponges
3. Bien imbiber les éponges de liquide physiologique sur la surface qui sera en contact avec le crâne (la partie plane)



Matériel prêt à être placé : les électrodes sont connectées aux câbles et insérées dans les éponges ; les éponges sont humidifiées avec du liquide physiologique

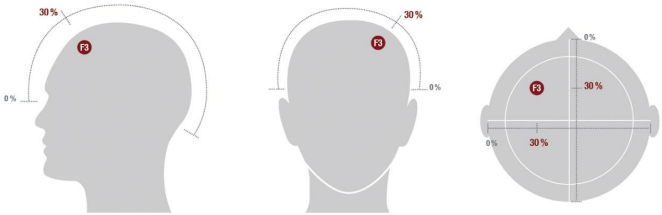
Positionnement

Positionnement des électrodes

La cathode (fil noir) doit être placée au-dessus de l'œil droit.

L'anode (fil rouge) doit être placée en F3, ce qui correspond à :

- 30% (en partant du haut du nez) de la distance séparant le haut du nez et l'occiput
- 30% en partant du haut de l'oreille gauche de la distance séparant le haut de l'oreille gauche et le haut de l'oreille droite.



Positionnement de l'anode en F3

2

Démarrage et exécution du programme

Pour démarrer la stimulation, appuyer simplement sur le bouton de l'appareil.

Un bip se fait entendre et la lumière commence à clignoter. La séance se déroule toute seule et dure 20 minutes. Au bout des 20 minutes, la stimulation s'arrête, un bip de fin se fait entendre et la lumière s'arrête de clignoter.

Problème éventuel

Si vous entendez un bruit répéter lors du démarrage de la session, et que la lumière ne se met pas à clignoter, cela signifie que la connexion n'est pas bonne. Cela peut-être dû à un fil déconnecté, ou plus probablement à un mauvais contact entre les électrodes et le crâne. Vérifiez que les éponges sont bien humidifiées et écartez éventuellement les cheveux au point de contact.

Si un bip prolongé (2 secondes) se fait entendre, cela signifie que les piles sont vides. Il faut donc les remplacer.

7.5. Appendix 5 – Case Report Form Adverse Event Reporting

Patient Number: _ _ _ _ _ _ _ _ _ _

D28: Visit 2 – End of the treatment 1 period

Date of the second visit: _ _ / _ _ / 20 _ _ (DD/MM/YYYY)

Collection of the adverse events from D0 to D28

Return of the form collecting the A.E. for the treatment 1 period:	**☐ Yes**	**☐ No**
Has the patient reported a severe A.E. since the first visit?	☐ Yes*	☐ No
Has the patient reported an A.E. since the first visit?	☐ Yes**	☐ No
Has the patient received an additional concomitant treatment since the first visit?	☐ Yes***	☐ No

* If "Yes", fill in the specific form for the severe adverse events (Appendix 3)

** If "Yes", fill in the specific form for the adverse events (Appendix 2)

*** If "Yes", update the form "Prior and/or associated treatments" (Appendix 1)

Has the patient reported redness of the skin during the treatment 1 period?	☐ Yes	☐ No
Has the patient reported irritation/injury of the skin during the treatment 1 period?	☐ Yes*	☐ No
Has the patient reported sign of pain or discomfort during the treatment 1 period?	☐ Yes*	☐ No
Has the patient reported epilepsy during the treatment 1 period?	☐ Yes*	☐ No
Has the patient reported sleepiness during the treatment 1 period?	☐ Yes	☐ No

*If "Yes", the patient must be removed from the study and the investigator has to fill in the form « Final Assessment ».

Investigator initials:

SCIENTIA

OPTIMVM