UCLouvain
Institute of NeuroScience

LIÈGE université
GIGA
Consciousness

ASSESSMENT OF PAIN PERCEPTION IN PATIENTS WITH DISORDERS OF CONSCIOUSNESS: A BEHAVIORAL AND NEUROPHYSIOLOGICAL BEDSIDE APPROACH

Nicolas LEJEUNE

UCLouvain - ULiège

Sous la direction des Professeurs

André MOURAUX et Steven LAUREYS

Thèse présentée en vue de l'obtention du grade de

Docteur en Sciences Médicales

Année académique 2019 – 2020

Cover photography

ASSESSMENT COMMITTEE

SUPERVISORS

Prof. André Mouraux (UCLouvain)

Prof. Steven Laureys (ULiège)

JURY MEMBERS

Prof. Emmanuel Hermans (**President of the jury** - UCLouvain)

Prof. Marie-Elisabeth Faymonville (**Secretary** – ULiège)

Prof. Marzia De Lucia (Université de Lausanne)

Prof. Andrea Truini (Sapienza Università di Roma)

Dr. Camille Chatelle (UCLouvain)

CONTENTS

ACKNOWLEDGMENTS

Ce manuscrit est le fruit de longues années de réflexion, d'apprentissage, de travail, de collaborations. Ces quatre années furent marquées par des moments exaltants mais aussi des moments de doute, où remise en question et résilience sont maîtres-mots. Je souhaiterais remercier toutes les personnes qui ont contribué, de près ou de loin, à cette entreprise passionnante que fût l'accomplissement de cette thèse.

Tout d'abord, je tiens à remercier mes promoteurs, les Professeurs André Mouraux et Steven Laureys, qui m'ont accordé leur confiance dès l'instant où nous nous sommes rencontrés, tandis que je leur soumettais mon projet de thèse. Merci à vous deux pour le soutien, merci de m'avoir fait découvrir le monde passionnant de la recherche. C'était un rêve d'enfant que j'avais laissé de côté au bénéfice d'une pratique neurologique que j'estime aujourd'hui indissociable de la recherche. Merci André pour tes précieux conseils, ta disponibilité, ta pédagogie, le partage de tes connaissances et ta capacité de réassurance. Merci Steven pour ton soutien sans faille, ton enthousiasme, ton optimisme, ton leadership inspirant et cette capacité que tu as à inciter ton équipe à croire en ses rêves. Je tiens également à remercier les membres de mon comité d'accompagnement et membres du jury de thèse, les Professeurs Emmanuel Hermans (Président), Marie-Elisabeth Faymonville et la Docteure Camille Chatelle. Merci à vous de m'avoir guidé au long de ces quatre années et merci pour les échanges toujours constructifs lors de mes comités d'accompagnement. I want also to thank Professors Marzia De Lucia and Andrea Truini for agreeing to be part of my external jury and to be present for my private defense. Je tiens également à remercier la Professeure Anne Jeanjean. C'est avec elle que j'ai fait mes premiers pas de stagiaire en neurologie en 2008, mes premiers pas d'assistant en neurologie en 2010 et a fait partie de mon comité d'accompagnement dès le début de ma thèse en 2015. Malgré la maladie, elle a tenu ses engagements jusqu'à mon comité d'écriture en septembre 2019 avant de malheureusement nous quitter il y a peu. Je tenais donc à lui rendre hommage pour la source d'inspiration qu'elle a été pour le clinicien-chercheur que je suis aujourd'hui.

Combiner pratique clinique et recherche est une merveilleuse opportunité. Cela m'a permis de m'inspirer des besoins de mes patients pour dessiner les axes de mes recherches.

Dans ce cadre, je ne peux que remercier l'ensemble de l'équipe du C2 du CHN William Lennox et de l'unité UCAre pour tout le travail effectué au quotidien auprès de nos patients. Merci tout particulièrement au Docteur Bruno Pierson et David Dikenstein. Merci Bruno et David pour votre enthousiasme à me suivre dans cette dynamique d'excellence tant clinique que scientifique, toujours au bénéfice de nos patients. Merci également au Docteur Anne Frédérick, directrice médicale du CHN ; Anne, merci de m'avoir accordé ta confiance depuis presque 8 ans, de m'avoir soutenu tant dans mes projets de thèse que dans les projets de cette unité de revalidation neurologique et d'éveil de coma que tu m'as confiée.

Pour assumer ces différentes responsabilités, il aura fallu être un peu acrobate également. En effet, non content de cumuler deux mi-temps, j'ai également dû diviser mon mi-temps de recherche entre Bruxelles et Liège. Cela a demandé une bonne dose d'organisation, mais également de sacrifices car il n'est pas aisé de s'intégrer dans deux groupes de recherche avec si peu de temps de présence. Merci à tous pour votre accueil et votre aide. Merci à toute l'équipe Nocions, et particulièrement à Dounia, Eva, Julien et Arthur pour votre collaboration, aide et disponibilité. Merci également à l'ensemble de l'équipe Coma pour ces belles années, ces collaborations, ces séances de team buildings et ces chouettes congrès. Merci à Jitka, Yorgos, Marie-Michèle et Leandro pour leur aide dans la relecture de ce travail. Je tiens également à adresser un remerciement plus particulier à Aurore et Camille. Merci à toutes les deux pour votre soutien, votre aide précieuse tout au long de mon parcours ; mais surtout merci de m'avoir inspiré, de m'avoir, peut-être sans le savoir, tellement motivé à entreprendre cette thèse. Vous avez été et êtes des modèles pour moi. Je crois que je peux dire que sans vous avoir rencontrées, je ne serais sans doute pas là où je suis aujourd'hui.

Je souhaiterais également remercier les patients ainsi que leurs familles qui nous accordent leur confiance dans ces moments tragiques de leur vie. Vous êtes notre principale source d'inspiration et de motivation.

Je souhaiterais remercier ma famille et mes amis. Merci à mes parents de m'avoir donné l'envie d'être curieux, de m'avoir incité à me questionner sur tout et rien à la fois, de m'avoir poussé à grandir intellectuellement. Merci également à mes amis sans qui je ne serais pas non plus la personne que je suis, et ces remerciements vont tout particulièrement aux membres du CDLF et leurs compagnes. Vous le savez déjà, vous faites partie de ma famille. Même si ces dernières années et certains de mes sacrifices nous ont un peu éloigné, je sais que je peux compter sur votre soutien en toute circonstance. Savoir que vous êtes présent pour les bons moments mais surtout pour les coups durs m'a souvent aidé à avancer. Merci également à Jules pour son soutien sans faille.

Et enfin, merci à celle qui me supporte au quotidien. Madisson, merci pour ton soutien dans les moments de découragement, merci pour ce rôle difficile et ingrat que tu as tenu durant de nombreux mois. Ta patience a été mise à rude épreuve ! Merci pour ton coaching et ton organisation, sans cela, je ne sais pas si j'aurais pu terminer en temps et en heure ! Merci pour les sacrifices que tu fais et d'avoir toléré ceux que je nous ai imposé. Merci d'être celle que tu es, de me comprendre si bien. Je me sens beaucoup moins bizarre depuis que je peux être bizarre avec toi. Alors, parce que je ne te l'ai sûrement pas assez dit et ne te le dirai jamais assez, encore merci…

ABSTRACT

Increased survival following a severe brain injury has led, in the last decades, to a dramatic increase of patients with a prolonged disorder of consciousness (DoC). The study of these patients faces today a paradigm shift, coming from the assessment of residual consciousness to that of residual specific cognitive processes. Pain is a subjective and, therefore, probably conscious experience. Regarding its close relationship with survival and consciousness, assessing residual ability to perceive pain is of major importance. Presenting both an impaired state of consciousness and a threat for their survival, patients with DoC constitute a pathological model of choice.

This thesis addresses several issues related to the assessment of pain perception in those non-communicative patients. Seminal studies on the topic used costly, hardly available and impractical neuroimaging paradigms. We here demonstrate that an approach based on behavioral and neurophysiological assessment (i.e., by means of electroencephalography [EEG]) complement well each other, is easily implementable in clinical practice and usable at bedside. Behavioral assessments, based on the Nociception Coma Scale – Revised (NCS-R) are thought to be useful for determining whether a patient is experiencing pain at a given moment, whereas EEG would help to determine whether the brain of the patient can process such a conscious experience of pain.

Behavioral studies of this thesis allowed to improve the understanding of the NCS-R scores and its applicability in a clinical context (e.g., in patients with a tracheostomy). Combined with an analysis of brain resting metabolism, they also allowed to identify patients with preserved neural basis for pain experience, which is the preservation of, at least, the left insula and the anterior cingulate cortex. Neurophysiological studies of this thesis demonstrate the possibility to identify brain

responses to selective somatosensory stimuli in some patients in a minimally conscious state. Unfortunately, brain responses displayed a very low signal-to-noise ratio. This highlighted the need of a dedicated approach for those patients. Hence, we developed and assessed several methods of stimulus presentation and validated an experimental setup which is easily transportable and usable at bedside.

The presented approach gives us, at the same time, the unique opportunity to look closely at each patient and to open up new perspectives. The strength of the relationship between pain and consciousness is such that it is required, to disentangle them, to go to the frontiers of consciousness; only in this way we will understand how pain experience emerges from the brain. Not only for science and knowledge, but also for ethical and clinical implications of these discoveries on the care of such highly vulnerable patients.

RÉSUMÉ

Au cours des dernières décennies, l'augmentation de la survie à la suite d'une lésion cérébrale sévère a mené à une augmentation spectaculaire du nombre de patients présentant de manière prolongée un état de conscience altérée (ECA). Aujourd'hui, l'étude de ces patients est confrontée à un changement de paradigme, passant de la recherche d'une conscience résiduelle à celle de processus cognitifs spécifiques préservés. La douleur, en tant qu'expérience subjective, et dès lors, probablement consciente, en fait partie. Étant donné les relations étroites à la fois entre douleur et conscience ainsi qu'entre douleur et survie, évaluer la capacité du cerveau à expérimenter consciemment la douleur est d'importance capitale. Présentant à la fois une altération de conscience et un état neurologique critique mettant en péril leur survie, les patients ECA constituent un modèle pathologique de choix dans cette optique.

Cette thèse aborde plusieurs questions liées à l'évaluation de la perception de la douleur chez ces patients non communicants. Les études princeps sur le sujet utilisaient des paradigmes de neuroimagerie coûteux, difficilement disponibles et peu pratiques en routine clinique. Nous démontrons ici que la complémentarité des évaluations comportementales et électroencéphalographiques (EEG) est probablement nécessaire mais également aisément implémentable en pratique clinique, au chevet du patient. Les évaluations comportementales, basées sur la Nociception Coma Scale – Revised (NCS-R), devraient permettre de déterminer si un patient ressent la douleur à un moment précis, tandis que l'approche EEG devrait aider à déterminer si le cerveau du patient a la capacité de traiter la perception douloureuse de manière consciente.

Les études comportementales de cette thèse ont permis d'améliorer la compréhension des scores obtenus à la NCS-R ainsi que son applicabilité dans un contexte clinique (par exemple, chez des patients porteurs d'une trachéostomie). Associées à une analyse du métabolisme au repos du cerveau, elles permettent

d’identifier les patients présentant les bases neurales pour expérimenter consciemment la douleur, qui sont la préservation de l'insula gauche et du cortex cingulaire antérieur. Les études neurophysiologiques de cette thèse ont permis d'identifier des réponses cérébrales à des stimuli somatosensoriels sélectifs chez certains patients en état de conscience minimale. Malheureusement, ces réponses cérébrales affichaient un très faible rapport signal-sur-bruit. Cela a permis de mettre en évidence la nécessité d'une approche spécifique pour ces patients. Dès lors, nous avons développé et évalué différentes méthodes de présentation de stimuli et validé une configuration expérimentale facilement transportable et utilisable au chevet des patients.

L'approche présentée donne à la fois l'occasion unique d’être au plus proche du patient tout en ouvrant de nouvelles perspectives. La force de la relation entre la douleur et la conscience est telle qu'il faut, pour les démêler, aller aux frontières de la conscience ; ce n'est qu'ainsi que nous comprendrons comment l'expérience de la douleur émerge du cerveau. Non seulement pour la science, mais aussi pour les implications éthiques et cliniques que ces découvertes ont et auront sur les soins de ces patients hautement vulnérables.

GLOSSARY

ACC	Anterior Cingulate Cortex. A brain area located on the medial part of the frontal lobe, involved in the pain experience
AMH	Mechano- and heat-sensitive Aδ-fiber nociceptors. Nociceptors whose activation generate the sensation of first pain. The signal is conveyed to the brain through thinly myelinated Aδ-fibers.
CEPs	Cool-evoked potentials. ERPs elicited by the activation of cool-sensitive afferents.
CHEPs	Contact-Heat Evoked Potentials. ERPs elicited by the activation of nociceptors through a heating contact thermode.
CMH	Mechano- and heat-sensitive C-fiber nociceptors. Nociceptors whose activation generate the sensation of second pain. The signal is conveyed to the brain through unmyelinated C-fibers.
CRS – R	Coma Recovery Scale – Revised. A clinical standardized evaluation of the level of consciousness.
DCML	Dorsal Column Medial Lemniscus. A sensory pathway conveying the sensation for tactile object recognition, light touch, and proprioception to the brain.
DoC	Disorders of Consciousness. This term refers to clinical entities of pathological altered consciousness and includes coma, vegetative/unresponsive wakefulness syndrome and minimally conscious state.
EEG	Electroencephalography. A method to record the electrical activity arising from the brain.
ERPs	Event - Related Potentials. Modification of the ongoing electrical activity of the brain, usually in response to a transient sensory event.

eMCS	Emergence of Minimally Conscious State. A clinical entity wherein patients show a reliable communication and/or a functional use of objects. They are therefore not considered to be in a DoC anymore.
GFP	Global Field Power. Quantifies the amount of activity at each time point in the field considering the data from all recording electrodes simultaneously resulting in a reference-independent descriptor of the potential field.
Harmonic	A harmonic is a signal whose frequency is an integral multiple of the fundamental frequency (i.e., the frequency of a given stimulus).
LEPs	Laser-Evoked Potentials. ERPs elicited by the activation of thermonociceptors by the use of a laser device.
MCS	Minimally Conscious State. A clinical entity wherein patients can be awake, display fluctuating signs of consciousness but without being able to functionally communicate.
MCS-	MCS minus. MCS patients without observed language-related behaviors.
MCS+	MCS plus. MCS patients with residual language abilities
MCS*	Non-behavioral MCS; also known as Cognitive Motor Dissociation (CMD). A clinical entity wherein patients do not exhibit any sign of consciousness at bedside while showing brain activity compatible with the presence of residual awareness or cognitive abilities.
MRI	Magnetic Resonance Imaging. This technique allows to analyze the structure of the brain or its activity (functional MRI [fMRI]).
NCS – R	Nociception Coma Scale – Revised. A clinical standardized scale to assess nociception in patients with a disorder of consciousness.
Nociception	Refers to the neural process of encoding noxious stimuli. Pain sensation is not necessarily implied.
PET	Positron Emission Tomography. A functional imaging technique allowing to observe metabolic processes using a radioactive tracer.

Salience	The salience of a stimulus refers to its quality, in an environment, to attract someone's attention.
S1	Primary somatosensory cortex**.**
SII	Secondary somatosensory cortex.
SC	Slowly-adapting CMH.
SNR	Signal-to-Noise Ratio. On the electroencephalographic recording, describes the ratio between the amplitude of the signal of interest and the amplitude of the background noise, unrelated to the signal of interest.
SS-EPs	Steady-State Evoked Potentials. Also known as Steady-State Responses (SSRs). SS-EPs reflect a sustained cortical response induced by the long-lasting periodic repetition of a sensory stimulus; unlike ERPs, SS-EPs are best identified in the frequency domain, as peaks appearing at the frequency of the stimulus and/or at harmonics of that frequency.
SSRs	*See SS-EPs.*
STT	Spino-Thalamic Tract. A sensory pathway conveying noxious, thermal and visceral information to the brain.
SUV	Standardized Uptake Value. The ratio between the imaged radioactive concentration (using PET) of a tracer and its injected concentration in the whole body.
TBI	Traumatic Brain Injury.
TCSII	A micro Peltier-based contact thermode able to generate very steep cooling/heating ramps of up to 300 °C/s.
UWS	Unresponsive Wakefulness Syndrome; also known as Vegetative State (VS). A clinical entity wherein patients can be awake but without any awareness of themselves and their surroundings.

CHAPTER 1. INTRODUCTION

"When your day is long and the night is yours alone,
When you're sure you've had enough of this life,
Well, hang on. Don't let yourself go.
Everybody cries and everybody hurts…sometimes."
- R.E.M.

The improvement of intensive care techniques, especially the accession to modern resuscitation techniques in the 1950s, has led to a dramatic increase in the number of patients with a prolonged disorder of consciousness (DoC). This raises numerous ethical, socio-economical and quality of life issues. Pain, one of the most important determinant of quality of life (Katz, 2002), is a subjective, and therefore, probably conscious experience. Unfortunately, given their state of impaired consciousness, patients with DoC are unable to communicate such a subjective experience. These patients, all along their care pathway, face a high rate of potentially painful medical complications (Ganesh et al., 2013; Whyte et al., 2013). Knowing their said great vulnerability, a crucial aspect in evaluating and improving the quality of life of these patients is to determine whether they have the ability to perceive pain.

The nature of the relationship between pain and consciousness remains barely known to date. Exploring the ability to process pain in patients with DoC is interesting both from a clinical and neuroscientist perspective: to understand a patient's ability to experience pain at an individual level and, ideally, at a given time point; and to question the link between consciousness and pain experience through a pathological model where those physiologically linked cognitive experiences could somehow be dissociated from each other.

This first chapter aimed at giving the necessary background to understand Chapters 3 and 4, the core chapters of this thesis. I will first go through the spectrum

of the pathological altered states of consciousness and the means to assess residual consciousness. Afterwards, I will review the neurophysiology of pain as well as the means to elicit brain responses following the activation of somatosensory pathways.

1.1. Disorders of Consciousness

Consciousness can be defined using two main components, arousal and awareness (Zeman, 2001). Arousal refers to the level of alertness or vigilance and involves the activity of the brainstem reticular formation, hypothalamus, and basal forebrain. Awareness refers to the contents of consciousness and is related to the activity of a widespread set of frontoparietal associative areas (Vanhaudenhuyse, Demertzi, et al., 2010). In healthy people, awareness and arousal are usually correlated, in the sense that the less aroused we get the less aware of our surroundings and ourselves we become (Steven Laureys, 2005). However, that assumption does not remain true in the case of patients with a prolonged DoC, for whom a dissociation between arousal and awareness components occur.

1.1.1. Clinical entities

In this section will be described the clinical entities to which the term "Disorders of Consciousness" refers, but also those which resemble them to avoid any misunderstanding in the further reading.

A - Brain death

Brain death is caused by an acute brain injury leading to an irreversible and complete absence of electric (i.e., iso-electric electroencephalogram) and metabolic activity in the brain and the brainstem (Figure 1.1) (Steven Laureys et al., 2004), in the absence of any confounding factor such as hypothermia or severe electrolytic disturbance (Wijdicks et al., 2010). Clinically, a patient in brain death will show an absence of motor response and an absence of brainstem reflexes, including spontaneous apnea. Brain dead patients are not able to maintain their autonomic functions by themselves and require the use of external technical support (such as mechanical ventilation). Stopping these technical

supports does not lead to death as the patient is already dead; it only signs the end of the functional preservation of the other organs, that could have eventually been used for organ transplantation.

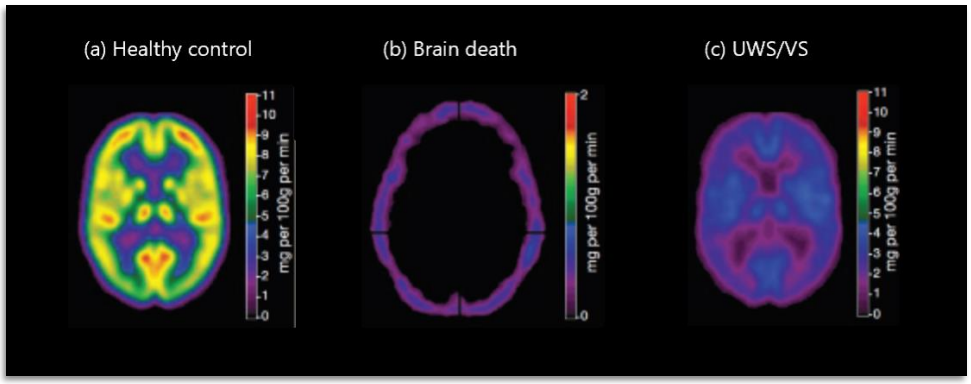


Figure 1.1. Cerebral resting brain metabolism acquired with ^{18}Fluorodeoxyglucose – positron emission tomography (FDG-PET) in (a) healthy control, patients with (b) brain death and (c) unresponsive wakefulness syndrome (UWS) or vegetative state (VS). In brain death, we can observe an "empty skull sign" whereas in UWS/VS, the cerebral metabolism is highly and globally decreased but not absent. The color scale shows the amount of glucose metabolized per 100 g of brain tissue per minute. Adapted from (Steven Laureys, 2005)

B - COMA

Coma is an acute, transient, but complete state of unresponsiveness lasting for more than one hour. Due to a complete failure of the arousal system, this unresponsiveness is accompanied by an absence of eye opening, even when the patients are intensively stimulated (Jennett & Plum, 1972). The patient has no awareness of self and surrounding while some reflexive movements can be observed (Posner et al., 2007). Autonomic function regulation is compromised and often requires a respiratory assistance. Transient by definition, coma rarely lasts longer than two to five weeks (Steven Laureys, 2007). Afterwards, if they survive this acute stage, patients will either recover or awaken to remain for an undetermined amount of time (days to years) in a

state of unresponsive wakefulness, called the vegetative state or the unresponsive wakefulness syndrome.

C - UNRESPONSIVE WAKEFULNESS SYNDROME

Unresponsive Wakefulness Syndrome (UWS) is somehow the European counterpart of the Vegetative State (VS), which remains much present in the American literature (Steven Laureys et al., 2010). Patients in a UWS/VS display three main features: (1) "sleep-wake-*like* cycles" consisting in periods of eye opening and closing (which is inconsistent with a diagnosis of coma); (2) at least a partial preservation of brainstem autonomic functions; and (3) the absence of awareness of self and surrounding, showing only reflexive movements (Monti, Laureys, et al., 2010). Reflexive movements can be, for instance, non-purposeful limb movements, a startle reflex to auditory or visual salient stimuli, orofacial chewing movements, eye movements without fixation. Accurate diagnosis of UWS patients is of capital importance as this diagnosis is associated with a less favorable outcome (Giacino et al., 2018), but also because the presence of consciousness will modify the rehabilitation as well as the interactions with these patients. If a patient does not regain any sign of consciousness after three months (from anoxic etiology) or twelve months (from traumatic injury), the patient is called to be in a "chronic UWS/VS", a term that should be followed by the duration of the DoC, because evidence supports that the likelihood of recovery decreases with the duration of unresponsiveness (Giacino et al., 2018)

D - MINIMALLY CONSCIOUS STATE

Patients in Minimally Conscious State (MCS) can show discernible, oriented, purposeful but fluctuating cognitively-mediated behaviors. Among behaviors associated with a diagnosis of MCS, fixation, visual pursuit and reproducible movement to command are each observed in more than 50% of patients. These items are also the most frequently observed when the patients only show one sign of consciousness (Wannez et al., 2018). The most common initial signs identified in MCS patients are

visual pursuit (41%), reproducible command-following (25%) and automatic movements (24%) (Martens et al., 2019).

Recently, a subcategorization of MCS patients was proposed regarding the presence (MCS+; MCS plus) or the absence (MCS-; MCS minus) of language-related behaviors (Bruno et al., 2012). MCS+ could be somehow defined as "MCS with residual language abilities" while MCS- could reflect either a lower level of consciousness and/or the presence of more severe aphasia in MCS patients (Aubinet et al., 2018). Therefore, MCS- patients cannot be defined *per se* as less conscious than MCS+ patients. This statement is of importance as it is suspected that the presence of aphasia leads to misdiagnosis of patients regarding their level of consciousness (Schnakers et al., 2015).

Misdiagnosis remains a huge problem in the field of DoC. A misdiagnosis rate of 41% has been reported, meaning that 41% of patients diagnosed as UWS by their caregivers were actually identified as MCS when using a standardized and validated behavioral scale (i.e., the Coma Recovery Scale – Revised; see next section) (Schnakers et al., 2009). There are several reasons to such a high misdiagnosis rate. It can be related to the (lack of) experience of the examiner (or caregivers) or the absence of use of systematic tools to assess residual awareness. Even with experienced examiners, there still remain misdiagnosed patients as demonstrated by the use of paraclinical exams (Stender et al., 2014). Indeed, the evaluation can be limited by the patients' disabilities (such as severe motor impairment, aphasia, etc.) preventing them to fully participate in the evaluation. This specific subset of patients that do not exhibit any sign of overt consciousness at bedside while showing brain activity compatible with the presence of residual cognition or awareness are labeled as "non-behavioral MCS" (MCS*) or as having a Cognitive Motor Dissociation (CMD) (Gosseries et al., 2014; Owen et al., 2006; Schiff, 2015).

E - Emergence from the Minimally Conscious State

The emergence from a MCS (eMCS) is defined by the appearance of a reliable and accurate functional communication and/or functional use of at least two different objects (Giacino et al., 2002). This state, also referred to as "confusional state" or

"post-traumatic amnesia", consists of a largely heterogeneous group of patients. Those patients can exhibit severe attentional deficit, disinhibition, labile behavior, hypokinetism or agitation. Therefore, they are often non-cooperative to rehabilitation therapy or systematic assessment of their impairments. Sometimes, the recovery of functional communication and object use does not occur simultaneously (Kalmar & Giacino, 2005; Taylor et al., 2007). When functional use of object recovers before functional communication, patients do not have the ability to report successfully subjective perception.

F - LOCKED-IN SYNDROME

Locked-in syndrome (LIS) patients do not suffer from impaired consciousness and this entity cannot be included in the DoC spectrum. This syndrome, usually caused by a stroke of the basilar artery, results in an ischemia of the anterior part of the pons, causing anarthria and quadriplegia (including head and neck), but usually sparing the vertical oculomotor movement. As the lesion is limited to the brainstem, cognitive impairment is very limited but the ability to communicate is lost by the absence of motor control. Since these patients look like patients with DoC, a delayed diagnosis is not unusual. Diagnosis is of extreme importance as those patients are fully conscious and can be taught to communicate using eye movements.

1.1.2. ASSESSMENT OF RESIDUAL AWARENESS

Although behavioral evaluation at bedside remains the gold-standard for assessing residual awareness in patients with DoC (Giacino et al., 2018), its use shows a significant remaining rate of misdiagnosis when confronted to paraclinical examination (Stender et al., 2014). Therefore, behavioral and paraclinical evaluations should be integrated. In this section, I will give an overview of the behavioral and paraclinical modalities available for the assessment of residual awareness in patients with DoC.

A – CLINICAL BEHAVIORAL EVALUATION

The Coma Recovery Scale – Revised (CRS-R; Table 1.1) (Giacino et al., 2004) was designed to allow a differential diagnosis between the different DoC entities, from coma to eMCS, and more specifically between UWS and MCS. The CRS-R consists of 23 hierarchically organized items in six subscales addressing (1) visual, (2) motor, (3) auditory, (4) oromotor/verbal, (5) communication and (6) arousal functions. The lowest item on each subscale represents reflexive activity while the highest item represents cognitively-mediated behaviors (Giacino et al., 2004). Because the CRS-R is not linear, the level of consciousness is imperfectly reflected by the total score (i.e., a UWS patient could have a higher score than an MCS patient, because the diagnosis of consciousness depends on the diagnosis associated with the highest-order behavior observed). Recent recommendations state that, in order to reduce the rate of misdiagnosis, patients should be serially assessed (Giacino et al., 2018) and at least five CRS-R should be performed within a short time interval (e.g., two weeks) (Wannez et al., 2017). Unfortunately, the complete protocol (30-45 minutes) of the CRS-R is not always suitable in clinical practice given time constraints. However, it was shown recently that limiting the CRS-R assessment to the five most frequently observed items in MCS patients (i.e., visual fixation, visual pursuit, reproducible movement to command, automatic motor response and localization to noxious stimulation) would allow to detect 99% of the patients diagnosed MCS at a behavioral level (Wannez et al., 2018). However, if the CRS-R is considered to date as the gold-standard for behavioral assessment of patients with DoC, we have to be aware that due to a lack of a diagnostic ground truth, criterion validity and diagnostic value (i.e., the scale's ability to establish an accurate diagnosis compared with the true diagnosis as measured by a reference standard) cannot be determined for any available scoring system (Seel et al., 2010).

Table 1.1. The Coma Recovery Scale – Revised

Auditory function scale		
4	Consistent movement to command	**MCS +**
3	Reproducible movement to command	**MCS +**
2	Localization to sound	
1	Auditory startle	
0	None	

Visual function scale		
5	Object recognition	**MCS -**
4	Object localization : reaching	**MCS -**
3	Visual Pursuit	**MCS -**
2	Fixation (*)	**MCS -**
1	Visual startle	
0	None	

Motor function scale		
6	Functional object use	**eMCS**
5	Automatic motor response	**MCS -**
4	Object manipulation	**MCS -**
3	Localization to noxious stimuli	**MCS -**
2	Flexion withdrawal	
1	Abnormal posturing	
0	None/Flaccid	

Oromotor/verbal function scale		
3	Intelligible verbalization	**MCS +**
2	Vocalization/Oral movement	
1	Oral reflexive movement	
0	None	

Communication scale		
2	Functional : accurate	**eMCS**
1	Non-functional : intentional	**MCS+**
0	None	

Arousal scale	
3	Attention
2	Eye opening without stimulation
1	Eye opening with stimulation
0	Unarousable

(*) Denotes MCS - except for anoxic etiologies

Denotes MCS

Denotes MCS +

Denotes eMCS

B – NEUROIMAGING TECHNIQUES

From a neuroscientific point of view, neuroimaging techniques are important means to study the neural correlates of consciousness, that is defined as the minimal neural mechanisms jointly sufficient for any one conscious experience (Crick & Koch, 1990). These are also interesting means to probe the residual awareness in patients with DoC. Neuroimaging can contribute to reduce misdiagnosis rate in these patients by identifying, or eventually quantifying their residual consciousness (Bodart et al., 2017; Stender et al., 2014, 2015).

In this section, we will discuss neuroimaging techniques by describing the most commonly used paradigms and the most commonly used techniques: (functional) magnetic resonance imaging (MRI), positron emission tomography (PET) and electroencephalography (EEG).

1. RESTING, PASSIVE AND ACTIVE PARADIGMS

Resting paradigms are paradigms in which patients are asked to mind-wander, without performing any specific task, allowing to detect spontaneous brain activity without the influence of any internal or external stimulation. Resting protocols are suitable means to study patients with impaired communication, such as patients with DoC. Most common techniques using resting paradigms are functional MRI (e.g., Soddu et al., 2011), PET (e.g., Steven Laureys et al., 2004) and EEG (e.g., Chennu et al., 2017).

Passive paradigms rely on the analysis of brain activity in response to external stimuli (e.g., somatosensory, visual, auditory), in the absence of any instruction to the patient. For instance, using functional MRI, it is possible to identify brain responses that are specific to the DoC patient's preferred music (Heine et al., 2017). In EEG, classical passive paradigms are constituted by the recording of time-locked brain responses to external stimuli, such as the patient's own-name (S. Laureys et al., 2004; Wang et al., 2015). However, one main limitation of all those passive paradigms is the heterogeneity of the widespread lesions of patients with DoC, potentially inhibiting the detection of those external stimuli (e.g., aphasia, blindness…). Another kind of passive paradigm

overcome this limitation: using the EEG, it is possible to analyze the modulation of brain activity in response to an external magnetic stimulus (combination of transcranial magnetic stimulation with EEG [TMS-EEG]). After stimulus onset, the complexity of the response is computed to obtain an index, the "Perturbational Complexity Index", a measurement of residual consciousness (Bodart et al., 2017).

Active paradigms require the subject to perform a specific task on request and are based on the analysis of willful modulation of brain activity in response to a command, for instance, using mental imagery tasks. Active paradigms suffer from the severe limitations inherent to the clinical state of patients with DoC, which may suffer from limited working memory or attention span. These techniques are resource costly for the patient, but when these paradigms work, they can eventually allow functional (not meaning easy) communication with the patient, as it was demonstrated with the famous "tennis playing motor imagery paradigm" (Bodien et al., 2017; Monti, Vanhaudenhuyse, et al., 2010; Owen et al., 2006). Motor imagery is also widely used in brain-computer interfaces using EEG (EEG-BCI) (Luauté et al., 2015), but other tasks can be used to elicit willful modulation of the EEG-responses, such as focusing on a lateralized somatosensory stimulus (Annen et al., 2018). Those methods, while resulting in a low rate of success are of major importance at an individual level, especially in patients not showing any sign of consciousness at bedside. Of course, when performing active paradigms, one should keep in mind that the absence of responsiveness does not equal to the absence of residual awareness (Sanders et al., 2012).

2. Positron Emission Tomography

Positron Emission Tomography is a neuroimaging technique based on the identification and the quantification of the uptake of a radioactive tracer. The most commonly used tracer is the fluorodeoxyglucose (abbreviated ^{18}F-FDG, or simply FDG), a glucose analog therefore taken-up by high-glucose-using cells, as neurons. The uptake of FDG during acquisition reflects the presence of active neurons.

In the field of DoC, the first FDG-PET was reported in 1987 and showed in a VS patient a decrease in global cerebral glucose uptake of 40-50% (Levy et al., 1987). Since

then, these results were reproduced in several studies (DeVolder et al., 1990; S. Laureys et al., 1999; Tommasino et al., 1995). However, global metabolism is not the only correlate of the level of consciousness. Indeed, at a regional level, it has been consistently shown that medial posterior cortex (encompassing the precuneus and adjacent posterior cingulate cortex) was more preserved in healthy subject and MCS patients than in UWS patients (Steven Laureys et al., 2004), potentially signing the importance of this area in the generation of consciousness. Furthermore, it was also demonstrated that the hypometabolism was more widespread and prominent in MCS patients when compared to eMCS patients (Nakayama et al., 2006). These studies allowed to identify a large fronto-parietal network whose functional integrity could be identified by machine-learning classifiers, allowing to calculate the probability for a patient to be conscious or not (Phillips et al., 2011). Unfortunately, those classifiers are not sufficiently powerful to classify correctly patients across the entire spectrum of the DoC. Therefore, to date, no standardized tool is available to classify patients according to their DoC. This classification still relies on the visual analysis by experienced researchers of images with the FDG Standardized Uptake Values (SUV). Despite this limitation, FDG-PET was recognized as the best tool allowing to disentangle UWS from MCS patients (Stender et al., 2014)

3. Magnetic Resonance Imaging

The study of consciousness owes a lot to MRI. Thanks to functional MRI (fMRI), it was shown that consciousness can be divided into two anatomical networks: (1) the large fronto-parietal lateral "external awareness network", devoted to the consciousness of the external world through the different senses (e.g., visual, auditory, somatosensory inputs); and (2) the mesial "internal awareness network" or "default mode network" (DMN), encompassing posterior cingulate/precuneus, anterior cingulate cortex and temporo-parietal junctions, which is more involved in self-related processes (such as mind-wandering, stimulus independent thoughts or self-related thoughts (Buckner et al., 2008; Fox et al., 2005; Mason et al., 2007)). Interestingly, those two networks are anti-correlated in healthy subjects, meaning that

when one is active, the other is not (Vanhaudenhuyse, Demertzi, et al., 2010). This anticorrelation is suspected to be linked to the recovery of consciousness since it has been demonstrated that patients who emerged from MCS showed a partial preservation of between-network anticorrelations, unlike patients remaining in a DoC that showed pathological between-network correlations (Di Perri et al., 2016).

From a technical and practical point of view, MRI has the double advantage to perform analysis on both brain structure and function into a single exam session and to display a high spatial resolution. Unfortunately, being able to record high-resolution images requires that the patients remain perfectly still, which is hardly the case in patients with DoC. Sedation of the patient increases the quality of acquisition, which is of particular interest for structural MRI. Unfortunately, it will inevitably affect the acquisition of fMRI data, which is an indirect measure of the blood flow, related to the regional consumption of blood oxygen. With sedation, the blood supply decreases inevitably in consciousness networks, affecting the acquired signal, regardless of the type of paradigm used (resting, passive or active). To avoid poor quality data acquisition due to the clinical condition of patients with DoC, it is necessary to adopt fast acquisition paradigms such as resting state fMRI. Passive or active paradigms (e.g., such as required for the study of pain) are much more challenging to achieve. Resting-state fMRI studies have detected reduced connectivity in the DMN of patients with DoC as compared to healthy subjects, mostly affecting the precuneus, a brain area considered to be a critical hub within this network (Cavanna, 2007; Vanhaudenhuyse, Noirhomme, et al., 2010). Passive or active paradigms, while technically challenging, are critical means to detect residual awareness in patients with DoC. In a famous cases series, researchers succeeded establishing a functional communication with patients, including patients evaluated UWS at bedside. For this active paradigm, the patients were asked to "imagine playing tennis" for responding "yes" and to "imagine walking into their house" for responding "no" to biographical questions (Monti, Vanhaudenhuyse, et al., 2010; Owen et al., 2006). Unfortunately, fMRI-based communication, because of the need of an MRI

scanner, is impractical and does not meet the needs of patients who would benefit much more from a bedside assessment.

4. ELECTROENCEPHALOGRAPHY

Electroencephalography is based on the recording of the electrical activity of the brain. This technique displays several advantages: portable, cost-effective and usable at patient's bedside, it can be easily used with any kind of paradigm (resting, passive or active). In this section, I will not discuss active paradigms since they are mainly used to attempt functional communication through a Brain-Computer Interface (BCI), which is out of the scope of this thesis.

Resting EEG paradigms has been used to assess the level of consciousness. It was shown that, in comparison to controls, UWS (but not MCS) patients show higher delta (brain activity in the 1-4 Hz range) and theta activity (4-8 Hz), while alpha activity (8-12 Hz) was strongly decreased in both UWS and MCS groups (Lechinger et al., 2013). Using a high-density EEG and applying network analysis tools, connectivity measures allow to identify specific spectral signatures of reorganized brain networks in patients with DoC. For instance, using different network metrics (e.g., such as alpha band connectivity), it seems possible to differentiate patients' level of consciousness across the whole spectrum of DoC (Chennu et al., 2014, 2017).

EEG-based passive paradigms allow to record brain responses to external stimuli, as mentioned in the preceding section. For instance, brain responses to stimuli or odd stimuli within a sequence (e.g., the subject own name or to deviant tones [mismatch negativity]) can be assessed (Fischer et al., 2008, 2010). Brain responses can also be elicited and analyzed following violations of auditory regularities in an oddball local-global paradigm (Bekinschtein et al., 2009; Faugeras et al., 2012). The main objectives of those paradigms are either to provide some prognostication of recovery or to differentiate UWS from MCS patients (Kotchoubey, 2017). An interesting and promising application of EEG-based passive paradigm using TMS-EEG has already been mentioned previously.

1.1.3. ASSESSMENT OF PAIN PERCEPTION

Nociception and pain are usually closely related to each other. However, pain can occur without the activation of nociceptors (Flor et al., 2006), while the activation of nociceptors can trigger reflex somatic and autonomic responses without necessarily generating a conscious experience of pain (Robert K. Hofbauer et al., 2004). As pain is a subjective cognitive process, emerging from cortical processing (R. D. Treede et al., 1999), and therefore probably constitutes a conscious experience, this dissociation between nociception and pain might occur in a state of impaired consciousness. Hence, the study of patients with DoC provides a unique opportunity to investigate the relationship between pain and consciousness.

Seminal studies on the topic used passive paradigm in neuroimagery, such as the recording of brain responses to non-specific noxious stimuli (i.e., electrical stimuli) using PET or fMRI. In two seminal studies on UWS patients, Laureys et al. (2002) and Kassubek et al. (2003) found slightly divergent activation patterns in these patients. In the first study, the activation pattern was limited to the brainstem and the primary somatosensory cortex (S1) without functional connection to other brain areas known to play a role in pain experience, suggesting that, in these patients, the painful electrical stimuli did not elicit a conscious percept. In the latter study, the activation of S1 was accompanied with an activation of the secondary somatosensory cortex (S2), the posterior insula and the anterior cingulate cortex (ACC), i.e., brain regions that are consistently activated during pain experience in healthy individuals. These contrasting results might eventually be explained by the population of the study. Indeed, at that time, the diagnosis of MCS was not well known and patients were indifferently described as being in a Persistent Vegetative State (PVS) while some of them might have exhibited signs of MCS. Hence, Boly et al. (2008) replicated these studies, including three well-defined group of UWS, MCS patients and healthy controls. In MCS and in healthy controls, the electrical stimuli elicited a similar pattern of brain activity, including the thalamus, S1, S2, the insula and the ACC, while UWS patients displayed a reduced activity in these areas. These results are compatible with the notion that functional brain

imaging could be able to distinguish different groups of patients with disorders of consciousness, having differing abilities to experience pain (André Mouraux, 2015).

Using fMRI, Markl et al. (2013) compared brain responses to electrical stimuli in a group of well-selected UWS patients (according to behavioral assessment using the CRS-R) to age-matched healthy controls. They observed an activation in different regions of interest, but in a low rate. S1 was activated in only 3/30, S2 in 5/30 and/or ACC in 5/30 patients. Noteworthily, significant activation was observed more frequently in healthy controls, but not systematically (e.g., activation of the ACC in 6/15 healthy controls).

An important and common drawback of those studies is the fact that they all rely on electrical stimuli which may not have been of a sufficient intensity to reliably activate nociceptive afferents. Moreover, these stimuli are neither specific for nociception nor for the spino-thalamic tract (STT), making impossible to isolate the contribution of the medial lemniscus pathway to the observed responses. Nevertheless, the finding that noxious stimulation elicited widespread cortical activity in this group of patients suggests, at least for some of them, a residual ability to experience pain. Most importantly, the different patterns of subcortical and cortical activation in different groups of patients with DoC suggests that functional brain imaging might be useful to identify patients more or less likely to perceive pain following nociceptive stimulation. Unfortunately, the accessibility, the availability and the cost of functional neuroimagery, as well as the technical difficulties encountered with those patients (from acquisition to data analysis) make the use of these techniques very challenging.

In the last years, a few studies tried to investigate the question of pain perception in patients with DoC using EEG methods. These findings will be described in detail in the introduction of Chapter 4, dedicated to the neurophysiological assessment of pain in those patients.

1.2. PAIN, NOCICEPTION AND THE SOMATOSENSORY SYSTEM

Nociception is not synonym of pain. Although intimately related, they are two distinct physiological phenomena. Nociception could be defined as a sensory modality whose aim would be to signal a potential threat to the body's integrity and trigger appropriate defensive reactions vital for survival (Legrain et al., 2011). Pain refers to a conscious percept and is defined as a *"distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive and social components"* (Williams & Craig, 2016). Interestingly, in its definition of pain, the International Association for the Study of Pain specifies that "*the inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment. Pain is always subjective"* (Merskey, 1994). In this section, I will first review the anatomy and physiology of the somatosensory and nociceptive pathways; then, I will review the different means available to probe these pathways.

1.2.1. ANATOMY AND PHYSIOLOGY

The somatosensory system consists of two major pathways: the dorsal column–medial lemniscus (DCML) and the spinothalamic tract (STT) (Figure 1.2). The DCML subserves mechanoreception (tactile object recognition, localization of skin contact, detection of vibration and texture) and proprioception (joint position, movement and force). The STT subserves thermoreception (coolness and warmth), nociception (impending tissue damage and pain) and viscероception (Cruccu et al., 2008).

A - SOMATOSENSORY RECEPTORS AND AFFERENT FIBERS

1. THE SPINO-THALAMIC TRACT

The spino-thalamic tract system is the part of the somatosensory system that conveys nociceptive and thermal inputs from the periphery (comprising thermoreceptors, nociceptors and nerve fibers) to the brain.

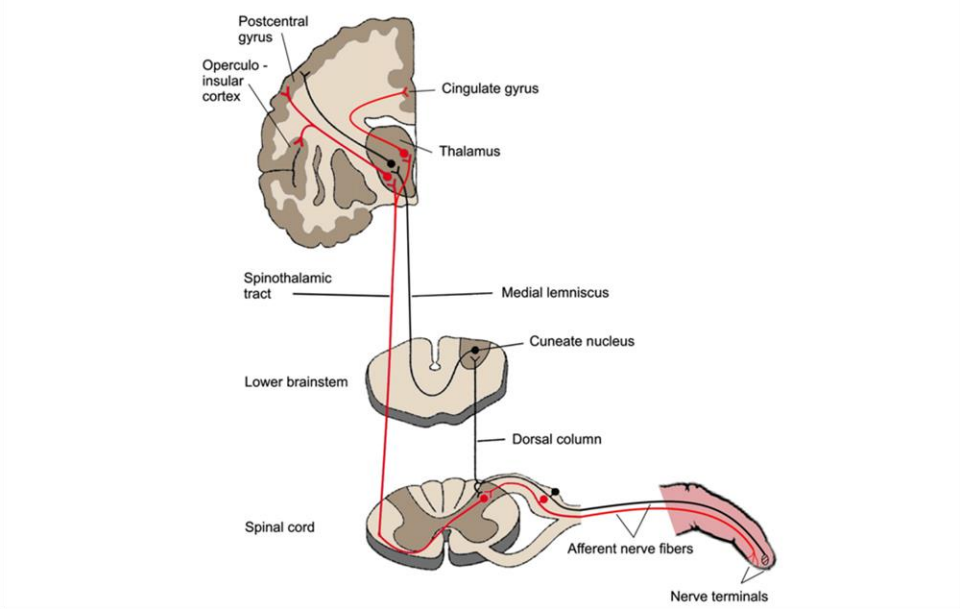


Figure 1.2. Schematic drawing of the somatosensory system. The somatosensory system consists of two major pathways. The DCML (black lines) subserves mechanoreception and proprioception. The STT (red lines) subserves nociception, thermoreception and viscероception. From (R.-D. Treede, 2007).

Nociceptors constitute a specific class of sensory receptors that are activated by stimuli considered as a potential threat for the body (Burgess & Perl, 1967). Their activation is more related to the intensity of the stimulus (i.e., the stimulus should be considered as noxious) than to its modality (mechanical, thermal or chemical stimuli). For this reason, they are considered as "polymodal receptors". Nociceptors are free nerve endings receptors, located in the skin (in the epidermis and dermal-epidermal junction (Novotny & Gommert-Novotny, 1988; Reilly et al., 1997)), bone, joint capsule, tendon, muscle, and many visceral organs. When nociceptors are activated, the signal is conveyed through thinly-myelinated or unmyelinated nerve fibers: Aδ- and C-fibers, respectively. Aδ- fibers, because of their myelination, are able to convey the signal with a velocity of 4 - 36 m/s, while unmyelinated C-fibers have a much lower conduction velocity of 0.4 - 2 m/s (Gardner & Johnson, 2014). Given the ability of those polymodal

receptors to respond to mechanical and heat stimuli, they are called "mechano- and heat-sensitive Aδ-fiber nociceptors" (AMH) and "mechano- and heat-sensitive C-fiber nociceptors" (CMH). Although they are activated by the same kind of stimuli, all of them do not respond in a uniform way.

- *AMH receptors* respond to mechanical input or thermal stimuli with an activation threshold around 46°C. (R. D. Treede et al., 1995).
    - *'Type 1' AMH (AMH-1)* show a tonic response only peaking several seconds after stimulus onset.
    - '*Type 2' AMH (AMH-2)*, are quickly adapting receptors that respond in a strong but very transient fashion, almost synchronous with stimulus onset.
- *CMH receptors* have a lower thermal activation threshold, around 41°C (Carmon et al., 1976; Cruccu et al., 2008; Julius & Basbaum, 2001).
    - '*Quick' CMH (QC)* respond vividly with a peak discharge occurring 0.4 second after the onset of the stimulus, and then rapidly adapt (within one second).
    - '*Slow' CMH (SC)* respond more gradually with a peak discharge occurring approximately two seconds after the onset of the stimulus and then tend to maintain a tonic level of activation throughout the whole stimulus period (Meyer & Campbell, 1981; Wooten et al., 2014).

Physiology of cool perception has been less extensively studied. Briefly lowering the skin temperature by a few degrees cannot be considered as nociceptive and, most importantly, the elicited percept is not painful. The main function of cool-sensitive afferents is probably to provide thermal cues for tactile discrimination (e.g., to discriminate two materials such as wood and metals based on their differing thermal conduction properties) (Ho & Jones, 2006). However, skin cooling, even by a few degrees cool will activate the free nerve endings of superficial layers of the skin and mucosae. Cool thermoreceptor neurons exhibit spontaneous electrical activity at resting

temperature (33°C), which increases in response to temperature reduction. Conversely, this basal action potential firing is suppressed by mild heating of the receptive field (Brock et al., 2001; Hensel & Zotterman, 1951). After signal transduction occurred, the action potential is conveyed to the brain by a specific set of Aδ- fibers (i.e., cool fibers), through the STT (as noxious heat) without eliciting a painful sensation. Cold nociceptors, whose activation threshold is around 14°C, are innervated by C-fibers (Harrison & Davis, 1999).

Spinothalamic projections travel a short distance within the ipsilateral spinal cord. They then enter the dorsal horn at segmental level, cross the midline and project, as the DCML, to the ventral posterolateral nucleus of the thalamus (VPL). VPL neurons receive nociceptive input and then project to the primary somatosensory cortex, the secondary somatosensory cortex, the insula and the anterior cingulate cortex (Cruccu et al., 2008). However, the STT is not the only ascending pathway; the spinoreticular tract, which is positioned closely to the STT, conveys nociceptive inputs to the reticular formation (and then to the thalamus) and some of its projections play a role in the arousal in response to nociceptive input.

2. THE DORSAL COLUMN-MEDIAL LEMNISCUS

Dorsal column-medial lemniscus pathway conveys from periphery to the brain the sensation for tactile object recognition, light touch, two-point discrimination, and proprioception.

Unlike for the STT, the peripheral terminals of the DCML are corpuscular nerve endings (and not free nerve endings) in the skin, joint capsule and muscle. Those receptors are sensitive to mechanical stimuli such as vibration, pressure or stretching of the receptor. When those mechanoreceptors are activated, the related-signal will be conveyed through the DCML by large diameters fibers, namely Aα- and Aβ-fibers. Those fibers have a low activation threshold, are highly myelinated and convey the signal with a high velocity of 72-120 m/s and 36-72 m/s, respectively (Gardner & Johnson, 2014). Large myelinated fibers do not convey nociceptive input, but can influence the spinal transmission of nociceptive stimuli, as exemplified by the gate

control theory of pain. In a few words, this theory explains the mechanism by which the perception of nociceptive input can be modulated by the concurrent activation of Aβ-fibers through the activation of inhibitory spinal interneurons (R. Melzack & Wall, 1965).

From periphery to the spinal cord, the DCML pathway is completely segregated from the STT. However, they both project onto the VPL of the thalamus and they probably share pathways to the cortical areas as nociceptive inputs do not appear to project onto a cortical area exclusively devoted to receiving and processing nociceptive inputs (G. D. Iannetti & Mouraux, 2010; R.-D. Treede et al., 2003).

B - CORTICAL REPRESENTATION OF NOCICEPTION AND PAIN

Nociceptive inputs are conveyed to the brain through multiple ascending pathways, with several thalamic relays, roughly divided in a medial and a lateral system, according to the localization of the relay (J. Brooks & Tracey, 2005; Willis, 1985). While other sensory modalities have a primary sensory cortex to receive and process their related sensory inputs (e.g., visual inputs projects on primary visual cortex), nociceptive inputs do not appear to project onto a dedicated cortical area (Andersson & Rydenhag, 1985; G. D. Iannetti & Mouraux, 2010). Instead, nociceptive-specific neurons (i.e., neurons responding exclusively to the activation of nociceptors) appear to be disseminated in cortical structures that are also involved in the processing of non-nociceptive sensory inputs and/or in other higher-order brain functions. Indeed, when a noxious sensory stimulus is applied on the human skin, neuronal activity is elicited in a vast network of brain regions, including S1, S2, the insula, and the ACC (Apkarian et al., 2005; Bushnell & Apkarian, 2006; L. Garcia-Larrea et al., 2003; Peyron et al., 2000; Tracey & Mantyh, 2007).

In this section, I will describe the involvement of those different structures in nociception and pain processing. Unless otherwise specified, the content of this paragraph is devoted to the cortical processing related to transient exteroceptive stimuli, using non-invasive approaches.

1. AN OBLIGATORY INVOLVEMENT OF THE PRIMARY SENSORY CORTEX?

Primary somatosensory cortex involvement in the processing of nociceptive input and the perception of pain remains controversial (Apkarian et al., 2005; Bushnell et al., 1999; Liang et al., 2013; Mountcastle, 2005; Ploner et al., 1999). fMRI studies have shown an activation of contralateral S1 following the application of nociceptive stimuli (Bingel et al., 2004; Bushnell et al., 1999; Hu et al., 2014; Valentini et al., 2012), but this activation is not systematic and could be modulated via a top-down attentional modulation related to the fact that the nociceptive stimulus is likely to attract attention (Jones et al., 1992). To date, there is no evidence that demonstrates unequivocally an obligatory involvement of S1 in extracting the sensory-discriminative dimension of pain perception (i.e., a role in evaluating the intensity, localization and duration of a nociceptive stimulus) (André Mouraux, 2015).

2. EVIDENT (BUT NOT SPECIFIC) INVOLVEMENT OF OPERCULO-INSULAR CORTEX

The operculo-insular cortex refers to the secondary somatosensory cortex, the posterior and anterior insula. These brain areas are consistently activated bilaterally following a nociceptive stimulus (Bushnell & Apkarian, 2006; L. Garcia-Larrea et al., 2003; Peyron et al., 2000; Tracey & Mantyh, 2007; R. D. Treede et al., 1999). Indeed, a large number of the STT afferents project onto a brain region called the "posterior insula medial operculum" (PIMO). PIMO is described by some authors as being pain specific and could therefore constitute some form of "primary nociceptive cortex" (Craig et al., 2000; L. Garcia-Larrea, 2012). This hypothesis was supported by at least three findings: (1) focal epilepsy originating in this region can elicit (but not systematically) painful seizures (Charlesworth et al., 2009); (2) several case reports suggest that lesions of the insula can alter pain perception (although the opposite has also been reported) (Baier et al., 2014; Feinstein et al., 2016); and (3) the direct electrical stimulation of this area induces pain-related experience. However, this latter finding is far from being systematic (14 out of 43 patients), and when pain was elicited, other sensations were often aroused concomitantly (Ostrowsky et al., 2002).

Recently, Evrard (2019) suggested an interesting and novel approach of the functional organization of the insula, that could be described in three axes: (1) the "sensory-motor axis", receiving e.g., sensory inputs from thalamus and having some control on the motoneuron of the spinal cord, directly affecting the contraction of striate muscles; (2) the "spino-cranial axis", comprising afferents and efferents from the sympathetic and parasympathetic branches of the autonomic nervous system; and (3) the "cognition-emotion axis", comprising two subdivisions of the anterior insular cortex, with the dorsal part being associated with cognitive tasks and the ventral part with emotional responses (for complete review, see Evrard, 2019). Hence, the insula is implicated in the processing of a wide range of sensory inputs, and contributes to a large number of cognitive, affective, interoceptive, and homeostatic functions, independently of sensory modality (zu Eulenburg et al., 2013). The insula appears to play an important role in the detection of salience (i.e., the property of a stimulus to stand out relative to neighboring stimuli) (Dowman et al., 2008; Downar et al., 2000), possibly constituting a hub connecting sensory areas to other networks involved in the processing and integration of external and internal information (André Mouraux, 2015).

3. SIGNIFICANCE OF THE SO-CALLED "PAIN MATRIX"

Probably because of the absence of evidence of a primary nociceptive cortex, it has been suggested that the perception of pain emerges from the joint activation of a network of brain structures, encompassing all structures shown to be consistently activated following nociceptive stimulation, in particular, somatosensory areas (S1, S2), the insula and the ACC (Luis Garcia-Larrea & Peyron, 2013; Tracey & Mantyh, 2007). It has been repeatedly demonstrated that, within this so-called "pain matrix", the magnitude of the brain responses elicited by a transient exteroceptive nociceptive stimulus correlate robustly with the intensity of perceived pain (Coghill et al., 1999; Derbyshire et al., 1997; G. D. Iannetti et al., 2005; Tölle et al., 1999). However, although probably necessary for the perception of pain, this "pain-matrix" is probably not specific for encoding pain experience and there are several arguments against that:

- The magnitude of the responses can be modulated by other factors than the intensity of pain perception. Indeed, the response habituates after a few trials without any difference in the elicited percept (i.e., the EEG response decreases without any decrease in the intensity of perception) (G. D. Iannetti et al., 2008). Moreover, the magnitude of those pain-evoked responses can also be differentially modulated in different subregions of this "pain matrix". For instance, hypnotic suggestion of increased intensity of perceived pain has been shown to increase selectively the response magnitude in somatosensory areas; on the other hand, hypnotic suggestion of increased pain unpleasantness has been shown to selectively increase the response magnitude in the ACC. This also supports the hypothesis that somatosensory cortices might be more implied in the sensory-discriminative aspects of pain, while insula and ACC might be more related to the cognitive-affective dimension of the pain experience (R. K. Hofbauer et al., 2001; Rainville et al., 1997).
- The fact that nociceptive stimuli consistently elicit the same pattern of activation (S1, S2, insula, ACC) does not imply that this pattern of activation is stimulus-specific. Indeed, the "pain matrix" response can be completely dissociated from pain perception (G. D. Iannetti et al., 2008; Lee et al., 2009; A. Mouraux et al., 2003). Furthermore, it has been demonstrated in an elegant set of experiments (A. Mouraux & Iannetti, 2009; André Mouraux, Diukova, et al., 2011) that the bulk of nociceptive ERPs could be explained by multimodal neural activity also contributing to the ERPs elicited by non-nociceptive somatosensory, auditory and visual stimuli. Importantly, all stimuli were presented within a random sequence, using a large and unpredictable inter-stimulus interval, such as to maximize their salience (A. Mouraux & Iannetti, 2009). Using fMRI and the same paradigm, it has also been demonstrated that nociceptive, somatosensory, auditory and visual stimuli elicited spatially indistinguishable responses in the cingulate, the insula and the largest part of S2. Furthermore, a matching pattern of activation was also observed for nociceptive and non-nociceptive somatosensory stimuli in S1 and in a small subregion of S2, whereas the magnitude of these responses was correlated with subjective ratings of stimulus

salience, independently of the modality of the eliciting stimuli. (André Mouraux, Diukova, et al., 2011).

The fact that stimulus salience appears to be one of the main determinants of these responses could suggest that they reflect cortical activity involved in the detection of salient events, or in triggering responses related to the occurrence of events such as an involuntary reorientation of attention. However, part of these brain responses could also be related, for example, to arousal reactions, autonomic activation or affective responses (André Mouraux, 2015).

For the aforementioned reasons, responses elicited by transient nociceptive stimuli are not necessarily adequate to explore the neural activity associated to the actual emergence of pain. The use of experimental designs involving long-lasting nociceptive stimulation which result in tonic pain sensations may constitute a promising strategy to enhance the likelihood of extracting neural activities more specifically related to the actual perception of pain and, possibly, more closely related to the pathophysiology of clinical pain conditions. In this view, the EEG analysis of steady-state evoked potentials elicited by tonic, periodically-modulated nociceptive stimuli could constitute an interesting approach (E. Colon et al., 2012; Elisabeth Colon et al., 2014; André Mouraux, Iannetti, et al., 2011).

1.2.2. PROBING THE SOMATOSENSORY PATHWAYS

A - RECORDING BRAIN RESPONSES TO SOMATOSENSORY STIMULI

Because EEG is a widely available and non-invasive technique with outstanding temporal resolution, the majority of studies have relied on this technique to study nociceptive brain processing following activation of nociceptors by noxious heat stimuli (Ulf Baumgärtner et al., 2005; André Mouraux & Iannetti, 2018). The ongoing electrical activity of the human brain can be recorded through the skull using an array of electrodes placed against the scalp. The recorded electrical activity, the electroencephalogram, reflects transient changes in scalp potential. Stimulus-evoked changes in the ongoing EEG can occur in response to a time-locked sensory, motor or

cognitive input: time- and phase-locked responses observed in the time domain are called Event-Related Potentials (ERPs); time-locked but non phase-locked modulation of the magnitude of ongoing rhythms are observable in the time-frequency domain and are referred to as Event-Related Synchronization, or Desynchronization. Delivering those inputs in a periodic fashion will induce periodic modulations of the ongoing EEG called "Steady-State Responses" (SSRs) or "Steady-State Evoked Potentials" (SS-EPs).

1. Event-Related Potentials

ERPs consist of brief monophasic deflections characterized by their polarity, peak latency (relative to the onset of the event), peak amplitude (relative to a baseline) and scalp distribution. It is generally accepted that these ERPs reflect synchronous changes of slow postsynaptic potential occurring within a large number of similarly oriented pyramidal neurons of a compact area of the cortex (A. Mouraux & Iannetti, 2009). Nevertheless, those changes in the ongoing electrical activity are of very small amplitude and will usually not stand out of the background EEG at a single-trial level. To identify stimulus-evoked deflections, it is required to repeat the stimulus a given number of times. The EEG is then segmented into epochs relative to the onset of each stimulus averaging all these epochs into a single waveform (A. Mouraux & Iannetti, 2008). Indeed, the stimulus-evoked activity is thought to be stationary across trials, while the ongoing electrical activity of the brain behaves as noise unrelated to the event. Therefore, the averaging procedure amplifies the stimulus-evoked activity whereas the background noise is largely canceled out by this procedure.

2. Event-related synchronization/desynchronization

In healthy individuals, it is well known that sensory, motor, and cognitive events elicit changes in ongoing rhythmic activities which are time-locked but not phase-locked to the event. This can be observed in the frequency domain as transient modulations of power of the ongoing EEG, referred to as event-related synchronization (ERS) and desynchronization (ERD) (Pfurtscheller & Da Silva, 1999). The terms "synchronization" / "desynchronization" reflect the view that the increase/reduction

in EEG oscillation power results from a synchronization/desynchronization of the activity of a population of neurons. For instance, ERS in the alpha frequency band (8-12 Hz) is often hypothesized to reflect cortical deactivation, or inhibition, whereas ERD in the same frequency band is hypothesized to reflect cortical activation, or disinhibition (Pfurtscheller & Da Silva, 1999). This interpretation is based on studies showing, for example, that the power of alpha band rhythms is enhanced over the hand area during visual processing, or during foot movements. In contrast, ERS in higher frequency bands (e.g., gamma-band oscillations [GBOs], 25-100 Hz) is thought to play an important role in cortical integration and perception (Zhang et al., 2012).

3. Steady-State Evoked Potentials

Steady-state evoked potentials were first described in 1966 by Regan (1966, 1989). Unlike conventional transient ERPs, which reflect a phasic cortical response triggered by the occurrence of a brief stimulus, SS-EPs reflect a sustained cortical response induced by the long-lasting periodic repetition of a sensory event. Unlike ERPs, SS-EPs are best identified in the frequency domain, as peaks appearing at the frequency of the stimulus and/or at harmonics of that frequency.

SS-EPs offers interesting advantages compared to ERPs : they usually exhibit a particularly high signal-to-noise ratio (SNR); they reflect neural activity originating mainly from modality-specific sensory cortices; they are less contaminated by cortical activity related to stimulus-triggered attentional reorientation (Elisabeth Colon et al., 2012). For these reasons, an increasing number of studies have used this approach successfully to explore the neural activity involved in the cortical processing of sensory modalities such as visual and auditory modalities.

However, how the SS-EPs emerge within the human EEG remains a matter of debate : in a first hypothesis, SS-EPs are thought to be the result of the linear summation of successive transient responses elicited by the repetition of the sensory stimulus, resulting therefore from the same neural activity underlying transient ERPs (Capilla et al., 2011). This hypothesis has been mainly demonstrated using visual and auditory stimuli. In a second hypothesis, SS-EPs would result from a stimulus-driven

entrainment of a network of neurons responding to the periodically modulated feature of the stimulus (Herrmann, 2001). According to this second hypothesis, SS-EPs would reflect the ability of the neurons to oscillate at particular frequencies, and to synchronize their activity to an external periodic event (Galambos, 1982; Herrmann, 2001). For instance, to support this hypothesis, it has been shown that the magnitude of the SS-EPs elicited by a flickering visual stimulus in the human visual cortex is markedly greater for particular frequencies of stimulation than for adjacent frequencies of stimulation. This indicates a preference of the underlying neuronal oscillators for given frequencies and their harmonics (Herrmann, 2001). None of those hypotheses can fully explain the generation of SS-EPs and it is possible that both co-exist.

B – SOMATOSENSORY NOCICEPTIVE AND NON-NOCICEPTIVE STIMULI TO ELICIT BRAIN RESPONSES

The design of a somatosensory stimulus for experiments should consider each characteristic of the somatosensory system. Regarding the differences between the two main somatosensory tracts, probing them requires different kind of stimuli that have a certain selectivity for receptors, fiber types, quality of percept and are conveyed by a single pathway. Here, I will review in a non-exhaustive way, several methods to generate such stimuli and the characteristics of their respective brain evoked responses.

1. ELECTRICAL STIMULATIONS

Electrical stimulation can be used either to activate afferents of the skin or to activate afferents of a nerve trunk by applying the stimulation on the skin over a selected nerve. The latter requires the delivery of monophasic square-wave electrical pulses of 0.1-0.2 ms, with a stimulation frequency of 3-5 Hz. Its intensity should be two to three times the sensory threshold, or, for mixed nerves, it should elicit a reproducible muscle twitch (Cruccu et al., 2008). The recorded brain responses are called "somatosensory-evoked potentials" (SEPs). Historically, SEPs were the first somatosensory ERPs recorded (Dawson, 1947) and remain to date the most widely studied in clinical practice. However, in a research context, SEPs suffer from the

important drawback of their lack of selectivity. Indeed, the use of a high-intensity electrical stimulus activates a wide range of fibers such as Aβ-, Aδ- and C-fibers (Figure 1.3). Ascertaining the selective activation of one type of fibers is either technically challenging (Dufour et al., 2011) or requires a subjective feedback of the evoked sensation from the patient in order to avoid a painful percept signaling the co-activation of Aδ- and/or C-fibers (A. Mouraux et al., 2010). Finally, the elicitation of SS-EPs related to periodic electrical stimulations of low intensity (i.e., the intensity to selectively activate large-diameter afferent) is of difficult interpretability; indeed, such stimuli generate a periodic artifact that is complicated to distinguish from the periodic neural response. Hence, the use of electrical stimulations does not meet our needs for the evaluation of patients with DoC.

2. MECHANICAL STIMULATIONS

Mechanical stimulations can be used either to activate AMH-1 receptors conveying the transduced signal by Aδ-fibers or to activate low-threshold mechanoreceptors whose action potentials are conveyed by Aβ-fibers.

AMH-1 receptors can be mechanically activated by the application onto the skin of a very thin stainless-steel probe with a flat tip (e.g., diameter 0.25 mm), ideally in a slow fashion, around one per second, to obtain a more robust recruitment of mechanosensitive nociceptors (G. Iannetti et al., 2013). According to this method, it is possible to record the so-called "pinprick-evoked potentials", consisting in a combination of responses corresponding to the activation of Aβ- and Aδ-afferents; this technique is mainly used to assess punctate hyperalgesia (van den Broeke & Mouraux, 2014).

Low-threshold mechanoreceptors, on their side, are most commonly activated by vibrotactile stimulations. Different techniques were used to generate those stimuli, such as inflatable membranes (Nangini et al., 2006), piezo-electric devices (Severens et al., 2010), solenoid vibrators (Adler et al., 2009), or vibrotactile transducers (Figure 1.4 ; Haptuator, Tactile Labs Inc., Canada).

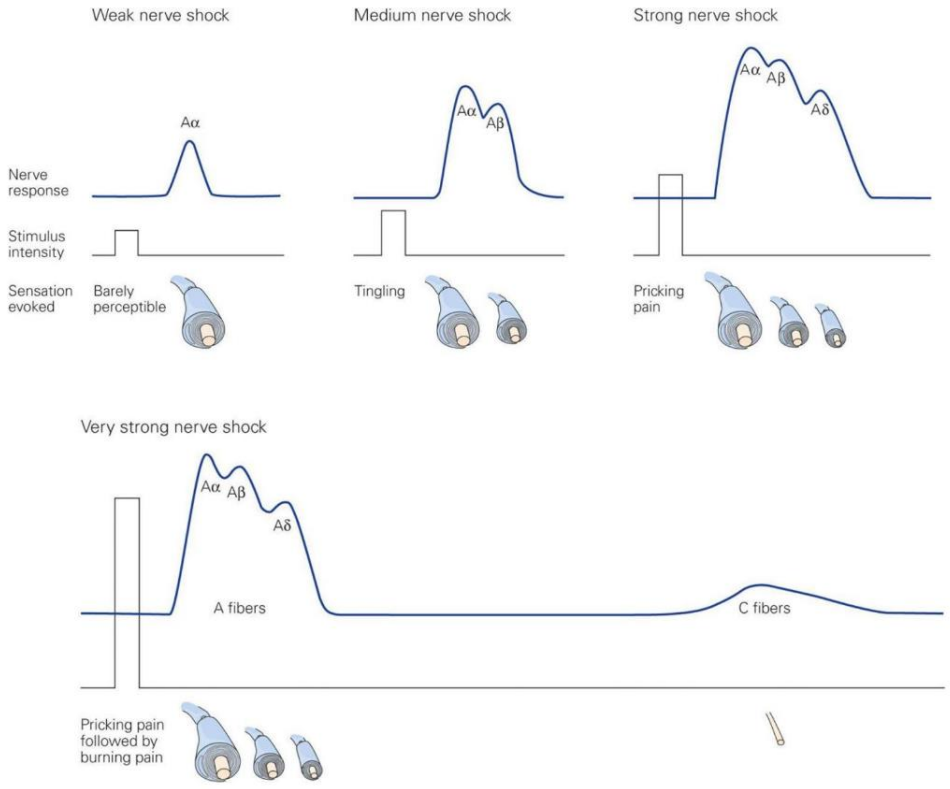


Figure 1.3. Representation of the pattern of nerve fibers activated by different intensities of electrical stimulations. Each deflection on the graphs represents a deflection generated by a specific type of nerve fiber. On the lower row, those deflections are put in relation with the diameter of the activated fibers. (From (Gardner & Johnson, 2014) itself adapted from (Erlanger & Gasser, 1937))

Unlike SS-EPs, time-locked ERPs elicited by the activation of low-threshold mechanoreceptors using vibrotactile stimulations are scarcely reported in the literature. Seminal studies faced a low SNR due to the strong habituation of the responses, probably related to the long duration of the stimulus (Hari, 1980; Kekoni et al., 1997). Later on, using vibratory pulses of shorter duration, vibrotactile-ERPs were characterized by a contra-lateralized, pre-centrally negative and post-centrally positive deflection named P50, followed by a bilateral P100 wave. Those waves seem to reflect

the sequential activity located first in the contralateral S1, and then in contra- and ipsilateral S2 (Hämäläinen et al., 1990; Münte et al., 1996; Severens et al., 2010). In a recent study, using a vibrotactile transducer (Figure 1.4), the delivery of short duration vibrotactile stimulation (50 ms) at a high frequency (300 Hz), repeated as low as twenty times with a sufficient interstimulus interval (five to ten seconds), allowed to elicit robust ERPs, identified at Cz electrode with two components : an early N1 peak which appears between 100 and 170 ms, maximal and negative at Cz, and a P2 peak which appears between 170 and 400 ms, maximal and positive at Cz (van den Broeke & Mouraux, 2014).

Figure 1.4. Illustration of the Haptuator (Tactile Labs Inc., Canada), a high-bandwidth vibrotactile transducer, driven as a loudspeaker by an audio amplifier (not seen on the picture). Thanks to its very low dimensions (29 mm length, 16 mm diameter) and weight (11 g), the Haptuator can be easily applied onto the skin without inconvenience for the subject. It can deliver vibrotactile stimulation in a 50-500 Hz bandwidth. Stimulus duration can be as short as 50 ms or as long as tens of seconds. The Haptuator is set at maximal input value (3 V) for the recording of ERPs. For the recording of SS-EPs, the carrier frequency is modulated in intensity by varying the input voltage in a periodic fashion at the desired modulation frequency. From Tactile Labs Inc. website (tactilelabs.com).

Vibrotactile SS-EPs were mostly studied using a carrier frequency (i.e., the vibration frequency that remains unchanged during the stimulus) greater than 100 Hz, periodically

modulated at frequencies ranging from 2 to 40 Hz. This allowed to identify that the preferred frequency to elicit somatosensory SS-EPs lies in the 20-30 Hz range (E. Colon et al., 2012; Tobimatsu et al., 1999). The observed SS-EPs when stimulating the hand display a clear maximum over the parietal region contralateral to the stimulated side, a topography compatible with an activity originating predominantly from S1 (Giabbiconi et al., 2007; Snyder, 1992). However, when low modulation frequencies are used (such as 3 Hz), the scalp topography differs (Elisabeth Colon et al., 2012). In this case, scalp topography was symmetrically distributed over both hemispheres, and maximal over the vertex and fronto-central regions. Such as the P2 wave recorded in laser-evoked potentials (LEPs; see next section), the magnitude of the 3 Hz-SS-EPs showed a marked habituation, suggesting that both responses reflect unspecific and non-obligatory stages of sensory processing, possibly related to stimulus evoked attentional capture (G.D. Iannetti et al., 2008; Legrain et al., 2011). Recently, using very low modulation frequency such as 0.2 Hz, robust SS-EPs were recorded at that frequency and its harmonic by means of intracranial insular electrodes (Liberati et al., 2019).

3. THERMAL STIMULATIONS

3.1. Thermal stimulation by infrared laser

The infrared laser was introduced in 1976 as an innovative method to activate heat-sensitive skin nociceptors and their spinothalamic projections (Carmon et al., 1976). Because it relies on thermal radiation to activate polymodal nociceptors, it avoids any skin contact and hence any confound related to the activation of low-threshold Aβ-fiber mechanoreceptors (Cruccu et al., 2008; L. Plaghki & Mouraux, 2003; R.-D. Treede et al., 2003). Those laser stimuli can be delivered by different type of laser, each of them characterized by their wavelength, depending on their active gain medium. Two main classes of laser are used to this purpose: solid-state lasers, such as Nd: YAP (Neodymium-doped Yttrium Aluminum Perovskite) with a short wavelength (λ = 1,34 μm), and CO_2-laser with a greater wavelength (λ = 10,6 μm).

The wavelength of the laser should be carefully considered, as it determines the skin reflectance and the absorption rate. At long wavelengths (i.e., $\lambda > 4$ μm), skin reflectance

is negligible and skin water causes nearly total absorption of the radiation (absorbance = 99.7%) within the superficial epidermal layers (Arendt-Nielsen & Chen, 2003; L. Plaghki & Mouraux, 2003; Truini et al., 2005). The calorific energy therefore remains confined to the upper skin layers where transducer nerve terminals sensitive to thermal variations are located (i.e., at the dermo-epidermal junction, between 20 and 500 μm deep (Novotny & Gommert-Novotny, 1988; Reilly et al., 1997)). At short wavelengths (i.e., $\lambda < 2$ μm), skin reflectance is lower in pigmented skin as compared to white skin, increasing the risk of severe burns (Arendt-Nielsen & Chen, 2003; Hardy & Muschenheim, 1934; L. Plaghki & Mouraux, 2003). Depending on these factors, a given amount of energy deposited on the skin generate very different heating profiles. For this reason, especially in the context of patients with DoC, it is probably very interesting to use a temperature-controlled laser which allows to actually know what the heating profile of the skin is, even without any feedback from the subject. CO_2-laser stimulator using closed loop feedback to control skin temperature are hardly used in practice because of their low availability and high cost. However, the advantages are huge (Meyer et al., 1976) since the closed loop implementation makes possible to deposit a well-defined amount of energy on a definite area of the skin and thus activate nociceptors by warming up the skin at the desired temperature. On the basis of mathematical laws allowing to calculate the evolution of the skin temperature under an energy supply, the device precisely determines the quantity of energy to deliver to reach a given temperature (Marchandise et al., 2014; SIFEC, 2009).

The EEG recordings of corresponding cortical responses are called laser-evoked potentials (LEPs). They are currently the gold-standard to assess spinothalamic function and the integrity of the nociceptive pathway (Cruccu et al., 2010; R.-D. Treede et al., 2003). They are thought to partially reflect the neural processes by which the perception of pain emerges from nociceptive input (Ulf Baumgärtner et al., 2006; R. D. Treede, Kief, et al., 1988). However, there are also increasing evidences indicating that the largest part of LEPs could reflect cortical activity unspecific for nociception, such as multimodal cognitive processes involved in the detection and the orientation of

attention toward the occurrence of a transient, salient sensory event (G. D. Iannetti & Mouraux, 2010; Legrain et al., 2011; André Mouraux, Diukova, et al., 2011).

LEPs result of the simultaneous co-activation of Aδ- and C-fibers (Bromm & Treede, 1984). This co-activation elicits both a sensation of first pain (rapid, acute, sharp, AMH-2-mediated) and a sensation of second pain (delayed, diffuse, dull, CMH-mediated) (Julius & Basbaum, 2001; Lewis & Pochin, 1937; Opsommer et al., 1999; Léon Plaghki & Mouraux, 2005; R. D. Treede et al., 1995). Since myelinated Aδ-fibers and unmyelinated C-fibers have very different conduction velocities, it can be expected to obtain two distinct responses at very distinct latencies (Opsommer et al., 1999; Léon Plaghki & Mouraux, 2002; R. D. Treede et al., 1995). However, the recorded cortical responses have latencies compatible with conduction velocity of Aδ-fibers activation exclusively, and not with the velocity of slow-conducting C-fibers (L. Garcia-Larrea et al., 2003; Luis Garcia-Larrea, 2004; Kakigi et al., 1989). LEPs may vary in latency with regards to the type of laser used, solid-state lasers showing shorter latencies than CO_2-laser. They may also vary in latency and topography according to the site of stimulation, with e.g., shorter latencies following facial stimulation as compared to forearm stimulation. Considering a stimulation using a CO_2-laser on the hand dorsum, the observed related-brain responses consist of a large negative-positive complex at the scalp vertex, named N2-P2, that peaks at approximately 240 ms for the N2 wave and 400 ms for the P2 wave. This complex is preceded by an earlier negative deflection, the N1 wave (±170 ms), appearing maximal over central and temporal regions contralateral to the stimulated side (Hu et al., 2010; R. D. Treede, Kief, et al., 1988; R. D. Treede, Meier, et al., 1988; Valentini et al., 2012). Given their large amplitude, the N2 and P2 waves are often visible at a single-trial level and can be identified reliably using only a few repeated stimuli, typically, two runs of 20 to 30 stimuli (Cruccu et al., 2008).

To record evoked potentials specifically related to the activation of C-fibers (C-LEPs), the Aδ component of the afferent volley must be suppressed. Several techniques exist to achieve this, such as the pressure-block of Aδ-fibers technique (thinly myelinated fibers are more sensitive to pressure block) (Nahra & Plaghki, 2003)

or the stimulation of tiny skin areas (the density of CMH at skin surface is higher than AMH receptors) (Bragard et al., 1996). Recently, the use of a temperature-controlled CO_2-laser stimulator to activate C-fibers afferents in a selective fashion allowed to reliably and consistently record C-LEPs, taking advantage of the lower temperature threshold of those C-fibers (Jankovski et al., 2013). C-LEPs are characterized by an "ultra-late" negative-positive complex (ultra-late N2-P2) occurring 750-1150 ms after a hand stimulus (Léon Plaghki & Mouraux, 2002).

Considering the cortical generators of LEPs, the N1 wave is thought to reflect activity originating from several temporally-overlapping sources, within the contralateral operculo-insular cortex and, possibly, the contralateral S1 (Perchet et al., 2008; Valentini et al., 2012; M. Valeriani et al., 2000). The N2-P2 complex reflects a combination of activity originating from bilateral opercular regions, that has been hypothesized to reflect neural activity originating from S2 and, possibly, from the deeper insular cortex (L. Garcia-Larrea et al., 2003; Valentini et al., 2012). Therefore, opercular activity seems to contribute to the generation of both N1 and N2-P2 waveforms. In addition, source-analysis studies have repeatedly proposed that LEPs also reflect activity originating from the anterior cingulate cortex (L. Garcia-Larrea et al., 2003). Regarding the C-LEPs, their scalp topography and morphology very much resembles those of LEPs. The "ultra-late" N1 and "ultra-late" N2-P2 probably share about the same generators than those of N1 and N2-P2 waveforms observed in LEPs (Léon Plaghki & Mouraux, 2002; Massimiliano Valeriani et al., 2002).

The recording of SS-EPs related to the application of long-lasting, periodically modulated, nociceptive stimuli could constitute a good complement to the study of LEPs. In a seminal study, it was shown that using rapid periodic stimulations ($f = 7$ Hz), the observed SSRs displayed a low SNR (André Mouraux, Iannetti, et al., 2011). However, lowering this modulation frequency at 0.2 Hz made possible the identification of nociceptive SS-EPs at a single-subject level, with a higher SNR (Elisabeth Colon et al., 2017). Interestingly, this study suggests that the generators of those nociceptive SS-EPs could be related to the activation of C-fibers (probably SC), as the presence of

an Aδ-fiber nerve block did not change the observed SSRs. Scalp topography of nociceptive SS-EPs is maximal at vertex, symmetrically distributed over both hemispheres. Those results contrast with the lateralized (contralateral to the stimulated side) scalp topography of the SS-EPs obtained by innocuous electrical stimulation of large-diameter Aβ-fibers. Those between-modalities differences in spatial distribution suggest that nociceptive SS-EPs reflect the activity of a cortical network spatially distinct from the somatotopically-organized cortical network involved in processing of innocuous vibro-tactile input and, possibly, preferentially involved in processing nociceptive input (André Mouraux, Iannetti, et al., 2011).

3.2. Thermal stimulation by contact

The most commonly used contact thermodes are based on the Peltier principle, allowing an increase or a decrease of temperature depending on the direction of the current (i.e., one direction of the current causes heating and the other way cooling) (Kenshalo & Bergen, 1975). A device which take the advantage of this principle would be able to generate cool as well as heat stimuli, which is not possible using a laser device. Such device, the Thermal Cutaneous Stimulator, has been developed and made commercially available very recently (TCSII, QST.Lab, Strasbourg, France). The TCSII is a contact thermode able to generate very steep cooling/heating ramps of up to 300 °C/s. It is composed of a very portable control unit (size: 350 x 300 x 120 mm; weight: 4kg; Figure 1.5a) connected to a stimulation probe (weight: 400 g; Figure 1.5b). The stimulation probe consists on its extremity of a flat 30 mm diameter surface containing 15 micro Peltier elements of 7.7 mm² each. The micro Peltier elements are organized in five zones (Figure 1.5b) in which the temperature can be controlled independently, allowing to vary the stimulated skin surface without displacing the stimulation probe. The neutral skin's temperature is automatically calibrated by keeping the stimulator on the skin of the patient. Feedback on the temperature is obtained via a thermocouple, present in each of the five zones, which drives the micro Peltier elements to the target temperature, allowing a precise control of the temperature at the skin surface (De Keyser et al., 2018). Active return to the baseline temperature can be

activated on request; noteworthy, the stimulus duration comprises "rising" time and "constant temperature" time, not the return to baseline, even if active.

The necessary contact with the skin constitutes the main disadvantage of the TCSII as it concomitantly activates low-threshold mechanosensitive Aβ-fibers. This is a potentially confounding factor interacting with the obtained responses as these fibers modulate the spinal transmission of both nociceptive and heat information (De Keyser et al., 2018; L. Plaghki & Mouraux, 2003). However, there are at least three strategies to limit those problems : (1) the different zones of the TCSII can be independently activated, meaning that the stimulated area can be varied without moving the stimulation probe, (2) when moving the probe, waiting a few seconds before delivering a stimulus limit the influence of the activation of mechanosensitive afferents and (3) the interstimulus interval (ISI) should vary between each stimulations.

(a) (b)

Figure 1.5. The thermal cutaneous stimulator (TCSII). (a) Control unit and stimulation probe. (b) Stimulation probe. The 5 stimulation surfaces that can be activated separately are delimited by white lines and labeled. (From QST lab website [qst-lab.eu], with permission of André Dufour)

Thanks to its characteristics, the TCSII is able to activate a sufficient afferent volley of cool-fibers to generate cool-evoked potentials (CEPs) (De Keyser et al., 2018; Leone et al., 2019). It is also likely to activate heat-sensitive afferents in a sufficiently

synchronous fashion to elicit measurable brain responses, the so-called "Contact Heat Evoked Potentials" (CHEPs). Finally, it is able to generate well-controlled periodic temperature variations, a prerequisite to make possible the recording of warm/cool SS-EPs.

3.2.1. Thermal heat stimulation by contact

Contact thermodes, by their application onto the skin, use the thermal conduction to increase the temperature of free nerve endings above their activation threshold. Such devices, while accessible and flexible in clinical context due to their limited size (Arendt-Nielsen & Chen, 2003), have a low heating ramp (less than 70°C/s): this allows to elicit measurable ERPs (Atherton et al., 2007), but with a significantly lower SNR than obtained with laser devices. The slow rise in temperature also accounts for an important jitter and the lack of synchronized activation of the receptors. A major challenge regarding the contact thermodes is to increase the heating slope while allowing a perfect temperature control. A device like the TCSII with such performances should open up promising perspectives by the recording of CHEPs with an increased SNR. However, the use of such device to record CHEPs has not been described yet.

3.2.2. Thermal cool stimulation by contact

Historically, Duclaux et al. were probably the first to record CEPs, showing a latency compatible with the conduction velocity of cool-sensitive Aδ-fibers (Duclaux et al., 1974). However, the low temperature drop (17°C/s) did not allow to record those responses with a high SNR. Different devices were subsequently developed, without being able to achieve significantly higher cooling ramps (10-21°C/s) (Hüllemann et al., 2016; Jamal et al., 1989).

Very recently, using the TCSII, De Keyser et al. achieved to record brain responses elicited by high-speed cooling of the skin (300°C/s). Those responses were identifiable at a single-subject level and with a good SNR (De Keyser et al., 2018). The elicited CEPs consisted in a series of three waves: an early-latency N1/P1 maximal over the hemisphere contralateral to the stimulated arm, followed by a large N2-P2 complex, maximal at scalp vertex. Their latencies were 189±18 ms, 216±37 ms and 395±58 ms

for N1/P1, N2 and P2, respectively. Scalp topography of the early-latency N1/P1 differs from the scalp topography of the N1 wave of LEPs, suggesting at least partially different cortical generators. The topography of CEP-N1 wave resembles the scalp topography of the early-latency responses of somatosensory-evoked potentials, such as the N20 wave elicited by stimulation of the median nerve (Lenoir et al., 2017). These scalp topographies are compatible with activity originating in S1. The implication of coldness in tactual perception, providing information to the material's heat capacity and thermal conductivity (Ho & Jones, 2006; Tiest, 2010) could account for the differential involvement of S1 in cool versus warm perception. However, other areas such as S2, the insular and the cingulate cortices are likely to also contribute to the activity underlying CEPs (Craig et al., 2000; Maihöfner et al., 2002).

CHAPTER 2. AIMS OF THE THESIS

"Pain is inevitable. Suffering is optional."
- Haruki Murakami

The first goal of this thesis is to develop non-invasive means to assess the ability of patients with DoC to perceive pain that can be used at bedside and are independent of the patient's communication ability. The proposed multimodal approach is based (1) on a comprehensive study of behavioral responses to nociceptive stimuli and how they are correlated to preserved brain activity patterns; and (2) on electroencephalographic (EEG) recordings of stimulus-evoked brain responses.

The second goal of this thesis is to examine whether, on average, the ability of the damaged human brain to respond to nociceptive stimuli is relatively preserved as compared to its ability to respond to non-nociceptive stimuli. Nociception is crucial for survival because it signals potential threats to the body. Probably for this reason, the projection of nociceptive input to the brain involves multiple ascending systems, some of which are highly conserved across species (Ronald Melzack & Katz, 2013). Probably also for this reason, numerous peripheral and central mechanisms exist to compensate the consequences of lesions of the nociceptive system. These mechanisms are thought to play a crucial role in the development of chronic neuropathic pain ("failsafe theory" (McCormack, 1999)). Therefore, assessing cortical responses to nociceptive stimuli could constitute a more robust means to assess residual cortical function in patients with DoC.

In order to achieve these goals, the approach is twofold: on the one hand, we need to understand whether a patient has the preserved neural resources to consciously experience pain. On the other hand, we should be able to determine if a patient is experiencing pain at a given moment. I decided to probe those abilities using a neurophysiological and a behavioral approach, respectively.

Considering the behavioral approach, I decided to focus on the only validated tool that has been specifically developed for assessing reaction to (potentially) painful stimuli in patients with DoC, namely the Nociception Coma Scale (NCS) and its revised version (NCS-R). This thesis aimed to gain a better understanding of the neural correlates of the scale as well as its clinical use.

Several neuroimaging techniques exist to record brain activity. However, they all have pros and cons, regarding their temporal and spatial resolution, their availability, usability at bedside but also their potential for applicability in a clinical setting. Functional MRI has the advantage to offer a very good spatial resolution, but a weak temporal resolution. The device is costly and not always easily available. Moreover, to record good quality data, the patient must remain still during several minutes, a challenge with patients with DoC. Sedation is not a solution in our cause as it will inevitably impair consciousness and therefore the pain experience. PET imaging allows to use a radioactive tracer that is selective to the aim and paradigm of the study. However, except for FDG, the other tracers (e.g., for passive paradigms) are hardly available. Its spatial resolution is lower than MRI and temporal resolution is weak. The device is costly and hardly available, making it unsuitable in a clinical setting. EEG has a very high temporal resolution (milliseconds), but its spatial resolution is low. Easily available and affordable, it is also highly portable and usable at bedside, making it potentially easy to translate in clinical practice. Given the widespread brain lesions of patients with DoC and the fact that probing the pain pathways integrity relies on the study of the brain responses to transient somatosensory stimuli, we decided to employ a device with a high temporal rather than a high spatial resolution. Usability at bedside is also important for assessing patients in, for them, comfortable conditions. Finally, the cost, the availability of the equipment and the potential clinical applicability of the technique were also considered. All of those criteria were best met by EEG.

Probing the somatosensory (including nociceptive) pathways using EEG requires to present different types of somatosensory stimuli in order to elicit a modulation of the ongoing electrical brain activity. First, we chose the stimuli according to their

selectivity for the somatosensory tract (and nerve fiber type) by which they are conveyed to the brain. Indeed, it is necessary to observe whether the recorded activity is specific for nociception or is indifferently observed with any other somatosensory stimulus. Therefore, we chose a purely nociceptive stimulus, namely a noxious-heat stimulus (i.e., a heat stimulus that raises the temperature of the free-nerve endings above their activation threshold) conveyed to the brain by the STT; and a mechanical vibrotactile stimulus that selectively activates large diameter Aβ-fibers, conveying the vibrotactile signal to the brain through the DCML. Second, if a brain response is observed following a nociceptive stimulus, it remains unknown whether the brain response is specific for the nociceptive characteristic of the stimulus or because it is conveyed to the brain by the same pathway, namely the STT. To disentangle this, we decided to record brain responses to cool stimuli, having the advantage to be conveyed by the STT without eliciting a painful percept in healthy subjects. Finally, stimuli can be delivered either in a transient fashion (a few hundred milliseconds) to record ERPs, or in prolonged periodic fashion, to allow the recording of SS-EPs. Indeed, due to the extensive brain damage, the abnormal large-amplitude EEG, and numerous sources of movement artifacts, it was suspected that the recording of SS-EPs (analyzed in the frequency domain and therefore insensitive to phase shifts) would allow to observe more robust brain responses than with ERPs.

In Chapter 3, I will focus on the behavioral approach and present two studies. In a first study, we will use the NCS-R to identify patients with preserved neural basis for pain perception and to refine the understanding of the scores obtained at the evaluation with this scale. In a second study, we will investigate whether the NCS-R is applicable in a clinical context, specifically in patients with a tracheostomy, a frequent condition in this population. **In Chapter 4**, I will focus on the neurophysiological approach and present three studies. In a first study, I will present an exploratory study aiming at recording selective somatosensory ERPs in patients with DoC. In a second study, I will present the validation of a novel contact thermode to elicit noxious-heat related brain responses against the gold-standard, namely the laser device. A third study will describe

a methodology to record SS-EPs in response to cool and warm periodic stimuli in healthy subjects. **Chapter 5** will summarize the findings and present the future perspectives offered by this work.

CHAPTER 3. BEHAVIORAL ASSESSMENT

"For there is nothing heavier than compassion.
Not even one's own pain weighs so heavy
As the pain one feels with someone, for someone,
A pain intensified by the imagination
And prolonged by a hundred echoes."
- Milan Kundera

This chapter is based on:

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.

Bonin, E. A., Lejeune, N., Thibaut, A., Cassol, H., Antonopoulos, G., Wannez, S., Martial, C., Schnakers, C., Laureys, S. & Chatelle, C. (2019). Nociception Coma Scale Revised allows to identify patients with preserved neural basis for pain experience. *The Journal of Pain.* doi: 10.1016/j.jpain.2019.11.004

3.1 Background: The Nociception Coma Scale

Pain is probably one of the most subjective experiences, and for this reason, the most consensual way to evaluate it is by asking how the subject rates the intensity of this experience. Unfortunately, as it is the case for any subjective experience, a means of communication is required. Communication might be impaired in many situations; transiently in physiological conditions, such as sleep or anesthesia, and prolonged or permanently in diseases such as neurodegenerative or consciousness disorders. Communication is intimately related to consciousness and therefore, when consciousness is impaired, little means exist to assess pain perception. To this aim, a few behavioral scales have been validated in specific non-communicative population such as neonates (the Neonatal Infant Pain Scale: NIPS; the Face, Legs, Activity, Cry, Consolability scale: FLACC) or demented patients (the Pain Assessment IN Advanced Dementia: PAINAD; the Checklist of Non-verbal Pain Indicators: CNPI). Those scales were used to develop a dedicated tool for patients with DoC: the Nociception Coma Scale (NCS) (Schnakers et al., 2010). The NCS aims to evaluate the behavioral responses observed in patients with DoC in response to an external noxious stimulus. The experimental noxious stimulus is the progressive pressure of the middle finger nailbed for five seconds, as this stimulus is widely implemented in different neurobehavioral scales to assess response to nociception (Giacino et al., 2004; Teasdale & Jennett, 1974). The initial version of the NCS comprised four subscales, evaluating motor, verbal, facial and visual responses. It showed a good concurrent validity with the above-mentioned scales (NIPS, FLACC, PAINAD and CNPI) and showed a good interrater agreement. Its specificity to pain (or at least nociception) was established in a validation study (Chatelle et al., 2012). In that study, the scores obtained in response to noxious stimuli (pressure on the nailbed) were compared to those in response to non-noxious and less salient stimuli (light touch on the shoulder). They were significantly different for total scores and for each subscale, except for the visual one. Interestingly, it was shown that discarding the visual subscale of the NCS increases its sensitivity, going from 46% to 73% for a cut-off score of 4 (i.e., a score allowing to distinguish a response to a noxious

from a non-noxious stimulus). Therefore, a new version of the scale was proposed, the Nociception Coma Scale – Revised (NCS-R; see Table 3.1) (Chatelle et al., 2012).

Hitherto, the NCS and NCS-R have been the subject of several studies aimed at characterizing the psychometric properties of the scale; identifying the neural correlates of the scale; and applying the scale to a clinical setting. This section reviews the literature on the topic and discuss investigations to undertake.

Table 3.1. The Nociception Coma Scale – Revised

	Motor response
3	Localization to painful stimulation
2	Flexion withdrawal
1	Abnormal posturing
0	None/flaccid

	Verbal response
3	Verbalization (intelligible)
2	Vocalization
1	Groaning
0	None

	Facial expression
3	Cry
2	Grimace
1	Oral reflexive movement
0	None

3.1.1. PSYCHOMETRIC PROPERTIES

Vink et al. (2017) performed a systematic review of the literature to evaluate the psychometric properties of the NCS (-R). Those properties encompasses reliability, validity and responsiveness (Mokkink et al., 2010), which, in the case of this scale, have

been studied with sufficient methodological quality. Among others, interrater agreement was mostly evaluated as excellent, while inter- and intra-rater agreement between different disciplines should be further investigated. Construct validity was difficult to evaluate as there is no gold-standard for the evaluation of pain in patients with DoC. It was also found that the NCS and the NCS-R are both valid and useful to evaluate nociception in patients with DoC. Main limitations were the small size samples, the lack of sample size calculations and the fact that almost all studies were performed by the same small groups of authors.

In the same systematic review, the authors also mentioned that a score below any given cut-off value is no guarantee for the absence of nociception. Moreover, any increase in NCS should give rise to assessment of possible discomfort, rather than waiting for a general threshold score to be reached. However, in the validation study of the scale (Chatelle et al., 2012), a cut-off score of 3 for UWS patients and 4 for MCS patients was first identified. On the other hand, two more recent studies showed a cut-off score of 2 with a high sensitivity and specificity, and without any difference between diagnosis of consciousness nor etiology, (Chatelle et al., 2018; Vink et al., 2014).

Although NCS (-R) was specifically designed for patients with DoC, its relation to the level of consciousness remains unclear. As the ability to perceive pain is presumably associated at some point with the level of consciousness, it may be difficult to disentangle both. Two studies found a correlation between CRS-R and NCS-R. The first one, a pilot study, showed that CRS-R and NCS-R were correlated at admission but failed to find a prognostic value at 6 months for the NCS-R (Bagnato et al., 2018). The second one, a large prospective multicentric study demonstrated the correlation between CRS-R total scores and NCS-R scores, showing that higher level of consciousness was associated with higher behavioral responsiveness to noxious stimuli (Chatelle et al., 2018). This correlation could be due to the overlap between the two scales, at least for motor and verbal subscales. The "facial expression" subscale is specific of NCS-R and its importance was highlighted by this study. Specifically, crying

and grimacing behaviors were more related to a noxious versus a non-noxious stimulation and more often observed in MCS than in UWS patients.

3.1.2. Neural correlates

Confidence put in a behavioral scale lies in its ability to correlate with an objective neurophysiological or neuroanatomical biomarker. The neural correlates of the NCS (-R) has been scarcely studied. Using FDG-PET imaging, a correlation between NCS-R scores and brain metabolism at rest in the posterior part of the ACC was shown, and, more interestingly, with no other region of interest (Chatelle et al., 2014). Importantly, metabolism of the ACC does not correlate with CRS-R total scores, suggesting that the NCS-R more closely reflects the process of nociceptive input (or pain experience) rather than the differences in patients' level of consciousness. As suggested by some authors, the posterior part of the ACC is implied in the cognitive-affective aspect of pain processing (Peyron et al., 2000). This region is considered to be a hub where information about negative reinforcers and pain can be linked to motor centers responsible for expressing affects and executing goal-directed behaviors (Shackman et al., 2011).

Some authors tried, using an EEG approach (further discussed in Chapter 4), to study the relationship between NCS-R scores and brain responses to specific nociceptive stimuli. Those studies suggested that the amplitude of those evoked brain responses (i.e., N2-P2 complex) did not correlate with the NCS-R scores (de Tommaso et al., 2015). A significant correlation between those parameters was found in a group of UWS patients from traumatic etiology (De Salvo et al., 2015). Unfortunately, the scarcity of data and potential methodological issues make it impossible to provide evidence of a relationship between the NCS-R scores and EEG biomarkers.

3.1.3. Applicability in a clinical setting

The NCS (-R) was first validated in an experimental setting, but it was first aimed to be used in a clinical setting. In this context, the validity, reliability and practicality of the NCS, as compared to other scales dedicated to non-communicative patients, were

assessed in a context of acute management following a craniotomy (Suraseranivongse et al., 2015). The three evaluated scales (FLACC, Revised-FLACC & NCS) showed a good validity and reliability, but the NCS was rated as the most practical for routine clinical practice. Moreover, the NCS and NCS-R showed both an excellent interrater reliability and were shown valid and reproducible. Importantly, they can be used by nurses regardless their experience with brain-injured patients (Vink et al., 2014). Given the amount of time that they spend with the patients, nurses have an important role regarding the clinical use of this scale. In a qualitative study, nurses agreed on the relevance of the content of the NCS-R and found the scale intuitively true based on their experience However, they felt a lack in the assessment of physiologic/autonomic signs of pain, as it is the case in the Pain Assessment Scale (PAS; Poulsen, Brix, Andersen, Westergaard, & Guldager, 2016). It should also be mentioned that some nurses felt uncomfortable to assess patients with the NCS-R due to the noxious stimuli with pressure on the nailbed, which is not consistent with their education (International Council of Nurses, 2012). Obviously, the use of this clinical tool should not cause more harm to the patient than benefit. In order to limit the discomfort caused by the procedure, Formisano et al. (2019) suggest the use of a personalized stimulus. This personalized stimulus is defined following the recording of all the maneuvers that induce motor/behavioral response and were reported by at least two different caregivers (among relatives, nurses, therapists and physicians). The use of a personalized stimulus showed, in this study, higher scores to the NCS-R, as compared to the experimental noxious stimulus, in 9 of 21 patients (42.8%) (Formisano et al., 2019).

In a clinical setting, patients with DoC are at high risk of medical complications that might be (potentially) painful (Ganesh et al., 2013; Whyte et al., 2013). Among them, spasticity is one of the most frequent. Indeed, according to the study of Thibaut et al. (2015), 61.5% of patients with DoC present a severe spasticity in at least one limb, as assessed with the modified Ashworth Scale (mAS) (a score ≥ 3, meaning at least a considerable increase in muscle tone with difficult passive movement). Interestingly, the NCS-R correlated positively with the mAS scores.

In the acute stage of a brain injury, in the intensive care unit, patients frequently face medical complications. For this reason, Chatelle et al. proposed to screen patients with a (potentially) painful condition, and to give them a dose of analgesics in the case of the patient displayed an NCS-R score ≥ 4 (Chatelle et al., 2016). In this study, a decrease in the NCS-R score was observed following the analgesic treatment, and importantly, the level of consciousness did not decrease, as assessed with the Glasgow Coma Scale (GCS). Even more surprising, the GCS increased in 25% of the patients, suggesting that the presence of pain may compromise the ability of the patient to display conscious behavior. This might be related to the interruptive function of pain, that direct attention towards the painful sensation and away from the task that the patient is asked to attend (Eccleston & Crombez, 1999). Due to the very low attentional span of patients with DoC, pain could easily prevent the patients from interacting with their surroundings or display any sign of consciousness.

3.1.4. LIMITATIONS

Although the NCS (-R) is a recent scale, it gains a lot of interest as being the only validated tool dedicated to the assessment of (potential) pain in patient with DoC. In order to add value to its use in a clinical context, a better understanding of the scale is required at different levels:

(1) The cut-off score aiming to identify a response to a nociceptive stimulus should be more clearly defined and/or replicated. This cut-off score appears to be low and its clinical significance/usefulness should be questioned as low scores can easily be achieved by purely reflexive behaviors, potentially limiting its interest in a clinical setting.

(2) The identification of strong neural correlates of the scale would increase the confidence in its use. A first step would be to determine the minimal NCS-R score that necessarily requires the preservation of brain regions involved in conscious pain processing.

(3) The applicability of the NCS-R seems feasible thanks to its ease of use, its good measurement properties and its minimal training requirement. However, the interpretability of the results has not yet been investigated with regard to medical

complications. Here, we will investigate the influence of a tracheostomy tube, one of the most common medical complication in those patients, on the NCS-R scores.

3.2. THE NCS-R TO IDENTIFY PATIENTS WITH PRESERVED NEURAL BASIS FOR PAIN EXPERIENCE

This section was adapted from the manuscript of Bonin et al. (2019)

One way to increase the confidence in the use of the NCS-R is to increase the knowledge on its neural correlates. To achieve this, differences were investigated in brain metabolism of UWS versus MCS patients with regards to their NCS-R responses. This would allow to identify brain regions necessarily involved in a conscious pain experience.

The NCS-R cut-off score of 2 (Chatelle et al., 2018), aiming to differentiate behavioral responses induced by noxious stimuli from those induced by non-noxious stimuli, is highly sensitive but not specific. With a neuroimaging-based approach, the possibility to determine a more specific cut-off score is investigated (i.e., a score above which the probability that the behavior results from a noxious stimulus is high).

For the clarity of the following sections, two groups of patients using the following criteria were defined:

1 - "FDG-PET confirmed UWS" group includes patients behaviorally diagnosed as UWS according to minimum five assessments to the CRS-R; and showing a global decrease of brain metabolism on FDG-PET based on visual examination of the FDG-PET Standardized Uptake Values (SUV). This global decrease consists, at least, in a severe bilateral hypometabolism of the associative frontoparietal cortex without preserved areas (Stender et al., 2014).

2 - "Patients with potential pain" group includes patients behaviorally diagnosed as UWS or MCS according to minimum five assessments to the CRS-R; and showing a preservation of brain metabolism on FDG-PET based on visual examination of the SUV images. Most importantly, those patients obtained a score

to the NCS-R that was greater than the highest score obtained in the *"FDG-PET confirmed UWS"* group (see below for details).

In this retrospective study, data from patients who underwent at least one NCS-R evaluation and an FDG-PET imaging were included. The study was then conducted in two successive steps:

(1) Using FDG-PET, the NCS-R score ranges obtained in the *"FDG-PET confirmed UWS"* group were investigated. The hypothesis was that patients with a global hypometabolism of the whole cortical area, including the network known to be involved in pain processing, cannot sustain conscious processing of potentially painful stimuli (Steven Laureys et al., 2002). Therefore, the highest behavioral responses observed in these *"FDG-PET confirmed UWS patients"* could be used as a conservative NCS-R cut-off score.

(2) Then, the global and local differences in brain metabolism between *"FDG-PET confirmed UWS",* "*Patients with potential pain"* and "*Healthy controls*" were considered. The hypothesis was that patients with perception of pain would have a minimal cortical preservation, particularly in areas involved in pain processing (i.e., ACC and insula (Boly et al., 2008)), as compared with patients without perception of pain.

3.2.1. MATERIAL AND METHODS

Using FDG-PET, we investigated the NCS-R score ranges obtained in *"FDG-PET confirmed UWS patients"*.

NCS-R scores were recorded at rest, following experimental noxious stimulation (deep pressure on the left and right nailbed of the middle finger for five seconds (Schnakers et al., 2010)) but also, to capture the patient's highest possible nociceptive response, they were considered during mobilization (passive mobilization of the upper and lower limbs during a physiotherapy session - stretching). If mobilization is not comparable to an experimental noxious stimulus, several studies reported that mobilization (i.e., care or physiotherapy) could be potentially painful for patients with brain injury (Formisano et al., 2019; Gélinas et al., 2017, 2019). The NCS-R was performed the same day as, but before FDG-PET.

FDG-PET was performed at rest after intravenous injection of 5 to 10 mCi (185-370 MBq) FDG on a Gemini Big Bore PET/CT scanner (Philips Medical Systems, Best, Netherlands). Data were spatially normalized to a stereotaxic space and smoothed using a 14 mm full width at half maximum Gaussian kernel. To overcome the problem of big deformations due to brain lesions as well as the fact that SPM has a default template based on $H_2{}^{15}O$ data, the normalization was performed using a customized template using the procedure as described in (Phillips et al., 2011).

We used the FDG-PET SUV to approximate the cerebral metabolic rate of glucose consumption at single subject level: $SUV = \frac{(Decay\ corrected\ Voxel\ Intensity)}{\frac{Injected\ Dose}{Body\ Weight}}$.

3.2.2. RESULTS

A- ALLOCATION TO THE DIFFERENT GROUPS

- For the *"FDG-PET confirmed UWS"* group, the 209 patients included in the database were screened, and among the 50 behaviorally diagnosed as UWS patients, 13 patients that fulfilled all the above mentioned requirements were included (5 female and 8 male, age range: 27-73 years, etiology: traumatic [n=1], post-anoxic [n=7], subarachnoid hemorrhage [n=1], stroke [n=2] and mixed etiology [n=2]). The SUV images were used to select FDG-PET confirmed UWS patients through visual observation by three different expert examiners (neuropsychologists and physiotherapist working with patients with DoC and PET imaging for at least five years), all blind to the clinical diagnosis and other examiners' assessment. Each expert provided a diagnosis based on two illustrative examples of UWS and MCS (Figure 3.1) Full agreement was observed for typical UWS (Figure 3.2; flow diagram is available in Appendix A, Figure A.1)
- The *"Patients with potential pain"* group was created after the first step of the study, in which the threshold score was set. The 13 patients included in this group were matched with the 13 patients in the *"FDG-PET confirmed UWS"* group according to gender, age and etiology, provided that they displayed NCS-R score ≥ to the threshold

score identified in the *"FDG-PET confirmed UWS"*. Of the 13 patients with *potential pain*, 10 were MCS and 3 were unresponsive at bedside (i.e., MCS* = UWS patients with atypical cortical metabolism preservation (Gosseries et al., 2014)).

- A third group named *"Healthy controls"* included healthy subjects that were matched according to gender and age.

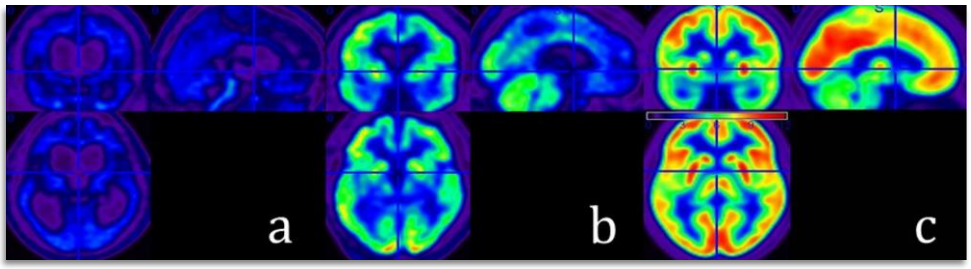


Figure 3.1. PET-FDG brain metabolism in (a) UWS patient with a global hypometabolism, (b) MCS patient and (c) healthy control. The scale represents the cerebral metabolic rate of glucose (CMRglc; µmoL/g per minute) from 0 (blue) to 12 (red). Figure adapted from Bonin et al. (2019).

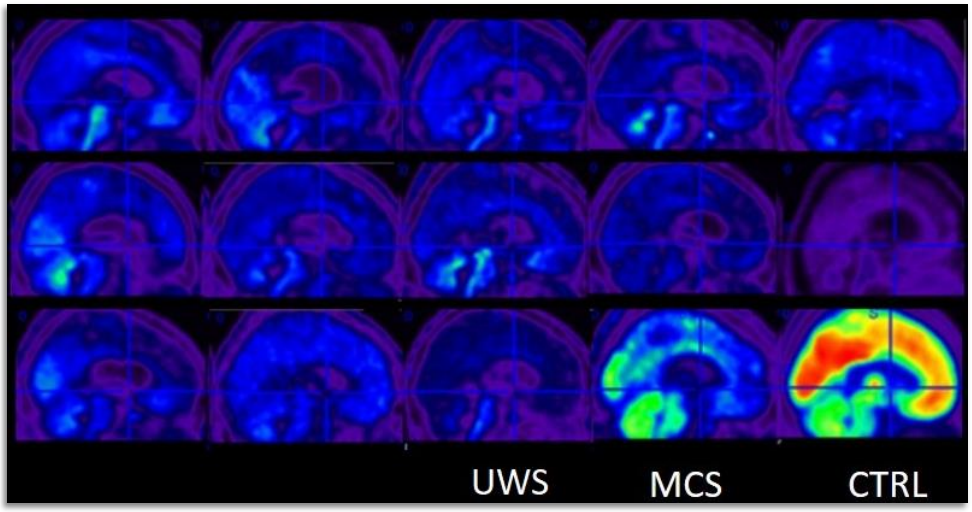


Figure 3.2. PET-FDG showing the brain metabolism of the 13 well-documented UWS selected for the study, from the left to the right. For comparison and illustrative purpose, a PET-FDG image of one MCS (Minimally Conscious State) patient and CTRL (Healthy subjects) on the rightest part of the lower row. Figure adapted from Bonin et al. (2019).

B - DETERMINING THE CUT-OFF SCORE

When looking at the range of NCS-R total scores obtained in the *"FDG-PET confirmed UWS",* scores between 0 and 4 were found after (potentially) painful conditions (i.e., noxious stimulation and/or mobilization). Therefore, the cut-off was set to ≥ 5.

C - BRAIN METABOLISM AND CUT-OFF SCORE

To investigate whether this cut-off score (i.e., NCS-R scores ≥ 5) can be used to support preserved neural basis for pain experience, we investigated the differences between groups in global and regional brain metabolism.

Global brain metabolism differences between the three groups was evaluated with the calculated global mean value. As the data was not normally distributed according to Shapiro-Wilk tests ($W < 1$, $p = 0.012$) and the sample size was small ($n < 30$), a non-parametric test of Kruskal-Wallis was used to compare global metabolism between the three groups. A higher global metabolism was found in *"Healthy controls"* compared to the group of *"FDG-PET confirmed UWS"* and the group of *"Patients with potential pain"* ($\chi^2 = 33.80$; $df = 2$; $p < .0001$), and in *"Patients with potential pain"* compared to *"FDG-PET confirmed UWS"* ($\chi^2 = 33.80$; $df = 2$; $p<.0001$; Figure 3.3).

Regional differences in brain metabolism were investigated using a design matrix including the same three groups. Brain regions with preserved metabolism were identified in the group of *"Patients with potential pain"* versus *"FDG-PET confirmed UWS patients"* and in *"Patients with potential pain"* versus *"Healthy controls"*. Brain regions with decreased metabolism were also identified in the group of *"Patients with potential pain"* versus *"Healthy controls"* and in *"FDG-PET confirmed UWS patients"* versus "*Healthy controls"*. Global normalization was performed by proportional scaling. Significant threshold of results was determined at $p < 0.05$ corrected for multiple comparisons within *a priori* defined regions of interest (using a 10 mm radius spherical small volume correction in SPM at voxel and cluster level) centered on *a priori* coordinates for areas previously identified as the most frequently identified in pain processing (i.e., ACC and bilateral insula were analyzed separately; respective MNI [Montreal Neurological

Institute] coordinates x = 12, y = 10, z =36; x = −34, y = −24, z = 36; and x = 34, y = −24, z = 36, were taken from two previous studies on pain perception in patients with DoC (Boly et al., 2008; Chatelle et al., 2014)). Locally, a preservation in brain metabolism was observed only in the left insula in *"Patients with potential pain"* compared to *"FDG-PET confirmed UWS"* (Z = 3.31; corrected p = 0.016; MNI coordinates x = - 38, y = -20, z = 44). This preservation was also observed in the group of *"patients with potential pain"* when compared to *"Healthy controls"* (Z = 5.95; corrected p = 0.004; MNI coordinates x = -32, y = -32, z = 40). No preservation in brain metabolism in the right insula was observed. In the ACC, a hypometabolism was observed in *"FDG-PET confirmed UWS"* as well as in *"Patients with potential pain"*, as compared to *"Healthy controls"*.

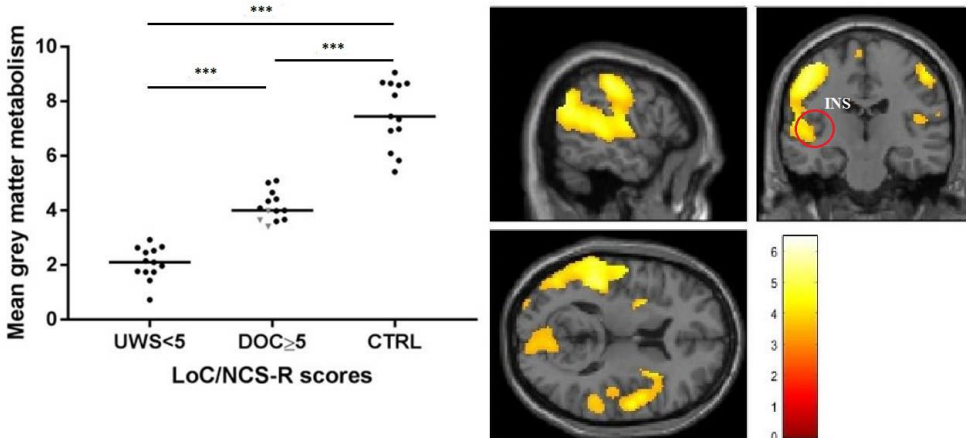


Figure 3.3. Left: Global brain metabolism preservation in *"FDG-PET confirmed unresponsive patients (UWS)"* with NCS-R score < 5, *"Patients with potential pain"* (i.e., patients with DoC and an NCS-R score ≥ 5) and *"Healthy controls"* (CTRL; grey triangles represent patients behaviorally diagnosed as UWS, Kruskal-Wallis, *** = p < .0001). Right: Regional brain metabolism preservation in *"Patients with potential pain"* compared to *"FDG-PET confirmed UWS"*. Preservation of the brain metabolism was observed in the left insula (INS, x = -55mm, y = -20mm, z = 12mm). Figure adapted from Bonin et al. (2019).

Regional brain metabolism preservation in three patients MCS* (i.e., behaviorally diagnosed in UWS but with an atypical cortical metabolism preservation

(Gosseries et al., 2014) who showed potential pain (i.e., a score ≥ 5)) were looked *a posteriori* at a single subject level, using the same approach as described above (compared with 33 healthy controls, 18 men, mean age 43 ± 15 years). A preservation in brain metabolism was observed in the insula bilaterally in all three patients, and in the ACC in two patients (see Figure 3.4)

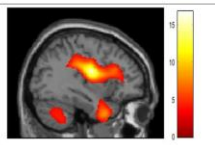
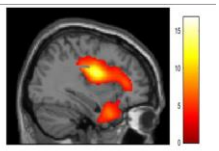
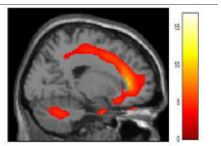
Region of interest	X (mm)	Y (mm)	Z (mm)	Z-value	P-value	
MCS*1 - preserved						
Left insula	-36	-20	28	6.65	0.002	
Right insula	36	-20	28	6.44	.003	
Anterior cingulate cortex	20	10	42	4.63	0.008	

Figure 3.4. *Illustration of the results for one MCS* patient*. Coordinates of peak voxels (in standardized stereotaxic MNI space) showing preserved metabolism in MCS* patients with a NCS-R score ≥ 5 compared to healthy controls (using a 10-mm radius spherical small volume correction in SPM - at voxel and cluster level, centered on *a priori* coordinates for areas previously identified as the most frequently identified in pain processing; i.e., ACC and bilateral insula; respective coordinates x = 12, y = 10, z =36; x = −34, y = −24, z = 36; and x = 34, y = −24, z = 36). P value corrected for multiple comparisons at cluster level. Figure adapted from Bonin et al. (2019).

D - SENSITIVITY AND SPECIFICITY

Sensitivity (i.e., proportion of patients who have received noxious stimulation and have an NCS-R score equal or above the threshold of 5) and *specificity* (i.e., proportion

of patients who have not received noxious stimulation and have a NCS-R score below the threshold of 5) of the defined threshold (i.e., NCS-R scores ≥ 5) were calculated for all MCS patients assessed with the NCS-R from 2011 to 2017 in the database, in order to determine the number of patients that may be underestimated by this threshold. For clinical interest, we also calculated sensitivity and specificity using the scores below that threshold.

When accounting for all MCS patients assessed with the NCS-R during an experimental noxious stimulation (n=65), we found a specificity of 98.4% (True Negative [TN] = 64/65, False Positive [FP] = 1/65) and a sensitivity of 16.9% (True Positive [TP] = 11/65, False Negative [FN] = 54/65) for detecting potential pain in this population with the NCS-R cut-off score ≥ 5.

When assessed for other NCS-R scores as cut-off, specificity and sensitivity were found to be the highest when the cut-off score was set to 4. Indeed, it allowed to obtain a specificity of 98.4% (TN = 64/65, FP = 1/65) and a sensitivity of 35.4% (TP = 23/65, FN = 42/65), meaning the same specificity and a higher sensitivity than a cut-off score set to 5 (for detailed results, see Figure 3.5).

3.2.3. DISCUSSION

The aim of this retrospective study was to determine a cut-off score at the NCS-R supporting preserved neural basis for pain experience, based on global and local brain metabolic activity.

As a first step, the NCS-R score ranges obtained in *"FDG-PET confirmed UWS patients"* were investigated (i.e., patients with critically low cortical metabolic activity, no access to consciousness and therefore no access to conscious process of pain). According to the results, the highest behavioral responses observed during noxious stimulation and mobilization were associated with an NCS-R total score of 4. These results suggest that a score ≥ 5 probably requires a certain degree of cortical integration of painful stimuli and therefore a potential experience of pain. Therefore, the conservative NCS-R cut-off score was set at 5, suggesting that careful attention should be given to treat with analgesics patients with score ≥ 5.

a)

	Specificity	Sensitivity
score ≥ 2	73,8	73,8
score ≥ 3	90,8	55,4
score ≥ 4	98.4	35,4
score ≥ 5	98.4	16.9

score ≥ 2	Noxious stimulation	At rest
Pain	48	17
No pain	17	48

score ≥ 3	Noxious stimulation	At rest
Pain	36	6
No pain	29	59

b)

	Noxious stimulation	At rest
Pain	True positive (TP)	False positive (FP)
No pain	False negative (FN)	True negative (TN)

score ≥ 4	Noxious stimulation	At rest
Pain	23	1
No pain	42	64

score ≥ 5	Noxious stimulation	At rest
Pain	11	1
No pain	54	64

Figure 3.5. Sensitivity and specificity of different NCS-R cut-off scores. The sensitivity and specificity of each cut-off scores are represented in a), confusion matrix is represented in b). Columns represent scores following noxious stimulations and at rest, respectively. Rows classify "patients with NCS-R score ≥ examined cut-off score" in "Pain" row, and "patients with NCS-R scores < cut-off score" in "No pain" row. Figure adapted from Bonin et al. (2019).

As a second step, brain metabolism at a global and a regional level were compared between the groups. *Global brain metabolism* comparison showed that *"Patients with potential pain"* had a significantly higher global metabolism than the *"FDG-PET confirmed UWS"* group. This agrees with previous findings on reduced metabolism in UWS as compared with MCS (Stender et al., 2016). When looking for *regional differences in brain metabolism* in two areas known to be involved in pain processing, the findings were as follows:

- In the insula, a preservation only in the left one was found in *"Patients with potential pain"* compared to patients with *"FDG-PET confirmed UWS"* and *"Healthy controls"* (Stender et al., 2015). Studies have suggested that this region could be involved in the affective dimension of pain by playing a mediating role between its posterior part (lateral system) and the rostral part of the ACC

(median system) (Coghill et al., 1999; Peyron et al., 2002). While some studies support a stronger involvement of the right hemisphere in pain sensation (Ostrowsky et al., 2002; Vogt et al., 1996), several of them have also shown a bilateral activation of the insula during a noxious stimulation (J. C. W. Brooks et al., 2002; Symonds et al., 2006).

- In the ACC, however, an hypometabolism was observed in *"Patients with potential pain"* and in *"FDG-PET confirmed UWS"* patients when compared to *"Healthy controls"*. In neuroimaging studies on pain processing, the ACC is a region commonly associated to pain. This region, most particularly the rostral part of the ACC, seems to be key for the affective dimension of pain processing (Youell et al., 2004). Neuroimaging studies have shown that an increase of brain activity in the ACC is correlated with an increase of pain unpleasantness (Ingvar, 1999; Rainville et al., 1997). Other studies demonstrate an activation of this region after a noxious stimulation in patients with MCS but also in some patients with UWS (Boly et al., 2008; Markl et al., 2013).

While these results may be due to the MCS patients included in the *"Patients with potential pain"* group (i.e., higher brain metabolism in MCS than UWS (Stender et al., 2016)), single subject analysis of the three MCS* patients with potential pain supports the idea that these patients also had a preserved brain metabolism in areas involved in pain processing (i.e., ACC and insula). This also suggests that the score of ≥ 5 could be used as a red flag for pain processing, even in patients behaviorally diagnosed in UWS and by extension could evoke the presence of covert cognitive abilities in these patients (Kassubek et al., 2003). Nevertheless, a threshold of 5, despite its significant advantage to be specific for a cortical process of pain and usable independent of the clinical diagnosis of the patient, has the disadvantage of leading to a large lack of sensitivity.

LIMITATIONS

These findings must be interpreted with regard of several limitations:

- The heterogeneity of the population (i.e., more anoxic patients in UWS than in MCS and time since injury);
- This is a retrospective study, so confounding factors such as motor abilities (within the two patient groups [n = 26], 18 of them showed moderate to high spasticity), intensity of the noxious stimulation or heterogeneity in threshold for pain in each patient might have influenced the results. Future studies should aim to control these factors as well as the stimulus intensity using, for instance, a Newton-meter as previously done in (Chatelle et al., 2012; Schnakers et al., 2010).
- The use of neuroimaging for patients with severe brain damage to target regions of interest may be challenging but also can be of limited interpretation due to normalization and smoothing issues. Indeed, following severe brain injury, patients with DoC may have serious brain damage such as widening of the ventricles or hydrocephalus. Hence, when neuroimaging is performed on severely damaged brains, it is sometimes difficult to target the regions of interest. Some areas like the insula or the ACC may be damaged or "shifted" from a healthy brain template making group analyses complex. In those analyses, a customized template built with patients and healthy subjects scans was used to limit the effect of these deformations.
- Only resting state brain metabolism data were analyzed, which is less sensitive and relevant than activation studies regarding pain processing which could also account for differences with some previously mentioned studies (e.g., painful stimulus activated the thalamus, the primary, the secondary somatosensory cortices and the fronto-parietal cortices (Boly et al., 2008; Steven Laureys et al., 2002)).
- Although the scores of 2, 3 and 4 showed better sensitivity results (4 and 5 showed the same specificity and close sensitivity values), the score of 5 is defined as a conservative threshold as it was shown that the *"FDG-PET confirmed UWS"* patients are able to have scores of 4 at the NCS-R. However, clinicians should use this threshold with caution as a better sensitivity was observed with lower scores (4, 3, 2, as also shown in another study (Chatelle et al., 2018)).

- FDG-PET during resting state and behavioral assessment can be influenced by the level of arousal of the patient and, even if the patient was maintained as awake as possible during these evaluations, awakening can fluctuate in some patients, influencing the result (Guenther et al., 2011; Monti, Vanhaudenhuyse, et al., 2010).
- One could also argue that one of the conditions used may not be nociceptive (i.e., mobilization). However, the first aim was to determine the highest score observable in *"FDG-PET confirmed UWS",* and as mobilization can be potentially painful (especially in this population suffering from various physical pathologies such as spasticity (Thibaut et al., 2015)), it is relevant to use such data in this study. The fact that some of the patients included showed higher scores during mobilization than during experimental pain supports this approach.
- The ability to perceive pain of these patients with DoC (and NCS-R score ≥ 5 cannot be inferred from brain metabolism preservation as brain metabolism preservation is used as evidence of the prerequisite to perceive pain (circular reasoning).

SUMMARY

Despite these limitations, behavioral (i.e., cut-off score ≥ 5) and neuroimaging results (i.e., higher global brain metabolism and better preservation of the metabolism in the left insula) suggest that patients with an NCS-R score ≥ 5 are potentially able to perceive pain or have at least the neural basis for experiencing it. However, this cut-off score must be interpreted with caution given its low sensitivity while being highly specific. Indeed, 74% of patients with MCS displayed a score below this threshold following an experimental noxious procedure. If some probably have impairment in pain processing, it is also likely that for others the NCS-R failed to detect potential painful stimuli due, for instance, to motor issues.

In summary, this retrospective study (1) provides the determination of a cut-off score of 5 at the NCS-R that permits the identification of patients with preserved neural basis for pain processing, (2) supports our hypothesis whereby behavioral responsiveness to pain necessitates a minimal preservation in regions involved in pain processing (left insula and ACC) in patients with DoC.

3.3. THE NCS-R IN PATIENTS WITH A TRACHEOSTOMY

Among medical complications occurring in patients with DoC, the presence of a tracheostomy is frequent. In those patients, because of the lack of an effective swallowing reflex, placement of a cuffed tracheostomy tube is often required to prevent the occurrence of aspiration pneumonia. A tracheostomy tube is basically a tube bypassing the vocal cords and, therefore, does not allow to emit any sound because of the lack of airflow passing by the vocal cords. As the verbal subscale of the NCS-R is dedicated to items involving the emission of sounds by the patients, it is suspected that the presence of a tracheostomy results in lower scores at the verbal subscale for patients with a tracheostomy and has an impact on the total score of the scale.

3.3.1. MATERIAL AND METHODS

In this retrospective study were included patients recruited from 2011 to 2017, diagnosed with UWS, MCS or eMCS without functional communication, based on behavioral assessment performed using the CRS-R (Giacino et al., 2004) and free from neuromuscular blockers or sedation 24 hours previous to enrollment. They had no past medical history of brain injury, developmental, psychiatric or neurologic illness resulting in documented functional disability up to time of the injury; they had also no upper limb contusions, fractures or flaccid paralysis. For the assessment of the patients, the NCS-R was administered in two different conditions: at baseline and in noxious condition. During baseline, we observed the patients' spontaneous behaviors for 60 seconds (Giacino et al., 2004). During the noxious condition, pressure was applied on the nailbed of the middle finger of the right and left hand (Giacino et al., 2004; Teasdale & Jennett, 1974) for a minimum of five seconds and stopped as soon as a behavioral response was observed. Behavioral responses were recorded ten seconds after each stimulation (Giacino et al., 2004). The highest score obtained across right and left side stimulation was considered. To ensure a sufficient level of arousal, each condition was administered while patients showed spontaneous eye opening. The entire

procedure lasted less than five minutes. Level of consciousness was assessed before or after this procedure with the CRS-R (Kalmar & Giacino, 2005).

3.3.2. RESULTS

A - DIFFERENCES ON THE VERBAL AND TOTAL SCORES BETWEEN PATIENTS WITH OR WITHOUT TRACHEOSTOMY

As a first step of the study, 65 patients were included (25 UWS, 35 MCS and 5 eMCS) for which the information on the presence or absence of a tracheostomy was available. 28 of them had tracheostomy (43%; UWS [61%], MCS [32%], eMCS [7%]). Mean age of the population was 42±13 years (time since injury was 1198±1495 days).

Differences in NCS-R subscores and total scores in response to noxious stimulation between patients with and without a tracheostomy tube were investigated using U Mann-Whitney tests. Statistical significance was set at $p<0.05$ (one-tailed). Statistically significant lower scores were observed on the NCS-R verbal subscores ($p = 0.002$) and total scores ($p = 0.039$) in the tracheostomy group compared to the group without tracheostomy in response to noxious stimulation (Figure 3.6). Moreover, there was no difference in facial ($p=0.241$) and motor subscores ($p=0.967$) between both groups.

B - INFLUENCE OF THE PRESENCE OF A TRACHEOSTOMY ON THE CUT-OFF SCORE

For the second step, we included any patient who underwent NCS-R assessments in our database. There were 125 patients with DoC (46 UWS [36,5%], 74 MCS [59,5%], 5 eMCS [4%]; mean age: 46±16 years; time since injury: 817±1280 days) assessed with the NCS-R. Receiver operating characteristic (ROC) analyses were used to determine the best cut-off score allowing to differentiate the noxious condition versus baseline using the NCS-R total scores and the sum of both the motor and facial expression subscales excluding the verbal one (see Figure 3.7).

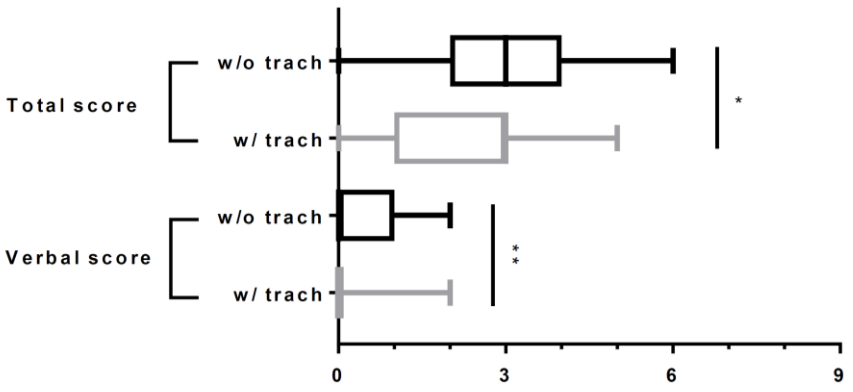


Figure 3.6. Median (and IQR) of NCS-R total scores (upper rows) and verbal subscores (lower rows) in response to noxious stimuli, in subgroups of patients without tracheostomy (n=37) (on the graph "w/o trach") or with a tracheostomy (n=28) (on the graph "w/ trach"). *one-tailed $p<0.05$ for total scores and **one-tailed $p= 0.01$ for verbal subscores).

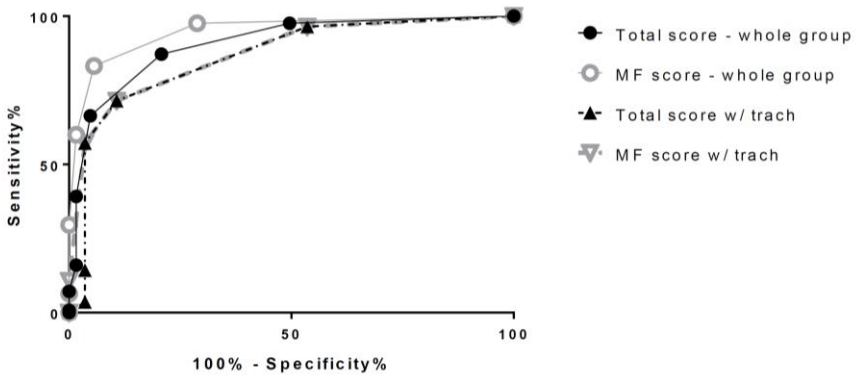


Figure 3.7. ROC curves representing the relation between sensitivity and specificity for the different potential threshold scores allowing to discriminate an NCS-R score at baseline (i.e., resting scores) and a NCS-R score after noxious stimulations using different scores (i.e., NCS-R total scores or total score without the verbal subscore [MF]) or population (i.e., the whole sample [n=125] and the subgroup of patients with tracheostomy [n=28]). Area Under Curve (AUC) values and values of each point of the curves are presented in Table 3.2.

The best cut-off score was determined by the method of adding sensitivity to specificity and keeping the cut-off score with the highest total, as described in (Krzanowski & Hand, 2009). According to this methodology, the ROC analyses reported a score of 2 or higher as the best threshold for nociception with a sensitivity of 87.2% and a specificity of 79.2% for differentiating noxious condition from baseline (Figure 3.6; Table 3.2). Removing the verbal subscale (MF score) did not modify the cut-off, with a score of 2 associated with a sensitivity of 83.2% and a specificity of 92.4% (Figure 3.6; Table 3.2).

Table 3.2. Values of sensitivity and specificity of the different cut-off scores.

NCS-R total - whole group

AUC	0,9057	
Cutoff	*Sensitivity%*	*Specificity%*
≥ 1	97,6	50,4
≥ 2	**87,2**	**79,2**
≥ 3	66,4	95,2
≥ 4	39,2	98,4
≥ 5	16	98,4
≥ 6	7,2	100
≥ 7	0,8	100

NCS-R total w/ trach

AUC	0,8622	
Cutoff	*Sensitivity%*	*Specificity%*
≥ 1	96,43	46,43
≥ 2	**71,43**	**89,29**
≥ 3	57,14	96,43
≥ 4	14,29	96,43
≥ 5	3,571	96,43

NCS-R (MF) - whole group

AUC	0,949	
Cutoff	*Sensitivity%*	*Specificity%*
≥ 1	97,6	71,2
≥ 2	**83,2**	**94,4**
≥ 3	60	98,4
≥ 4	29,6	100
≥ 5	6,4	100

NCS-R (MF) w/ trach

AUC	0,8737	
Cutoff	*Sensitivity%*	*Specificity%*
≥ 1	96,43	46,43
≥ 2	**71,43**	**89,29**
≥ 3	57,14	96,43
≥ 4	10,71	100

Note: cut-off values allowing to discriminate NCS-R scores at baseline from NCS-R scores measured after a noxious stimulus. The AUC and the best threshold identified are highlighted in light grey. AUC = Area Under Curve; MF = Motor + Facial Expressions subscores of the NCS-R.

Then, those analyses were performed on a subset of 28 patients with tracheostomy. This led to the same cut-off score of 2 at the NCS-R total score, with a lower sensitivity (71.43%) and specificity (89.29%). Removing the verbal subscale led to the exact same results (Figure 3.6; Table 3.2).

3.3.3. DISCUSSION

This study suggests that the presence of a tracheostomy is associated with a lower score on the verbal subscale, resulting also in a lower total score. Patients with tracheostomy tended to display similar pattern of responses at the motor and facial subscales of the NCS-R when compared to non-tracheostomized patients. These findings are in line with the proposed hypothesis that the NCS-R scores are mainly influenced by the presence of a tracheostomy itself.

The previously reported cut-off score of 2 (Chatelle et al., 2018) allowing to differentiate behaviors in response to noxious versus non-noxious stimulation was replicated. Most importantly, this cut-off score remained the same after removal of the verbal subscore or when focusing on a subgroup of patients with tracheostomy.

In the future, it could be of interest to compare three groups of patients: a first group including patients without tracheostomy, a second including patients with a non-speaking tracheostomy and a third including patients with a speaking tracheostomy. The hypothesis is that the group with a speaking tracheostomy would have no significant different score when compared to the group without a tracheostomy, but significantly higher scores when compared to patients with a non-speaking tracheostomy. It would help to conclude that lower scores observed with a non-speaking tracheostomy are solely due to the medical device itself.

In addition, future studies should use a Newton-meter to control stimulus intensity and limit interrater variability on the amount of pressure administered during noxious condition.

The significance of the cut-off score of 2 should also be further investigated, as the score of 2 can easily be reached with only reflexive behaviors (i.e., flexion withdrawal), limiting its interest in a clinical setting to identify a (potentially) painful condition.

In conclusion, this study highlights the fact that the presence of a tracheostomy still allows the use of the NCS-R. However, the presence of a non-speaking tracheostomy should be clearly mentioned when assessing patients with the NCS-R as it clearly lowers the verbal subscore. In addition, an increase of the NCS-R scores, even if low, should be cautiously interpreted, especially in those patients.

3.4. CONCLUSION

The studies in this chapter aimed to improve the characterization of the Nociception Coma Scale – Revised in three domains: its measurement properties, neural correlates, and applicability in a clinical setting. These studies encountered their objectives as so:

- The previously defined cut-off score of 2 was replicated. This score aims, with a high sensitivity, to disentangle behavioral responses from non-noxious versus noxious stimulations. Unfortunately, because this score can easily be achieved by purely reflexive behaviors, its clinical significance/usefulness should be questioned.

Most importantly, a very specific and conservative cut-off score of 5 or more was identified. Hence, an NCS-R score ≥ 5 occurs almost always and only following a noxious stimulation. However, the drawback of this very specific score is a high rate of false negatives, meaning that a lot of patients that received a noxious stimulation are not identified using this cut-off score.

- Regarding the neural correlates of the NCS-R, we found that patients with scores ≥ 5 have a metabolic preservation of the left insula (and the ACC). These brain regions are involved in the cognitive-affective experience of pain. In other words, this means that patients with NCS-R scores ≥ 5 have the preserved neural basis for pain experience.
- Additionally, we found that no UWS patient display an NCS-R score ≥ 5. This means that a patient with such high score is probably "at least" in an MCS*.

- The use of the NCS-R was validated in the frequent clinical context of patients with a tracheostomy. Although the presence of a tracheostomy lowered significantly the verbal subscale and total scores, it does not impact the cut-off score.

LIMITATIONS

These studies suffer from several limitations that should be accounted for future experimental design:

- The intensity of noxious stimulations should be controlled using a Newton-meter to limit the interrater variability on the amount of pressure administered.
- The noxious stimuli (i.e., nailbed pressure) used in these studies are not nociceptive specific as they inevitably also activate low-threshold mechanoreceptors. Using specific stimuli would allow to characterize the relation between the NCS-R and the activation of specific somatosensory pathways.
- Studies using resting paradigms have a limited interest to study the phasic responses to noxious stimuli. An approach using a high time-resolution technique such as EEG would be interesting.
- Using neuroimaging techniques such as PET or fMRI, data analysis can be challenging in those severely brain injured patients due to normalization and smoothing issues, making it difficult to target precisely the regions of interest.
- The population of patients with DoC is very heterogeneous (etiology, topography of brain lesions, age, level of consciousness) but also limited in number, making it difficult to generalize results.
- The presence of medical complications is frequent in this population and might represent confounding factors in the study of pain process in those patients.
- The interest of adding other parameters should be questioned, such as autonomic responses (heart rate variability, skin conductance, pupillary response,…). However, the challenge is to identify whether these parameters vary in response to nociception or pain(Constant & Sabourdin, 2015; Riganello et al., 2019).

- Finally, the risk of circular reasoning should be strictly assessed, although this might be challenging in this specific context of pain assessment in patients with impaired consciousness.

CHAPTER 3: BEHAVIORAL ASSESSMENT
SUMMARY OF FINDINGS

A. TWO CUT-OFF SCORES WERE IDENTIFIED FOR THE NCS-R:
   1. NCS-R < 2: BEHAVIORAL PATTERN NOT RELATED TO A NOXIOUS STIMULUS.
   2. NCS-R ≥ 5: REQUIRES A CORTICAL PROCESS OF NOXIOUS STIMULATIONS.

B. PATIENTS WITH NCS-R SCORES ≥ 5 DISPLAY A PRESERVATION OF METABOLIC ACTIVITY IN, AT LEAST, THE LEFT INSULA (AND THE ANTERIOR CINGULATE CORTEX).

C. RED FLAG: A PATIENT DISPLAYING AN NCS-R SCORE ≥ 5
   1. PROBABLY HAS THE NEURAL BASIS FOR EXPERIENCING PAIN.
   2. PROBABLY IS AT LEAST IN AN MCS*.

D. THE PRESENCE OF A TRACHEOSTOMY INFLUENCES, BUT STILL ALLOWS THE USE OF, THE NCS-R.

LIMITATIONS

A. NOXIOUS STIMULATIONS SHOULD BE STANDARDIZED AND CONTROLLED FOR THEIR INTENSITY AND THEIR SELECTIVITY FOR NOCICEPTION.

B. SEVERE MOTOR IMPAIRMENT AND AGITATION PROBABLY INFLUENCE THE NCS-R SCORES. THEREFORE, VERY LOW OR VERY HIGH NCS-R SCORES SHOULD BE CAUTIOUSLY INTERPRETED.

C. THE HETEROGENEITY OF THE PATIENTS MAKE DIFFICULT THE GENERALIZATION OF THE RESULTS.

CHAPTER 4. NEUROPHYSIOLOGICAL ASSESSMENT

"The art of life is the art of avoiding pain"

- Thomas Jefferson

This chapter is based on:

Lejeune, N., Chatelle, C., Laureys, S. & Mouraux, A. Cold evoked potentials in patients with disorders of consciousness: a new bedside approach to probe spino-thalamic pathways in non-communicative patients. *22th Congress of the Association for the Scientific Study of Consciousness (Krakow, Poland, 2018)*

Mulders, D., De Bodt, C., Lejeune, N., Courtin, A., Liberati, G., Verleysen, M. & Mouraux, A. (2020). Dynamics of the perception and EEG signals triggered by tonic warm and cool stimulation. *PLOS One; in press*

Lejeune, N., Petrossova, E., Dufour, A., & Mouraux, A. High-speed heating of the skin using a contact thermode elicits comparable brain responses to those elicited by a CO_2-laser device *(in prep)*

4.1. BACKGROUND: EEG RESPONSES TO NOCICEPTIVE INPUTS IN DOC

Recently, it has been demonstrated that some patients, while considered unresponsive (i.e., UWS/VS) at bedside, could actually display a willful modulation of neurophysiological signals as assessed by fMRI (Monti, Vanhaudenhuyse, et al., 2010; Owen et al., 2006) or by an EEG-based brain-computer interface (Cruse et al., 2011; Luauté et al., 2015). Those patients with residual conscious awareness without motor control are referred to as patients with a "Cognitive Motor Dissociation" (Schiff, 2015). Since these findings, it has become clear that the absence of (behavioral) evidence of consciousness does not equal to the evidence of its absence.

As for conscious awareness, a similar axiom can be formulated regarding pain experience among patients with DoC: the absence of behavioral response to a noxious stimulus does not equal to the absence of a conscious pain experience (and the absence of a conscious pain experience does not imply an absence of behavioral response to a noxious stimulus). Therefore, we cannot rely solely on behavioral assessments to evaluate the ability of patients to experience pain. Hence, by directly recording the brain electrical activity, EEG could constitute an interesting means to assess this ability. This section will review investigations already done by means of EEG to this purpose, highlight the limitations of these studies and suggest means to overcome them.

4.1.1. RECORDING OF LASER-EVOKED POTENTIALS

To our knowledge, De Tommaso et al. were the first to report EEG brain responses related to the selective activation of nociceptors in patients with DoC (de Tommaso et al., 2013). They used a CO_2-laser to generate 30 ms pulses, repeated 40 times on the hand dorsum. The output power of the laser beam was set to 9 W according to a pre-analysis in healthy subjects and was therefore not controlled for temperature. In this seminal study, 7 patients with DoC (3 UWS, 3 MCS and 1 eMCS) were assessed and all displayed a response suggestive of the N2-P2 complex that can be recorded in

healthy subjects. This N2-P2 complex displayed a greater latency than in healthy controls but a consistent topography.

In order to understand if the brain responses were more related to the salience of the stimulus rather than its nociceptive characteristic, the same group replicated a study of Mouraux & Iannetti (2009) in a set of 9 patients with DoC (5 UWS and 4 MCS). They recorded the brain activity evoked in response to randomly delivered stimuli from different sensory modalities (namely auditory, visual, non-noxious somatosensory and noxious laser stimuli). The presence of an N2-P2 complex was systematic in response to noxious laser stimuli. One UWS patient did not show any response belonging to the other sensory modalities, while only one MCS patient showed a response for each modality. The amplitude of the N2 and P2 waves were not correlated with the level of consciousness assessed with the CRS-R nor with the NCS-R scores. N2-P2 latencies were greater among DoC patients than among healthy controls (N2: 188-377 ms; P2: 285-469 ms; mean latencies in healthy subjects: N2: 240 ms, P2: 400 ms), while their amplitudes were not significantly different (except for one UWS patient), illustrating a surprisingly good preservation of the brain responses in the severely brain-injured patients. Interestingly, neither the CRS-R score nor the NCS-R score were correlated with LEPs. We should also note that the effective presence of an N2-P2 complex at a single-subject level should be cautiously interpreted due to the low SNR.

Nd: YAP-laser has been also used in patients with DoC to elicit nociceptive stimuli (De Salvo et al., 2015), with an energy of 2.75 J delivered through a 5mm diameter laser beam. This energy was set to correspond to a pin-prick sensation followed by a heat one, in at least three of five consecutive stimuli and to a Visual Analogue Scale score of 4/10, according to Perchet et al. (2008). The study showed, in a set of 23 patients (13 UWS and 10 MCS) preserved N1 and N2-P2 responses with greater latencies than in healthy subjects (N1: 310-508 ms; N2-P2: 392-570 ms). Interestingly, the authors found that patients with no severely altered N2-P2 latencies and amplitudes were able, according to the NCS scale, to localize the painful stimulation site, which is a well described sign of consciousness (Giacino et al., 2002). The authors tried to describe

correlations between N2-P2 parameters and behavioral assessment (CRS-R and NCS-R) but those should be considered cautiously given the small number of patients included in the study and the possible misclassification of patients according to their diagnosis of consciousness (e.g., in this paper, at least one patient classified as UWS displayed a localization to painful stimuli, which is incompatible with the diagnosis of UWS as this behavior constitutes an operational criteria for MCS).

RECORDING OF C-FIBER SPECIFIC RESPONSES

As previously mentioned, it is well known that brain responses elicited by a laser are the reflect of the activation of Aδ-fibers only, while a co-activation of C-fibers inevitably occurs. Characterizing C-fibers responses has always been challenging in healthy subject and is probably even more difficult in patients with DoC. However, Naro et al. (2015) used the technique of Bragard et al. (1996) to generate those stimuli with a Nd: YAP-laser, applied onto the peri-oral region (Massimiliano Valeriani et al., 2002). This way, they succeeded to elicit C-LEPs in all MCS patients (15/15) and in 65.2% of UWS patients (15/23). Interestingly, they achieved to record C-LEPs in 6 patients for whom Aδ-LEPs were not recorded. This suggests that in the absence of Aδ-LEPs, C-LEPs can remain preserved in severely brain-injured patients. However, a rather low number of stimuli (two runs of 30 stimuli) was used, which can be a limiting factor. Moreover, using an Nd: YAP-laser, a given amount of energy deposited on the skin can result in very different heating profiles. Therefore, it may be difficult to ascertain that the responses recorded are related to C-fibers activation in the absence of subjective feedback such as the recording of reaction times.

4.1.2. LIMITATIONS

ABSENCE OF WELL-CONTROLLED STIMULI

The characteristics, advantages and drawbacks of the different stimulus generators have been discussed in Section 1.2.2, and the importance of using selective and well-controlled stimuli has already been stressed out. In addition to the control of the

stimulus itself, the reliability with which it is applied on the skin should not be overlooked. For instance, using the temperature-controlled CO_2-laser, the laser beam needs to be applied perpendicularly to the skin making difficult to deliver the stimulus if the patient is not perfectly still. Conversely, the Nd: YAP-laser can be activated regardless of the position of the laser onto the skin, such that it is impossible to verify that the stimulus has been correctly delivered. Using a contact thermode, the experimenter has a direct control and can ensure that the thermode is firmly in contact with the skin at the time of stimulation.

POOR QUALITY OF THE SIGNAL IN PATIENTS WITH DOC

Electroencephalographic recording of healthy subjects classically displays different rhythmic pattern. In normal awakening conditions, eyes closed, we can observe a bilateral, symmetric, posterior alpha rhythm (8-12 Hz) that decreases in favor of a faster rhythm of lower amplitude, the beta rhythm (13-30 Hz) when the eyes are open. The faster gamma rhythm (31-100 Hz) is usually not seen with the naked eye. Those rhythmic activities constitute the so-called background EEG. Slower theta (4-7 Hz) and delta (1-3 Hz) rhythms are usually not seen in normal awakening conditions.

However, after a severe brain lesion, drastic changes can occur in the normal background activity : it displays larger amplitudes and can be asymmetric, in relationship with the localization of the brain lesion (Forgacs et al., 2014). These factors inevitably impact the genesis and recordability of ERPs; they also have an influence on the averaging procedure, especially the large amplitudes slow waves of the ongoing EEG.

In addition to those changes in ongoing electrical activity, external factors will also influence the brain signals recording quality. In patients with DoC, movement or muscle artifacts can occur for several reasons. Preparation of the session: even taking precautions to make the recording session comfortable, conditions are rarely optimal. At the time the recording begins, the patient has already received a lot of sensory stimulations, e.g., instructions from the experimenter, cold electrode gel on the EEG cap or movement to put the patient in the correct position in the bed or wheelchair. This leads to the activation of head and neck muscles, especially scalp muscles, jaws and

sternocleidomastoid muscles, which can result in substantial muscle artifacts on the EEG. Spontaneous movements: those patients remain rarely completely still and exhibit frequent movements, either goal or non-goal oriented, such as oromotor automatisms, eye blinking, movement of the limbs toward their tracheostomy, gastrostomy or even toward the experimenter. They are of course unable to refrain these movements. Movements in response to the stimulus: the influence of the two preceding movements can be somewhat limited by waiting the right time to deliver the stimulus, movements appearing in response to the stimulus itself are unavoidable.

Therefore, due to all these factors, it can be expected that more stimulations than in healthy controls will be required in order to observe a robust response after averaging.

AVAILABILITY, SAFETY AND TRANSPORTABILITY OF THE DEVICES

Regarding their availability: temperature-controlled CO_2-lasers are only available in a low number of centers, due to their high cost. Open-loop solid-state lasers (such as the Nd: YAP-laser) are more readily available because they are less expensive.

Regarding their safety: any kind of laser device might be dangerous in non-collaborative patients displaying unpredictable movements. Strict safety regulations are required as the laser beam can cause serious eye injury if it is moved unintentionally towards the eyes of the patient or examiner. Moreover, as already mentioned, solid-state lasers cannot be used on pigmented skin given the higher risk of burn injuries.

Regarding their transportability: given the large size and fragility of its components, the transport of the CO_2-laser is not recommended, which makes it difficult to bring at patient's bedside. The Nd: YAP-laser is more transportable but remain cumbersome in a small hospital room. Contact thermodes, on their side, are easily transportable.

PROPOSALS TO OVERCOME THESE LIMITATIONS

(1) The use of well-controlled stimuli is required, and the assessment should not be limited to the recording of nociceptive ERPs. In the absence of a control stimulus, the presence or absence of these nociceptive-related brain responses does not allow to infer the presence or absence of a pain-related experience. In Section 4.2, a novel

approach is proposed, based on the recording of brain responses following three type of stimuli : a nociceptive stimulus conveyed by the STT; an innocuous mechanical vibrotactile stimulus conveyed by the DCML; and an innocuous cool stimulus that is non-noxious but nevertheless conveyed by the STT. This approach should allow to determine whether the responses to nociceptive inputs are more preserved as compared to non-nociceptive inputs.

(2) Given the aforementioned limitations, the potential for using laser devices in a clinical context at the bedside of patients is low. Therefore, it is necessary to validate tools capable of overcoming them. The TCSII (see Section 1.2.2.) is a novel contact thermode having interesting characteristics to generate contact-heat evoked potentials (CHEPs) with a good SNR. Given its low weight and high transportability, the TCSII would be ideal for bedside assessments of patients with DoC. However, CHEPs using the TCSII have not yet been recorded. In Section 4.3., the use of this novel contact-thermode will be validated against the gold-standard to record those noxious-heat ERPs in healthy subjects, namely the CO_2-laser.

(3) Given the poor quality of the EEG signal in these patients, methods to increase SNR should be investigated. To this aim, we will record brain responses to long-lasting periodic thermal (warm and cool) stimuli, to identify related steady-state responses. The periodicity of the applied stimulus on a non-periodic EEG background should allow the signal to stand out from the background noise. However, the recording of such thermal SS-EPs in response to warm/cool stimuli is hardly/not known in healthy subjects, respectively. Therefore, a method to record those SSRs in healthy subjects is developed in Section 4.4, before assessing its potential for use in patients with DoC.

4.2. MULTIMODAL ASSESSMENT OF SOMATOSENSORY PATHWAYS IN PATIENTS WITH DOC

Neuropathological studies have reported thalamic lesions in 62-80% (62/100 and 28/35, respectively) of the patients with DoC from traumatic brain injury (TBI) etiology and in all cases of DoC from anoxic etiology (14/14) (Adams et al., 2000; Jellinger,

2013). Therefore, we can state that spino-thalamic tract lesions are frequent in patients with DoC. To date, the functional integrity of the STT is best assessed using selective-nociceptive stimuli such as laser stimuli. The elicited brain responses, the so-called laser-evoked potentials (LEPs), if they are not a biomarker for pain experience, are probably the reflect of brain activity required for such experience. LEPs are known to be decreased or absent in small-fiber neuropathy (Cruccu et al., 2010) but also in STT lesions (Haanpää et al., 2011). However, it is not clear whether those LEPs are more a reflection of spino-thalamic integrity, a marker of the stimulus salience or a correlate of the conscious processes underlying a perceptual experience.

As suggested by seminal studies (De Salvo et al., 2015; Tommaso et al., 2013), LEPs seem to be highly preserved in patients with DoC, but the meaning of such preservation remains unknown. To date, it is impossible to know whether the ability of the damaged human brain to respond to nociceptive stimuli is relatively preserved as compared to its ability to respond to non-nociceptive stimuli. It is also impossible to know whether this preservation is related to the salience of the stimulus, its nociceptive characteristic or because it is conveyed by the STT.

For this reason, brain responses elicited by a specific nociceptive stimulus will be compared to those elicited by two other types of somatosensory stimuli: a purely non-nociceptive stimulus conveyed by the DCML, namely mechanical vibratory stimulus and a stimulus that is conveyed by the STT but unrelated to nociception or pain, namely an innocuous cool stimulus (Table 4.1).

The aim of this exploratory study is to replicate previous findings regarding the EEG responses to laser stimuli (i.e., their presence/absence, latencies, amplitudes and SNR), but also to assess the ability to identify brain responses at a single-subject level. This study will also provide indications on the feasibility of recording cool- and vibrotactile-evoked brain responses in patients with DoC.

Table 4.1. Types of stimuli and their characteristics.

Stimulus	*Fibers*	*Sensory pathway*	*Percept elicited in HC*
Noxious heat	Aδ & C	STT	Painful
Cool	Aδ & C	STT	Non-Painful
Mechanical vibratory	Aβ	DCML	Non-Painful

Note: Shared characteristics are highlighted in light- and dark-grey. Cool stimuli share at least one characteristic with the other two stimuli. STT = Spino-Thalamic Tract; DCML = Dorsal Column - Medial Lemniscus; HC = Healthy Controls

4.2.1. MATERIAL AND METHODS

A - STIMULI

Noxious-heat stimulation – Noxious-heat stimuli were delivered by an Nd: YAP-laser (Nd: YAP, Electronical Engineering, Florence, Italy). An energy of 2.5 J was applied onto the skin within a pulse duration of 5 ms through a 5 mm-diameter laser beam.

Cool stimulation - Cool stimuli were delivered by the TCSII. Baseline temperature of the skin was actively set to 30°C. The stimulus lasted for 100 ms at maximal cooling speed (i.e., 300°C/s) to reach a skin temperature of 15°C (Δ=15°C), followed by a passive return to baseline temperature.

Mechanical vibratory stimulation - Mechanical vibratory stimuli were delivered through a recoil-type vibrotactile transducer driven by a standard audio amplifier (Haptuator, Tactile Labs Inc., Canada). The stimulus consisted in a vibration lasting 50 ms with a frequency of 250 Hz, delivered at maximal output voltage (3 V).

B - PROCEDURE

Patients were evaluated in their hospital room and installed either in their bed or in their wheelchair. They were instructed to remain still knowing that they might not consider the instruction due to their DoC. Their level of consciousness was assessed

with the CRS-R just before the session. They were evaluated in a single session. The side of stimulation was contralateral to the most spared brain hemisphere; if this could not be determined, the side with less spontaneous motor activity was chosen. The stimuli were delivered on the hand dorsum; two runs of stimulation (20-30 stimuli per run) were administered for each type of stimulus. The inter-stimulus interval was self-paced by the examiner but was over seven seconds. Block order was pseudo-randomized with the constraint not to begin the session with laser stimuli. Indeed, in a small pilot study, patients became much more agitated following the laser condition so that it was nearly impossible to pursue the recording afterwards.

C- EEG RECORDING AND ANALYSIS

EEG was recorded using 32 Ag-AgCl electrodes placed on the scalp according to the international 10-20 system (WaveGuard 32-channel cap; Advanced Neuro Technology, The Netherlands). Signals were amplified and digitized using a sampling rate of 1024 Hz. The continuous EEG recording was filtered using a 0.5-30 Hz bandpass 4^{th} degree Butterworth filter. The EEG was then segmented in epochs of two seconds, starting 0.5 second before stimulus onset. The signal was baseline corrected regarding the time interval -0.5 to 0 second relative to the stimulus onset. Channel selection was done visually, allowing to remove electrodes with bad recordings. When possible, the removed channels were interpolated with the EEG signal of three neighboring channels. Artefacted epochs were first rejected by visual inspection (4.9 ± 6.0, 4.3 ± 3.5 and 2.0 ± 2.1 were rejected for laser, cool and vibrotactile stimuli, respectively). Artifact removal was subsequently done using an Independent Component Analysis (FastICA algorithm). Due to the general bad signal recorded at both mastoids, M1 and M2 channels were deleted and the signal was re-referenced to an average reference for identification of N2 and P2 waves and re-referenced to Fz for identification of potential N1 responses.

Identification of brain responses relied on the presence, by visual inspection, of an N2-P2 complex. Whenever the presence of a response was uncertain, topographical maps were generated, and global field power (GFP) calculated. GFP was calculated by

rectifying (by computing the absolute value of all samples in the dataset) the pooled signal of all the channels of the average waveforms. The coherence of the suspected peak, taken together with consistent topography and increased GFP at the latencies of interest determined whether a response was observed or not.

Signal-to-noise ratio was computed as the ratio between peak-to-peak signal amplitude in the post-stimulus time window (0 to 1 second relative to stimulation onset) and the peak-to-peak signal amplitude in the pre-stimulus time window (-0.5 to 0 second relative to stimulation onset). The SNR was calculated at Cz versus an average reference.

4.2.2. RESULTS

Ten brain injured patients (etiology: TBI [n=7], hemorrhage [n=2] and anoxia [n=1]) with various level of consciousness (3 UWS, 4 MCS, 3 eMCS without functional communication) were included in the study (2 females, 8 males; aged 32.5±16.0) during their in-hospital rehabilitation (5.9±3.5 months post-injury), in a convenience sample.

EEG responses were identified in 30%, 40% and 20% of the patients for laser, cool and vibrotactile stimulations, respectively (Tables 4.2 and 4.3). Patients with higher CRS-R scores seemed to show more consistent responses (Figure 4.1).

Table 4.2. Number of patients and EEG responses observed in each modality according to their diagnosis.

Level of consc.	N	Laser stimuli	Cool stimuli	Vibratory stimuli
MCS	4	2/4 (50%)	3/4 (75%)	1/4 (25%)
UWS	3	0/3 (0%)	0/3 (0%)	0/3 (0%)
eMCS	3	1/3 (33%)	1/3 (33%)	1/3 (33%)
Total	**10**	**3/10 (30%)**	**4/10 (40%)**	**2/10 (20%)**

Note: N = total number of patients in each group according to their level of consciousness.

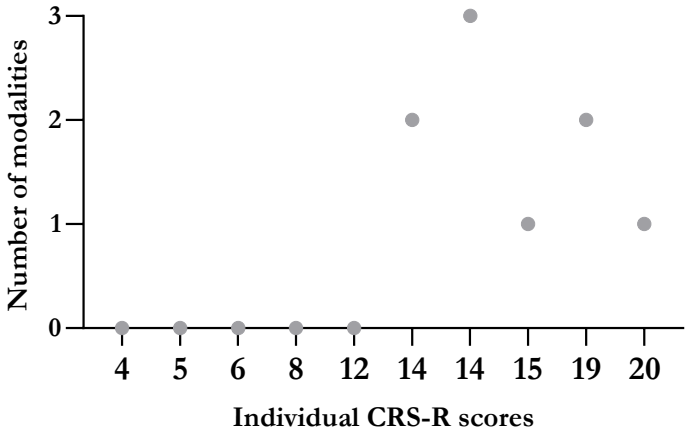


Figure 4.1. Representation of the number of modalities for which an EEG response was identified according to the CRS-R total score of each individual.

Table 4.3. Details of the presence or absence of EEG responses for each patient according to stimulation modalities.

Diagnosis	CRS-R	TSI	Etiology	Laser stim.	Cool stim.	Vibro. stim.	Total of mod.
UWS	4	180	Anoxic	0	0	0	**0**
UWS	5	366	TBI	0	0	0	**0**
UWS	6	104	TBI	0	0	0	**0**
MCS-	8	224	TBI	0	0	0	**0**
MCS+	14	146	Vascular	1	1	1	**3**
MCS+	14	100	TBI	1	1	0	**2**
MCS+	15	209	Vascular	0	1	0	**1**
eMCS	12	367	TBI	0	0	0	**0**
eMCS	19	182	TBI	1	1	0	**2**
eMCS	20	59	TBI	0	0	1	**1**
n = 10				**3/10**	**4/10**	**2/10**	

Note: Diagnosis = Diagnosis of consciousness; CRS-R = CRS-R total score; TSI = Time Since Injury (days); Total of mod. = number of modalities in which a response is observed for each patient. In the columns "Laser stim(ulations)", "Cool stim(ulations)" and "Vibro(tactile) stim(ulations)", results equal either 0 or 1, defined as the absence or the presence of an EEG response, respectively.

A - NOXIOUS HEAT STIMULATION

For patients displaying identifiable responses, individual values for amplitudes, latencies and SNR are reported in Table 4.4.

UWS patients - No brain evoked responses to noxious heat stimuli were recorded in any of the three UWS patients.

MCS patients - Noxious heat stimuli appeared to evoke identifiable EEG brain responses in 2/4 (50%) MCS patients, consisting essentially in N2-P2 complex, maximal at vertex. Responses in one MCS patient are illustrated in Figure 4.2. Global field power (GFP) and corresponding topographic maps of LEPs are represented as well as the recorded LEPs. Responses in the other MCS patient are shown in Appendix A, Figure A.2.

eMCS patients – Only one eMCS patient appeared to display N1 and N2-P2 responses with, however, a limited confidence due to major movements artifacts that could only be partially removed during the preprocessing. A representation of GFP and corresponding topographic maps of LEPs are available in Appendix A, Figure A.3. No responses were identified in the two other eMCS patients due to the large contamination by artifacts, mostly related to the great agitation of the patients in response to those salient nociceptive stimuli.

Table 4.4. Details of EEG responses elicited by noxious-heat stimuli

Noxious heat stimuli	Subj. 1	Subj. 7	Subj. 8
Level of consciousness	**MCS+**	**MCS+**	**eMCS**
N1 Amplitude (μV)	-6.95	N/A	-16.1
N2 Amplitude (μV)	-4.97	-1.61	-11.25
P2 Amplitude (μV)	5.79	2.56	7.94
N2-P2 Amplitude (μV)	10.76	4.17	19.19
Signal-to-Noise Ratio	0.84	2.30	2.84
N1 Latency (ms)	209	N/A	183
N2 Latency (ms)	214	474	318
P2 Latency (ms)	443	669	560

Note: Amplitudes and latencies area calculated at Cz with an average reference for N2 and P2 waves while N1 waves are identified at T7 using Fz as reference.

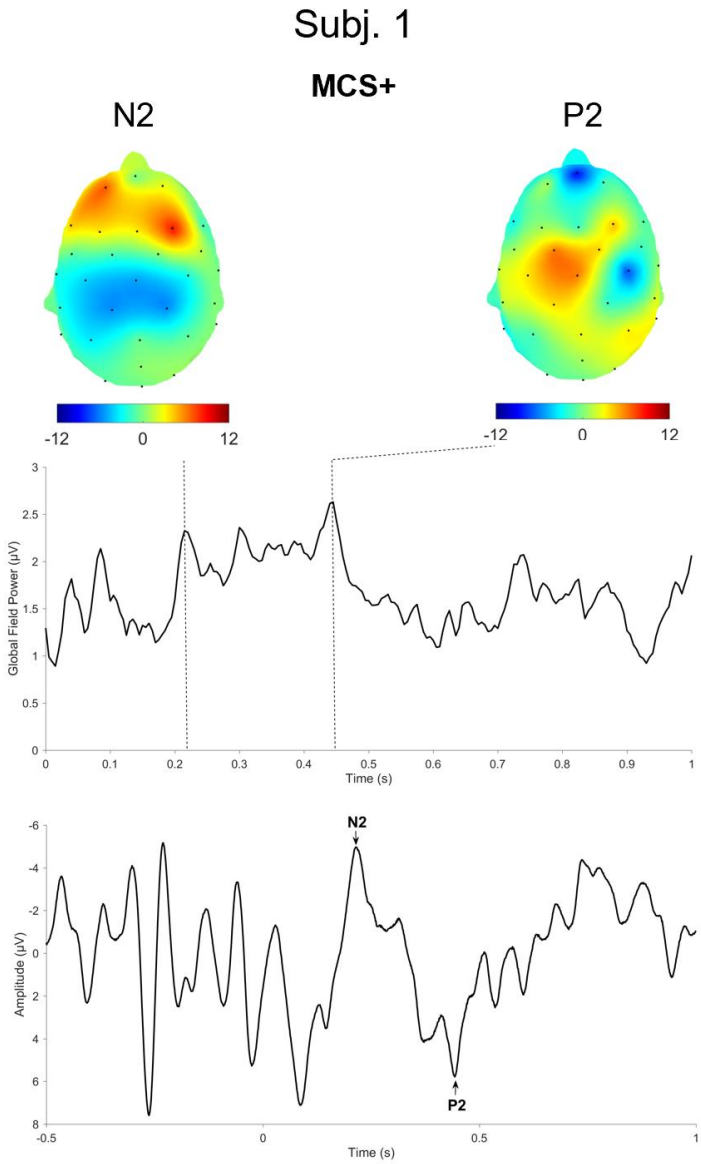


Figure 4.2. Global field power (GFP) and corresponding topographic maps of nociceptive event-related brain potentials (**LEPs**). The last row corresponds to the LEPs recorded in one MCS patient (Subj. 1), using Cz versus an average reference. Despite its low SNR, an N2-P2 complex is identifiable.

B - COOL STIMULATION

For patients displaying identifiable responses, individual values for amplitudes, latencies and SNR are reported in Table 4.5.

UWS patients - No brain evoked responses to cool stimuli were recorded in any of the three UWS patients.

MCS patients - Cool stimuli appeared to evoke identifiable EEG brain responses in 3/4 (75%) MCS patients, consisting mainly in a P2 wave, while the N2 wave was only identifiable in two of those patients. Those responses are maximal at vertex. Interestingly, the MCS patient without identifiable response was also the patient with the lowest CRS-R score. GFP and corresponding topographic maps of cool-evoked potentials (CEPs) are represented as well as the recorded CEPs in Figure 4.3. Brain responses of the two other MCS patients are illustrated in Appendix A, Figures A.4 and A.5.

eMCS patients - Only one eMCS patient displayed a clear P2 wave, while the N2 component was not identifiable. For this patient, a representation of GFP and corresponding topographic maps of CEPs is available in Appendix A, Figure A.6. No responses were identified in the two other eMCS patients.

Table 4.5. Characteristics of EEG responses elicited by cool stimuli

Cool stimuli	Subj. 1	Subj. 6	Subj. 7	Subj. 8
Level of consciousness	**MCS+**	**MCS+**	**MCS+**	**eMCS**
N1 Amplitude (μV)	N/A	N/A	N/A	N/A
N2 Amplitude (μV)	-4.44	-0.42	N/A	N/A
P2 Amplitude (μV)	5.28	2.05	3.43	5.17
N2-P2 Amplitude (μV)	9.72	2.47	N/A	N/A
Signal-to-Noise Ratio	1.00	0.97	N/A	N/A
N1 Latency (ms)	N/A	N/A	N/A	N/A
N2 Latency (ms)	205	221	N/A	N/A
P2 Latency (ms)	499	306	505	448

Note: Amplitudes and latencies are calculated at Cz with an average reference for N2 and P2 waves while N1 waves are identified at T7 using Fz as reference.

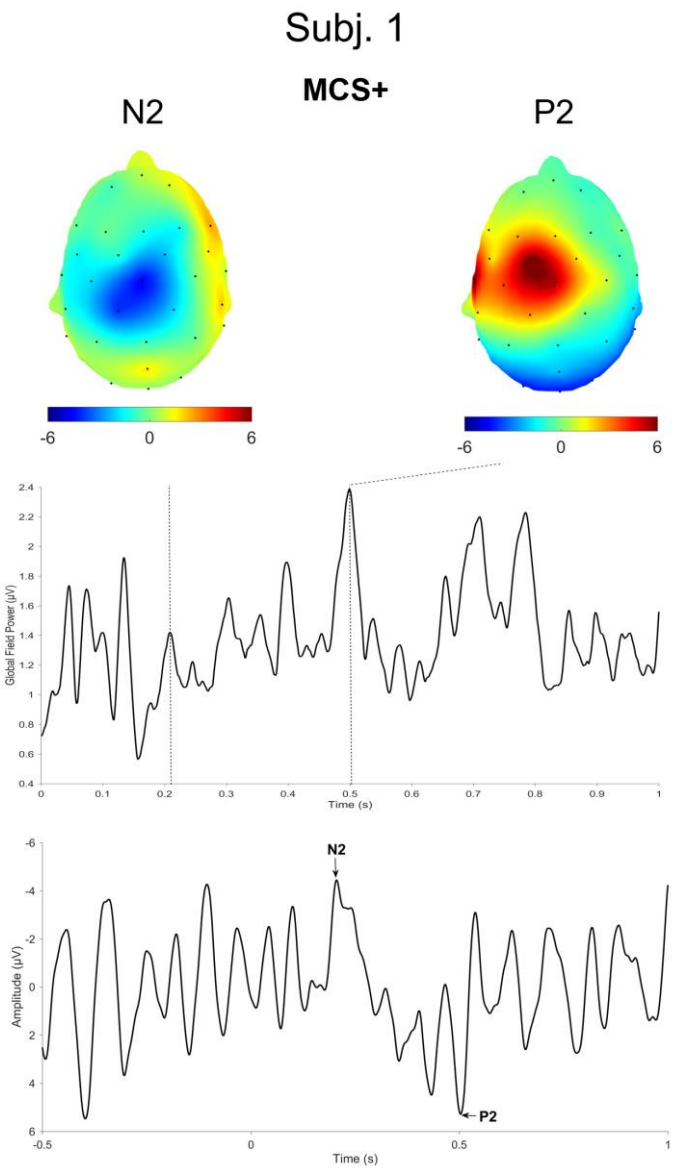


Figure 4.3. GFP and corresponding topographic maps of cool event-related brain potentials (**CEPs**). The last row corresponds to the CEPs recorded in one MCS patient (Subj. 1), using Cz versus an average reference. Despite its very low SNR, an N2-P2 complex is identifiable.

C - MECHANICAL VIBRATORY STIMULATION

For patients displaying identifiable responses, individual values for amplitudes, latencies and SNR are reported in Table 4.6.

UWS patients - No brain evoked responses to mechanical vibratory stimuli were recorded in any of the three UWS patients.

MCS patients - Mechanical vibrotactile stimuli appeared to evoke an identifiable EEG brain response in 1/4 (25%) MCS patients. However, the N1 wave is not identified with a high degree of confidence. The evoked response consists therefore in a P2 wave, with a lateralized topography (i.e., contralateral to the side of stimulation; see Figure 4.4). GFP and corresponding topographic maps of vibrotactile-evoked potentials (Vibro-EPs) are represented as well as the recorded Vibro-EPs in Figure 4.4.

eMCS patients - Only one eMCS patient displayed clear N1 and P2 responses. Nevertheless, amplitudes and SNR remains very low. For this patient, a representation of GFP and corresponding topographic maps of Vibro-EPs is available in Appendix A, Figure A.7. No responses were identified in the two other eMCS patients.

Table 4.6. Characteristics of EEG responses elicited by mechanical vibratory stimuli

Mechanical vibratory stimuli	Subj. 1	Subj.10
Level of consciousness	**MCS+**	**eMCS**
N1 Amplitude (μV)	-4.50	-1.08
P2 Amplitude (μV)	3.07	0.56
N1-P2 Amplitude (μV)	7.57	1.64
Signal-to-Noise Ratio	0.84	1.2
N1 Latency (ms)	168	288
P2 Latency (ms)	240	373

Note: Amplitudes and latencies are calculated at Cz with a mean reference for N1 and P2 waves.

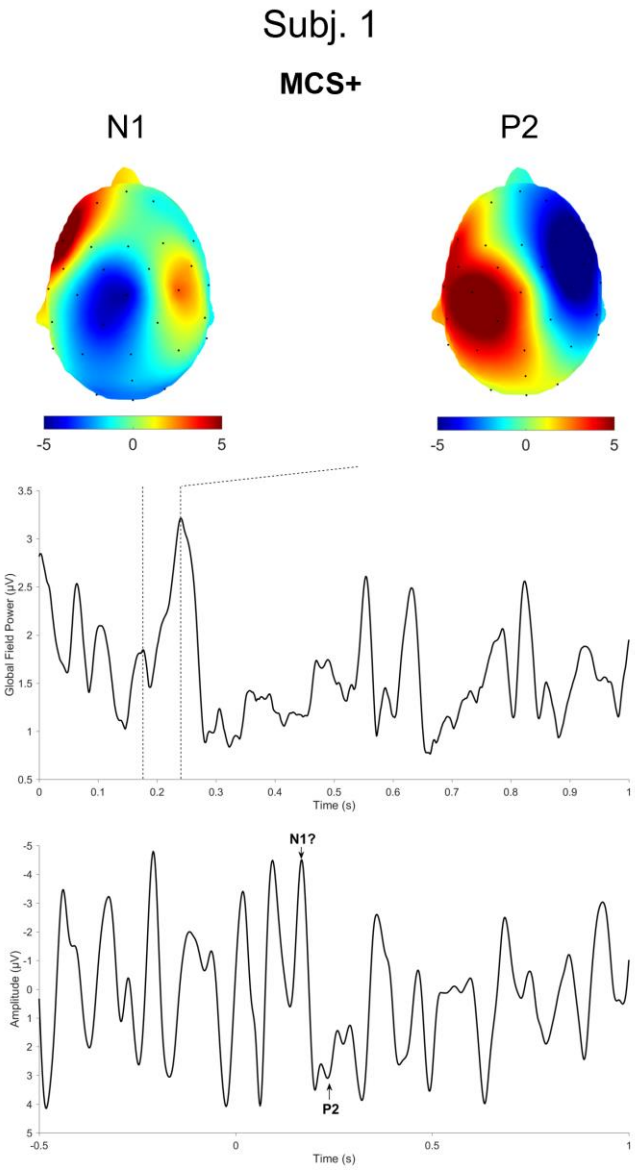


Figure 4.4. GFP and corresponding topographic maps of vibrotactile event-related brain potentials (**Vibro-EPs**). The last row corresponds to the Vibro-EPs recorded in one MCS patient (Subj. 1), using Cz versus an average reference. A P2 wave appears to be identifiable despite the very low SNR.

4.2.3. DISCUSSION

In this exploratory study, we aimed to replicate previous findings regarding the brain responses to laser stimuli and to assess the feasibility to record cool- and mechanical vibrotactile- evoked potentials in patients with DoC at a single-subject level.

We identified brain responses in the time-domain in 30%, 40% and 20% of the patients for laser, cool and vibrotactile stimulations, respectively. The presence of those brain responses seemed to increase together with the CRS-R scores. Given the strong relationship between consciousness and pain experience, we hypothesized that the presence of brain responses correlates with the level of consciousness. Interestingly, no brain response to any kind of stimulus was observed in UWS patients, while 3/4 MCS patients displayed possible responses in at least one modality. On its side, the low presence of identifiable responses in eMCS patients could be simply related to the presence of large movement artifacts. Indeed, those patients with a recovered level of consciousness (while being unable to communicate) were much more agitated, or even aggressive following laser stimulations. In addition, we should keep in mind that the patients were only assessed once for their level of consciousness, while it is recommended to assess serially patients with the CRS-R in order to lower the rate of misdiagnosis (Wannez et al., 2017)

The observed EEG responses had a very low SNR, sometimes even close to 1. To identify those responses, we sometimes needed to rely on the increases in GFP and the related scalp topography of the ERP signal, meaning that the confidence in observing those responses was low. Therefore, it is difficult to determine whether the absence of identified brain response is related to its very low SNR or to the effective absence of it. Further analyses should be undertaken to quantify the presence of an evoked response following each stimulation type by different means such as topographic consistency analysis (Koenig & Melie-García, 2010) or as cluster permutation test across electrode montage (Maris & Oostenveld, 2007).

Regarding noxious-heat laser evoked potentials, we failed to replicate previous findings of de Tommaso et al. (2013, 2015), who observed LEPs in almost every patient, even in UWS. In our case series, LEPs were identified in only 30% of the patients, and in no UWS. Scalp topographies of the observed responses were maximal at vertex for both N2 and P2 waves. Latencies were heterogeneous in the four patients, with no trend regarding their level of consciousness, varying from normal values to delayed responses. The reason for such discrepancy between those studies could be related to the different type of laser device used. In this study was used a Nd: YAP laser instead of a CO_2-laser so the stimuli were not matched to those used by de Tommaso et al. However, the shorter pulse duration of the Nd: YAP laser should have been an advantage to recruit in a more synchronized fashion the fibers afferent volley. Anyhow, an important drawback and limitations of both studies is the absence of temperature-controlled stimuli that would have allowed a perfect control of the heating profile of the skin and hence, a better control of the effective activation of the thermonociceptors.

Cool-evoked responses in patients with DoC are described for the first time. As for noxious-heat stimuli, no response was observed in UWS patients. Three over four MCS patients displayed an identifiable response consisting mainly in a P2 wave, while the N2 wave was only identifiable in two of those patients. Scalp topographies were maximal at vertex for both N2 (when identifiable) and P2.

ERPs related to the selective activation of low-threshold mechanoreceptors using vibrotactile stimuli are also described for the first time in patients with DoC. As for noxious-heat and cool stimulations, no brain response was observed in UWS patients. Only one MCS patients displayed identifiable responses with a very poor SNR. N1 was not confidently identified and the evoked response consisted mainly in a P2 wave, whose scalp topography was lateralized (i.e., contralateral to the side of stimulation).

The absence of nerve conduction study is another limitation of this study. Indeed, these severely brain-injured patients have been bedridden for a long time and are therefore at high risk of critical illness polyneuropathy, that could prevent the stimuli to be conveyed to the brain. Therefore, for future studies, it is recommended to determine

nerve conduction studies before recording these somatosensory ERPs. Applying the stimulations on the face could also be an alternative as sensory afferents from the face are less likely to be affected by this critical illness polyneuropathy.

Finally, given the heterogeneity of brain lesions and level of consciousness resulting in large differences in amplitudes and latencies of the ERPs across subjects, the brain responses were more easily identified at a single-subject level than after grand averaging procedure.

In summary, the aim of this exploratory study was to assess the feasibility of recording brain responses to noxious-heat, cool and vibrotactile stimuli in patients with DoC. We showed that, while it was possible to record such responses, they were not systematically identified and, if they were, displayed a very poor SNR. The findings of previous studies were not replicated regarding the recording of LEPs, but for the first time, brain responses to cool and vibrotactile stimuli were recorded. The presence of identifiable brain responses in all three somatosensory modalities and the fact that these responses seem to be more visible in MCS patients than in UWS patients is promising. However, the very low SNR of these responses is somewhat discouraging. For this reason, and also because laser stimuli were poorly tolerated by the patients, it was necessary to investigate alternative means to counter these important drawbacks (e.g., low potential for clinical application of laser device and low SNR) before further assessing the integrity of the somatosensory pathways in patients with DoC.

4.3. ELICITING ROBUST BRAIN RESPONSES TO NOXIOUS STIMULI USING A HIGH-SPEED HEATING THERMODE

The gold-standard to assess the integrity of the STT remains to date the CO_2-laser (Cruccu et al., 2008). However, given their limitations, laser devices have a low potential for use in a clinical context at the bedside of patients. Conversely, contact thermodes are readily available and ideal for bedside assessments. Hitherto, the recording of CHEPs has shown a poor SNR due to the low heating slope of the thermodes. But the

TCSII, a novel type of contact thermode (see Section 1.2.2.), displays characteristics compatible with the ability to record such CHEPs with a higher SNR.

This experiment aims to validate the TCSII against the gold-standard to evaluate the integrity of the STT, namely the CO_2-laser. This validation process should lead to the use of the TCSII as an alternative means to elicit noxious-heat related brain responses interpretable at a single-subject level with a decent SNR. To that end, this experiment consists in three parts: In Part 1 we will compare brain-responses elicited by matching noxious-heat stimuli generated by the temperature-controlled laser device and the TCSII; in Part 2 we will match the intensity of perception of the stimuli generated by both devices using a staircase algorithm; and in Part 3 we will compare brain responses elicited by both devices using the perception-matched stimuli identified in Part 2.

4.3.1. PART 1 - BRAIN RESPONSES TO LASER AND CONTACT HEAT STIMULI

A – MATERIAL AND METHODS

1. DEVICES

- The CO_2 Laser Stimulation Device (LSD; SIFEC, Belgium) used for this experiment is temperature controlled. The control of temperature is provided thanks to a thermal sensor that continuously records temperature at the skin target surface with a sampling rate of 10000 Hz. The measured data are sent to a controller that regulates the laser output power to maintain the temperature as close as possible to the target temperature throughout the duration of the stimulus.
- The TCSII, as already mentioned, is also temperature controlled thanks to thermocouples located at the center of each of the five stimulation surfaces. Those thermocouples measure the skin temperature with a sampling rate of 100-200 Hz and drive micro-Peltier elements to the defined target skin temperature.

2. STIMULI CHARACTERISTICS

Based on the aim of the study, the stimuli generated by the two devices were designed to be as comparable as possible to each other. However, the heating slopes were set at their maximum for both devices: 300°C/s and over 1000°C/s for the TCSII and the LSD, respectively. Three other characteristics were matched between the LSD and the TCSII:

- Target temperature was set to 55°C. This temperature is known to be above the activation threshold of Aδ- and C-fibers.
- Duration of the stimulus was set to 200ms. This ensures a sufficient time for the skin temperature to rise to 55°C with the TCSII. Using the LSD, this target temperature is easily reached within this stimulus duration.
- Stimulation surface: the whole surface of the TCSII was used. The stimulation surface equal the sum of the surface of all the micro Peltier elements of the TCSII, which is 115,5mm² (i.e., 15*7.7mm²). The stimulation surface of the laser beam was accordingly set to 113,04 mm², using a 12 mm diameter lens.
- Temperature profiles were recorded during stimulations for both devices. The actual maximal heating slopes of the devices were of 1041±122 °C/s and 388±15 °C/s and were achieved 17±2 ms and 30±1 ms after the stimulus onset for the LSD and the TCSII, respectively. The target temperature was 55±1°C for the TCSII but the LSD reached a supra-target temperature 58±1°C. This problem of overshoot is intrinsic to laser stimulation devices. Note that active post-stimulus cooling of the TCSII for return to baseline temperature was activated (Figure 4.5).

3. PROCEDURE

The stimuli were delivered to the left or right volar forearm. The order of the blocks was counterbalanced across subjects. The first stimulated arm was randomized between participants and the stimulated arm was interchanged between each block. The subjects were given a five minutes break after each stimulation block. Each stimulation block consisted in 20 stimuli. There were two blocks of stimulation for each condition (i.e., 40 stimuli per condition). To avoid skin damage, fatigue or sensitization of

nociceptors, and central habituation, the interstimulus interval (ISI) was self-paced by the experimenter (with a minimum of seven seconds) and the stimulated spot of the laser or the probe of the TCSII was slightly shifted between consecutives stimulus at different places on the forearm (Cruccu et al., 2008). This ISI also allowed us to place the probe of the TCSII on the skin for a time before providing heat stimulation in the aim to avoid artifactual potentials evoked by the activation of Aβ mechano-sensitive fibers when the probe is applied on the skin. Finally, to reduce the novelty effect of heat stimulations, we applied two stimuli with each device before beginning the recording of ERPs in order to familiarize the subject with the stimuli.

After each stimulation block, the subjects were asked to rate the average intensity of the stimuli across the block, using a numerical rating scale (NRS). They were asked to rate the intensity of the stimulation from 0 to 100, with 0 signifying that the stimulus was not perceived, 50 being the transition from a painless to a painful stimulus and 100 being the most painful percept imaginable. This adaptation of the numerical pain rating scale allows the subject to rate, under 50, the intensity of a stimulus eliciting a percept without evoking a painful experience.

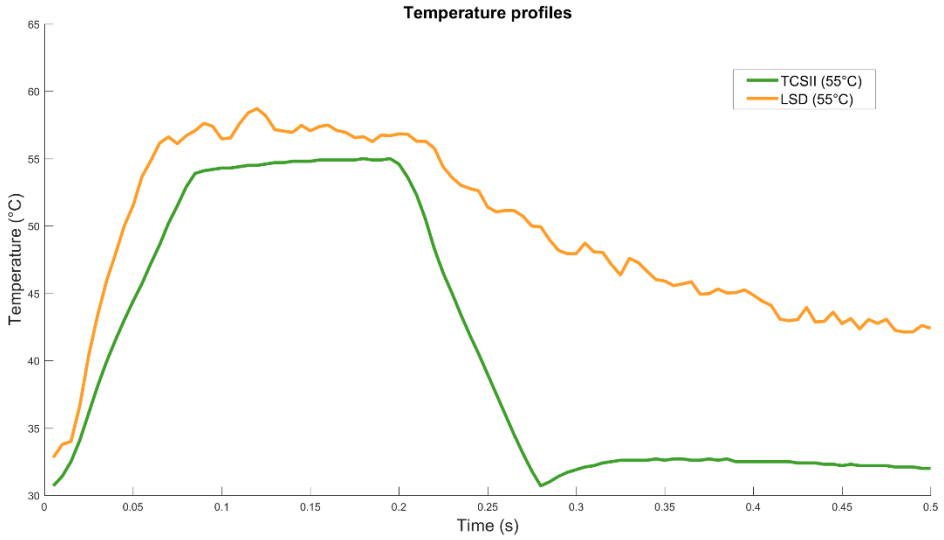


Figure 4.5. Temperature profile of the LSD at 55°C and of the TCSII at 55°C as target temperatures. On the x-axis, time since onset of the stimulus.

4. DATA RECORDING, PROCESSING AND ANALYSIS

The EEG was recorded using 64 Ag-AgCl electrodes, whose impedances were kept below 10 kΩ, placed on the scalp according to the international 10-10 system (WaveGuard 64-channel cap; Advanced Neuro Technologies). Signals were amplified and digitized at 1000 Hz. The EEG recordings were analyzed offline using Matlab R2017a (The MathWorks), and the preprocessing was performed using Letswave 6 (http: //1etswave.org) (A. Mouraux & Iannetti, 2008). The continuous EEG recording was filtered using a 0.5-30 Hz bandpass 4^{th} degree Butterworth filter. The EEG was then segmented in epochs of 1.5 second, starting 0.5 second before stimulus onset. Removal of artifact was done through the analysis of independent components using the FastICA algorithm. Finally, before averaging, the signal was baseline corrected regarding the time interval -0.5 to 0 seconds relative to the stimulus onset. The EEG was re-referenced to joint ears (A1-A2) to identify N2-P2 complex and to Fz for identification of N1 wave.

Signal-to-noise ratio SNR was obtained by computing the ratio between peak-to-peak signal amplitude in the post-stimulus time window (0 to 1 second relative to stimulus onset) and the peak-to-peak signal amplitude in the pre-stimulus time window (-0.5 to 0 second relative to stimulation onset). The SNR was calculated at electrode Cz versus A1-A2 using the EEG signal obtained by averaging the 40 trials recorded using each device.

B – RESULTS

Twelve healthy volunteers (5 males and 7 females, aged 20-34) were included in the Part 1 of the study. After averaging, a clear N2-P2 complex was identified in 100% (12/12) and in 75% (9/12) of the participants following stimulations using the LSD and the TCSII, respectively. Paired-sample *t*-tests were performed to compare intensities of perception, peak latencies and amplitudes of the N1, N2 and P2 waves between conditions (LSD versus TCSII). Statistical significance was set at $p < 0.05$. Results for

amplitudes, latencies, intensity of perception and p value of the paired-sample *t*-tests are all represented in Table 4.7.

Amplitudes – Mean amplitudes of the N2-P2 complex were 34.22±14.57 μV and 14.64±6.45 μV for the LSD and the TCSII, respectively. Differences in amplitudes between both devices were statistically significant for the N2-P2 complex.

Mean amplitudes of the N1 wave were -6.33±3.11 μV and -2.50 ± 1.17 μV for the LSD and the TCSII, respectively. Differences in amplitude between both devices were statistically significant for the N1 wave.

Latencies – Mean latencies of the N2 wave were 238±57 ms and 297±77 ms for the LSD and the TCSII, respectively. Mean latencies of the P2 wave were 382±56 ms and 478±83 ms for the LSD and the TCSII, respectively. Differences in latencies between both devices are statistically significant for both N2 and P2 waves.

Mean latencies of the N1 wave were 202±59 ms and 255±63 ms for the LSD and the TCSII, respectively. Differences in latencies between both devices were not statistically significant for the N1 wave.

Signal to Noise Ratio – Averaged SNR was 6.04 and 2.50 for the LSD and the TCSII, respectively. The SNR was 2.42 times greater with the LSD than with the TCSII.

Grand average – see Figure 4.6

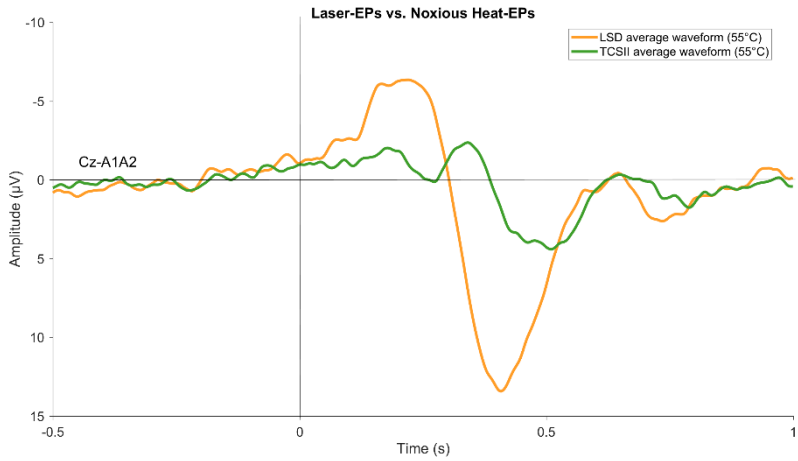


Figure 4.6. ERPs recorded after stimulation with LSD at 55°C and TCSII at 55°C. Waveforms correspond to the group-level average recorded at electrode Cz versus A1-A2.

Table 4.7. Intensity of perception, amplitude and latencies obtained with LSD and TCSII both at 55°C.

	LSD (55°C)	TCSII (55°C)	Value of p
N1 Amplitude (μV)	-6.33 ± 3.11	-2,50 ± 1.17	p = 0.0164 *
N2 Amplitude (μV)	-13.27 ± 6.56	-5.49 ± 1.36	p = 0.0015 **
P2 Amplitude (μV)	16.68 ± 7.03	7.43 ± 5.04	p < 0.0001 ***
N2-P2 Amplitude (μV)	29.95 ± 11.05	12.92 ± 5.50	p = 0.0001 ***
N1 Latency (ms)	202 ± 59	255 ± 63	p = 0,0851 (NS)
N2 Latency (ms)	238 ± 57	297 ± 77	p = 0,0050 **
P2 Latency (ms)	382 ± 56	478 ± 83	p = 0.0008 ***
Intensity of perception (NRS)	61.04 ±18.90	45.38 ± 15.22	p < 0.0001 ***

Note: Average values and standard deviation of intensity of perception, amplitude and latencies obtained in the experiment. The last column indicates the p value of the paired-sample *t*-tests testing for the difference between the TCSII and the LSD. *** p < .001, ** p < .01), *p<.05 (paired-sample *t*-tests). μV = microvolts; ms = milliseconds; NRS = NRS score.

Intensity of perception - After each block, participants were asked to report the average intensity of perception elicited by the stimuli on a Numerical Rating Scale (NRS). The scale ranges from 0 (no perception) to 100 (maximal pain), with 50 representing the transition from non-painful to painful sensation. All stimuli were clearly perceived, but the intensity of the elicited sensations was greater for the LSD than for the TCSII. The average rating of the intensity of perception was 61.04±18.90 and 45.38±15.22 for the LSD and the TCSII, respectively (Table 4.7 and Figure 4.7). A paired-sample *t*-test was performed to compare the average ratings of intensity of perception reported by subjects after stimulation by both devices. Differences in intensity of perception between both devices are statistically significant ($p < 0.001$). The subjects were also asked to describe their sensation in qualitative terms: 91.7% (11/12) and only 50% (6/12) qualified the stimuli as painful when delivered using the LSD and the TCSII, respectively. The qualifiers used to describe LSD stimulations were sharp, pricking and well localized in time and space. TCSII stimulations, on their side, were described as unpleasant and more diffuse.

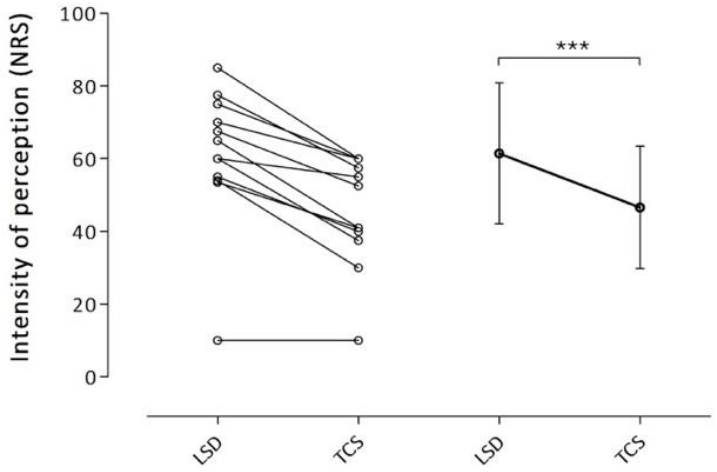


Figure 4.7. Intensity of perception of stimuli delivered with LSD and TCSII both at 55°C. Left side of the graph shows individual data and right side, the group-level average. *** $p < .001$ (paired sample *t*-test).

C - DISCUSSION OF THE RESULTS AND DIRECTIONS

Heat stimuli delivered by the TCSII evokes brain responses that resemble those elicited by the stimuli delivered by the LSD. Typical N1, N2 and P2 responses were identifiable with the TCSII, but with four main issues: increased latencies; lower amplitudes; lower SNR; and the absence of CHEPs identified at a single-subject level in 25% of the 12 healthy subjects.

Those differences could be explained by at least two factors. First, the lower heating slope of the TCSII versus the LSD results in a less synchronized recruitment of the afferent volley of nerve fibers. Second, the differences in heating mechanisms between both devices: in the case of the CO_2-laser, the irradiated energy is absorbed by the superficial layers of the skin, so there is a direct heating below the surface, where the thermonociceptors are located. In the case of the TCSII, heating at the depth of the free nerve endings relies entirely on thermal conduction. This could lead to a stronger gradient between surface and depth temperature and a stronger delay between peak

temperature at surface and depth. Those factors could explain the observed differences in term of amplitudes and latencies on the EEG recordings.

In addition, the stimulations delivered by the TCSII were also evaluated less painful than those delivered by the LSD. Importantly, TCSII stimuli were also depicted most of the time as not being painful. These differences observed in term of intensity of perception could also be explained by the differences in skin heating at the level of free nerve endings.

Part 2 of this experiment will describe how we matched the intensity of perception for the two types of stimuli. Finally, Part 3 will describe how we, subsequently, compared LEPs and CHEPs elicited by perception-matched heat stimuli.

4.3.2. PART 2 - PERCEPTION-MATCHED LASER AND CONTACT HEAT STIMULI

An adaptative staircase method was used to identify the temperature generated by the TCSII eliciting a similar intensity of perception than that provided by the 55°C-LSD stimuli. The only variable is the temperature while the other parameters remained unchanged from those used in Part 1 of the experiment.

Thermal stimuli were applied to both volar forearm of the subjects in two sessions. Initial side (right or left) and device (TCSII or LSD) were randomized across participants. The LSD target temperature was set to 55°C and remained unchanged throughout the experiment, while the subjects were blinded to this. At the beginning of each session, the target temperature of the TCSII was arbitrarily set to 60°C. Stimuli were delivered by pairs; a pair of stimuli is defined as the consecutive administration of one stimulus of each device. After each trial, the target of the LSD and the probe of the TCSII were slightly displaced in order to avoid nociceptor sensitization and/or habituation. Each session was as follows: pairs of stimuli were delivered to the skin of the subject; after each pair, the subject was asked which stimulus was the most or the less painful among the two. If the TCSII stimulus was more intensely perceived than the LSD stimulus, its temperature was decreased by 1°C, else, it was increased by 1°C. For safety issues regarding potential skin lesions, the maximum temperature delivered

by the TCSII was 70°C. The pairs of stimuli were repeated until achievement of four staircase reversals. A reversal is defined as the occurrence of a change in the comparative perception after a modification of the TCSII stimulus temperature, i.e., when a stimulus comes from being described as less (or more) painful to being described as more (or less) painful (than the comparison stimulus). The threshold temperature (i.e., the temperature at which stimuli delivered with the TCSII match the perception of the 55°C LSD-stimulus) was obtained at single-subject level by averaging the temperatures at which the four staircase reversals occurred.

Ten healthy volunteers (4 females and 6 males, aged 22-33) were recruited for this part of the study. The average threshold temperature obtained across the participants was 61.5±1.8 °C. This temperature has been rounded to 62°C for Part 3 of this experiment.

4.3.3. PART 3 - COMPARING BRAIN RESPONSES ELICITED BY PERCEPTION-MATCHED STIMULI

In this Part 3, the experimental design of Part 1 was replicated to compare the brain-responses elicited by the two devices at the temperature of 62°C for the TCSII (i.e., the temperature determined in Part 2) and 55°C for the LSD. Stimulation surface, stimulus duration and heating slopes remained unchanged. Active post-stimulus cooling was disabled from TCSII to better match the temperature profile of the LSD (see Figure 4.8). For further details regarding the EEG recording and experimental design, please refer to Part 1 of this experiment.

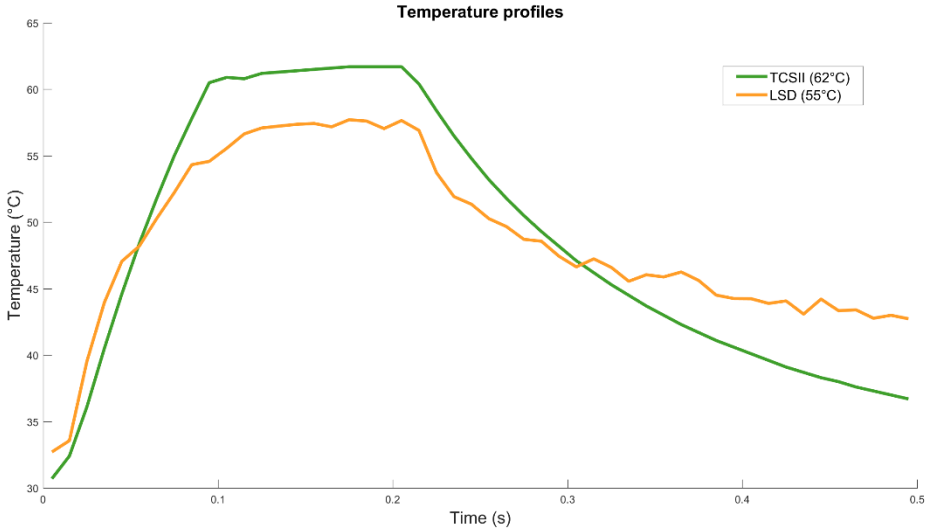


Figure 4.8. Temperature profiles of the LSD at 55°C and of the TCSII at 62°C as target temperatures. On the x-axis, time since onset of the stimulus.

Twelve healthy subjects were included in the study (7 females and 5 males, 10 right-handed, aged 20-28). After averaging, a clear N2-P2 complex was identified in 91.7% (11/12) and 100% of the participants using the LSD and the TCSII, respectively, contrasting with the results of Part 1. Paired-sample *t*-tests were performed to compare intensities of perception, peak latencies and amplitudes of the N1, N2 and P2 waves between conditions (LSD versus TCSII). Statistical significance was set at $p < 0.05$. Results for amplitudes, latencies, intensity of perception and p value of the paired-sample *t*-tests are all represented in Table 4.8.

Amplitudes - Mean amplitudes of the N2-P2 complex were 37.21±15.16 µV and 36.53±2.82 µV for the LSD and the TCSII, respectively. Differences in amplitudes between both devices were statistically not significant for the N2-P2 complex.

Mean amplitudes of the N1 wave were -2.83±3.72 µV and -2.84±2.93 µV for the LSD and the TCSII, respectively. Differences in amplitudes between both devices were not statistically significant for the N1 wave.

Latencies - Mean latencies of the N2 wave were 273.2±19.43 ms and 328.1±15.23 ms for the LSD and the TCSII, respectively. Mean latencies of the P2 wave

were 412.5±32.48 ms and 448.5±39.95 ms for the LSD and the TCSII, respectively. Differences in latencies between both devices are statistically significant for both N2 and P2 waves.

Mean latencies of the N1 wave were 217.5±29.02 ms and 271.8±7.43 ms for the LSD and the TCSII, respectively. Differences in latencies between both devices are statistically significant for the N1 wave.

Signal to Noise Ratio – Averaged SNR was 4.79 and 4.76 for the LSD and the TCSII, respectively. The SNR is almost identical with both devices (i.e., the ratio of SNR between LSD and TCSII is 1.006).

Intensity of perception – It was assessed using an NRS scale, using the same methodology as in Part 1. The average rating of the intensity of perception was 61.29±10.23 for the LSD and 58.67±8.74 for the TCSII. Differences in intensity of perception were assessed using a paired-sample *t*-test. The difference was not statistically significant.

Grand average – See Figure 4.9

Table 4.8. Intensity of perception, amplitude and latencies obtained with LSD at 55°C and TCSII at 62°C

	LSD (55°C)	TCSII (62°C)	Value of p
N1 Amplitude (μV)	-2.83 ± 3.72	-2.84 ± 2.93	p = 0.991 (ns)
N2 Amplitude (μV)	-18.19 ± 8.67	-16.09 ± 6.23	p = 0.157 (ns)
P2 Amplitude (μV)	19.01 ± 8.426	20.43 ± 7.56	p = 0.310 (ns)
N2-P2 Amplitude (μV)	37.21 ± 15.16	36.53 ± 12.82	p = 0.711 (ns)
N1 Latency (ms)	217.5 ± 29.02	271.8 ± 27.43	p < 0.001 ***
N2 Latency (ms)	273.2 ± 19.43	328.1 ± 15.23	p < 0.001 ***
P2 Latency (ms)	412.5 ± 32.48	448.5 ± 39.95	p < 0.001 ***
Intensity of perception (NRS)	61.29 ± 10.23	58.67 ± 8.74	p = 0.080 (ns)

Note: Average values and standard deviation of intensity of perception, amplitude and latencies obtained in the experiment. The last column indicates the p value of the paired-sample *t*-test testing for the difference between the TCSII and the LSD. *** p < .001, (ns) Nonsignificant for p value threshold set at .05. μV = microvolts; ms = milliseconds; NRS = NRS score.

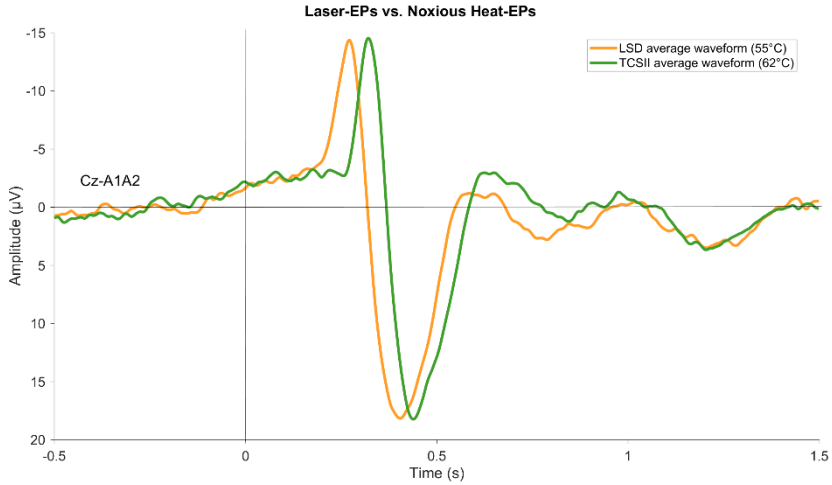


Figure 4.9. ERPs recorded after stimulation with LSD at 55°C and TCSII at 62°C. Waveforms correspond to the group-level average recorded at electrode Cz versus A1-A2.

4.3.4. Discussion

The aim of this three-step experiment was to characterize the brain responses elicited by a contact thermode (i.e., the TCSII) as compared to those elicited by a temperature-controlled CO_2-laser device (i.e., the LSD). Eliciting comparable responses with both devices would allow the TCSII to be used in a clinical setting for bedside assessment of noxious-heat related brain responses.

In the first step of the study, using stimuli matched for diameter, duration of the stimulus and temperature (55°C, i.e., above the activation threshold of the Aδ-fibers) and using their maximal heating slopes, evoked brain responses were elicited using both devices. However, the responses elicited with the TCSII showed increased latencies, lower amplitudes, lower SNR and were absent in 25% of the subjects.

In the second step of the study, we determined the temperature of the TCSII matching the perception of the 55°C laser stimulation. Using a staircase adaptative algorithm, this matching temperature was set at 62°C.

In the third step of the study, we replicated the first one using a temperature of 62°C for the TCSII, versus 55°C for the LSD. Using this temperature, we obtained visible EEG-responses in 100% of subjects with TCSII and in 92% with the LSD. The

amplitudes of the N1 wave and the N2-P2 complex as well as the SNR were not statistically different between both devices. However, a significant difference between the devices remained with regard to the latencies of those brain responses.

Regarding those findings, we can state that the differences observed in the first part of the experiment are probably due to a stronger difference between surface and depth temperature when skin is heated using contact as compared to radiant heat. A better understanding of those differences could be provided by modeling the transfer of heat through the layers of the skin to the free nerve endings according to the heating mechanism of both devices.

In summary, this validation study shows that it is possible to record robust noxious-heat ERPs using a high-speed heating contact-thermode, namely the TCSII. The observed brain responses are comparable to those obtained with the gold-standard, namely the CO_2-laser, at least in term of amplitude and SNR. Latencies are however a bit delayed using the TCSII, probably because of differences in heating mechanisms that should be further investigated in the future.

4.4. BRAIN RESPONSES TO PERIODIC THERMAL STIMULATIONS IN HEALTHY SUBJECTS

This section is adapted from the manuscript of Mulders et al. (2020)[1].

Periodic modulation of a sensory stimulus can be expected to elicit synchronized periodic activity in the neuronal population responding to the stimulus, sometimes referred to as steady-state response (SSR) (Elisabeth Colon et al., 2014). In the frequency domain, the stimulus evokes a "frequency-tagged" activity that concentrates at the frequency of stimulation and its harmonics, making it easy to isolate from non-stimulus-related activity. Interestingly, depending on the frequency of stimulation

[1] Mulders, D., De Bodt, C., Lejeune, N., Courtin, A., Liberati, G., Verleysen, M. & Mouraux, A. (2020). Dynamics of the perception and EEG signals triggered by tonic warm and cool stimulation. *PLOS One, in press.*

and, possibly, the shape of the periodic stimulus, periodic thermal stimulation can be expected to preferentially activate different types of thermonociceptors. Microneurography studies suggest that a very slow modulation frequency of 0.2 Hz elicit periodic activity in both slowly- and quickly-adapting thermonociceptors (R.D. Treede et al., 1995). Using periodic radiant heat stimulation of the skin to 50°C at this very slow oscillation frequency of 0.2 Hz, it is possible to generate a periodic EEG response predominantly conveyed by unmyelinated C-fibers, possibly by activation of slowly-adapting CMH (SC) (Elisabeth Colon et al., 2017; Meyer & Campbell, 1981). However, this technique requires the use of a temperature-controlled CO_2-laser, allowing a precise control of the skin-heating, a device which is not compatible with the use in patients with DoC.

In the present study, the TCSII, which allows to generate well-controlled cooling and warming ramps, will be used to characterize for the first time SSRs related to the sustained periodic activation of cool-sensitive afferents and compare them to those elicited by periodic noxious heat stimulations. Moreover, the temporal dynamics of the elicited SSRs will be related to the temporal dynamics of the stimulus-induced cold and heat sensations. Sustained or repeated stimulation of thermonociceptors can be expected to induce some amount of peripheral habituation (U. Baumgärtner et al., 2012) and/or activity dependent slowing. Therefore, taking advantage of the fact that the thermal stimulator allows separately controlling five different zones of the probe contacting the skin, we also assessed the effect of displacing the stimulated skin area across the stimulation cycles. All stimuli consisted in a sinusoidal temperature profile, repeated 15 times, oscillating at 0.2 Hz between a neutral temperature of 31°C and either 14°C (cool stimulation) or 48°C (heat stimulation). These temperatures were selected for two main reasons: first, the amplitude of the cool and heat stimuli was thus identical (Δ = 17°C); second, the temperature used with the TCSII in the previous experiment (i.e., 62°C) was too high for such long-lasting stimuli (i.e., 75 s), resulting in skin burns.

In a first experiment, we collected continuous subjective intensity ratings from healthy subjects exposed to warm and cool stimuli applied to a fixed or varying area of

the skin along the stimulation cycles. In a second experiment, we recorded the EEG of healthy subjects exposed to the same stimuli, allowing to confront the observed EEG features with subjective perception.

4.4.1. MATERIAL AND METHODS

All the stimuli considered in this study were delivered using the TCSII (for further details, see Section 1.2.2). As a reminder, the stimulation probe contains 15 micro Peltier elements organized in five zones of around 24mm². The temperature is controlled independently in each zone, allowing to vary the stimulated skin surface without displacing the probe. Temperature of the skin is continuously monitored during the delivery of the stimulus through thermocouples present in each of the five stimulation zones.

All stimuli consisted in 15 periods of 0.2 Hz sinusoidal cooling (between 31°C and 14°C) or warming (between 31°C and 48°C) of the skin. Each stimulus thus lasted 75 seconds (15 cycles of 5 seconds). The maximal warm temperature of 48°C was chosen such as to recruit the largest amount of heat-sensitive thermonociceptors while avoiding burn lesions due to stimulus duration (Wooten et al., 2014). The frequency of 0.2 Hz was selected because previous studies showed that, at this frequency, radiant heat stimuli generate clear SSRs related to the activation of heat-sensitive C-fiber afferents (Elisabeth Colon et al., 2017). The same frequency, stimulus duration and amplitude of temperature variation was used for cool stimulation, such as to allow a direct comparison of heat and cool-evoked SSRs.

A - EXPERIMENT 1: TIME COURSE OF HEAT AND COOL PERCEPTION

The goal of this first experiment was to assess the time course of the perception elicited by long-lasting periodic cool and warm stimuli oscillating at a frequency of 0.2 Hz. In order to study the effect of displacing the stimulated skin surface, three types of stimulation profiles were used for both cool and warm stimulation: (1) synchronous activation of all five zones of the probe ("large-fixed"). (2) two alternating zones per cycle ("small-variable"), and (3) two fixed zones during the entire stimulation

("small-fixed"). We chose to stimulate using 2/5 of the probe because the sensation elicited by only one zone was very weak. The temperature profiles are illustrated in Figure 4.10a Two epochs were delivered for each condition, in a randomized order across participants, on either the left or the right volar forearm. The probe was manually displaced after each stimulus of 75 seconds to avoid trial-to-trial habituation or sensitization.

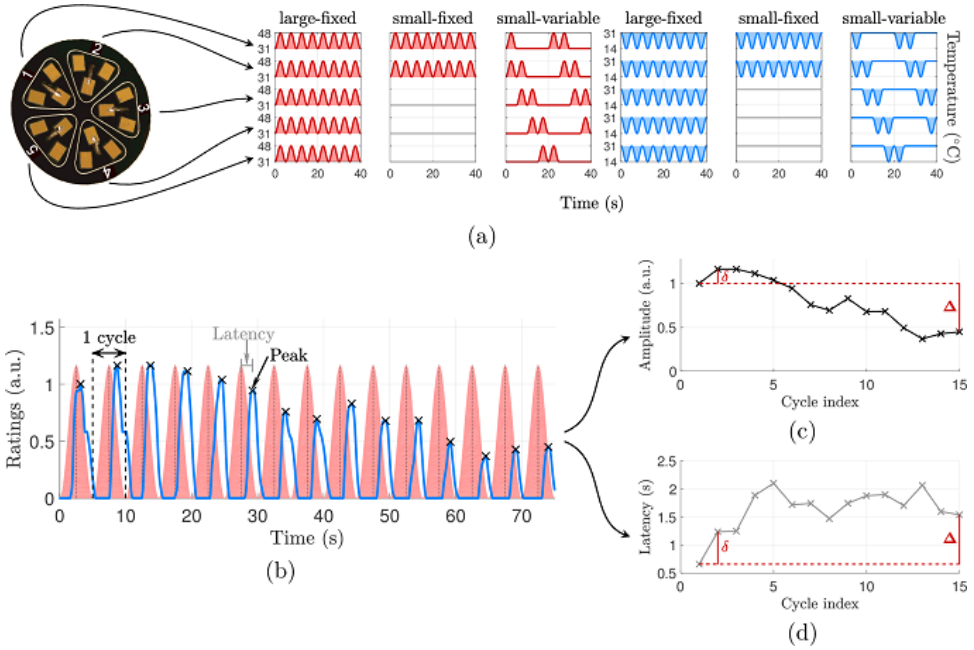


Figure 4.10. Periodic cool and warm stimulations employed, and intensity ratings collected. (a) Stimulation surface of the TCSII and stimulation temperature profiles considered in Experiment 1, depicted in red (resp. blue) for the warm (resp. cool) stimuli and truncated to 40 seconds for readability. The temperature profiles of the small-fixed and small-variable conditions were also employed in Experiment 2. (b) Example of intensity ratings from one subject during one stimulus (warm large-fixed). The temperature waveform is shaded in red and the blue curve is the intensity rating. In each cycle of 5 seconds, the amplitude of the rating peak and its latency compared to the corresponding temperature peak were computed. (c-d) Definition of the first and final differences, denoted respectively by δ and Δ, of (c) the rating peaks amplitude and (d) latencies along cycles. Figure adapted from Mulders et al. (2020)

During stimulation, the subject was asked to continuously rate the perceived intensity of the stimulus on a visual analog scale (VAS). The instructions were the same for both warm and cool stimulations (i.e., the subjects were instructed to focus more on the intensity than on the painful percept elicited by warm stimuli). The participant had to displace continuously, all along the duration of the stimulation, a 10 cm vertical slider with the contralateral hand. The extremities of the slider were defined as "lowest reported intensity" and "highest reported intensity". The continuous ratings were digitized at 1000 Hz with an analog/digital converter (USB-6343, National Instruments, Texas). The two epochs of each condition were then averaged.

To study the time courses of the intensity of the ratings and how they were affected by the temperature (warm or cool) and the way the stimulation probe was employed, some features were extracted and analyzed as follows. For all statistical tests mentioned, significance level was set to 5%.

- Average features. For each intensity rating waveform, per subject and type of stimulus, the point of maximum rating was identified in each of the 15 stimulation cycles. These maximum ratings and their latencies relative to the stimulation cycle (0 second corresponding to when the temperature change relative to baseline was maximal) were averaged across stimulation periods. These two measures were then compared across stimulation conditions using a two-way ANOVA with the factors temperature (cool or warm) and surface (large-fixed, small-fixed, small-variable). Whenever the effect of one of the factors was significant, post-hoc paired sample *t*-tests were conducted with Holm-Bonferroni correction (Shaffer, 1995). Furthermore, for each type of stimulus, the relative delay between the maximum intensity rating and stimulation was assessed by comparing the average latencies of maximum rating against 0 using one-sample *t*-tests.

- Temporal dynamics of heat and cool perception across stimulation cycles. Each rating time course was then normalized such that the maximum and minimum ratings during the first stimulation cycle correspond to 0 and 1 respectively, as illustrated in Figure 4.10b. This allowed characterizing the temporal dynamics of intensity ratings

along the stimulation cycles without being affected by initial differences in rating amplitude across conditions. These waveforms normalized to the ratings of the first stimulation cycles were used to extract the maximum rating of each stimulation cycle (expressed relative to the rating of the first stimulation cycle) and its latency. Within these normalized ratings, if the intensity of the stimulus-evoked sensation tended to increase (resp. decrease) across stimulation cycles, this would result in maximum ratings becoming greater (resp. smaller) than 1. Since the evolution of the intensity of a long-lasting sensation is typically nonlinear, with an early strong decrease (resp. increase) rate when habituation (resp. sensitization) occurs (Greffrath et al., 2007; Kleinböhl et al., 2006; Mancini et al., 2018; Smith et al., 2008) the changes in maximum ratings and in latencies of the maximum ratings across stimulation cycles were characterized both between the first and the second cycle (to assess immediate changes in perception already occurring after the first cycle), and between the first and the last cycle (to assess global changes in perception occurring across the 15 cycles). The immediate and global changes in rating intensities and latencies, denoted respectively by δ and Δ, are illustrated in Figure 4.10c-d. These quantities were compared across stimulation conditions using two-way repeated measures ANOVAs with the factors 'temperature' (cool or warm) and 'surface' (large-fixed, small-fixed or small-variable) as within-subject fixed factors. Post-hoc paired *t*-tests were conducted when justified. In addition, one sample *t*-tests were employed to assess the significance of each of these changes against 0.

B - Experiment 2: electroencephalographic recordings

In this second experiment, the EEG was recorded while participants were exposed to small-surface cool and heat stimuli delivered using two zones of the contact probe at either a fixed or variable skin location, resulting in four conditions: cool-variable, cool-fixed, warm-variable and warm-fixed. Such as in Experiment 1, each stimulus lasted 75 seconds (15 periods of a 0.2 Hz sinusoidal waveform). Each type of stimulus was repeated 12 times, presented in a randomized order. Inter-stimulus interval was self-paced by the experimenter and varied between 10 and 20 seconds. The TCSII probe

was manually displaced after each stimulus on the volar forearm of the subject. Such as in Experiment 1, a significance level of 5% was used for all statistical tests.

- EEG recording and preprocessing. The EEG was recorded using 64 Ag-AgCl electrodes, whose impedances were kept below 10 kΩ, placed on the scalp according to the international 10/10 system (WaveGuard 64-channel cap; Advanced Neuro Technologies). Signals were amplified and digitized at 1000 Hz, with an average reference. The EEG recordings were analyzed offline using Matlab R2017a (The MathWorks), and the preprocessing was performed using Letswave 6 (http://1etswave.org) (A. Mouraux & Iannetti, 2008). All signals were high-pass filtered above 0.05 Hz to remove slow drifts with a 4th order Butterworth filter. The epochs were defined by segmenting the EEG from 0 to 75 seconds after each stimulation onset. Epochs containing large artifacts were rejected by visual inspection, leading to an average (± standard deviation) of 9.1±2.5, 8.9±2.0, 9.1±2.1, and 9.4±2.4 epochs remaining for the analyses of the responses elicited by cool-variable, cool-fixed, warm-variable and warm-fixed stimulation, respectively.

- Frequency domain analysis. Average waveforms were computed for each subject and condition. To identify the presence of a periodic EEG response at the frequency of stimulation and its harmonics, the Fourier Transform (FT) of each 75 seconds average waveform was then computed per subject, stimulation condition and electrode, using an average reference. The significance of the signal amplitude at the stimulation frequency and its first harmonics was then assessed. To do so, the noise level at each frequency was estimated using the average amplitude at eight neighboring frequencies (four higher and four lower frequencies) and was removed from the spectrum, resulting in a noise-subtracted spectrum (Bach & Meigen, 1999; André Mouraux, Iannetti, et al., 2011). The significance of the noise-subtracted amplitudes at the frequencies of interest (i.e., the harmonics) $f_k = k \cdot 0.2$ Hz, for $k = 1, \ldots, 5$, was then tested using one sample *t*-tests against 0. The noise-subtracted amplitudes at the stimulation frequency were also compared across stimulation conditions using a

two-way repeated measures ANOVA with the factors "temperature" (cool or warm) and "surface" (fixed or variable). Post-hoc paired *t*-tests were conducted when justified.

- Time domain analysis. The 75 seconds average waveforms were further segmented in 15 periods of 5 seconds and averaged to analyze the stimulus-induced periodic EEG waveform and assess its latency across the different conditions. Response latency was defined as the difference between the peak of the EEG response and the latency of maximum temperature change. Latencies were compared across conditions using a two-way repeated measures ANOVA with the factors "temperature" (cool or warm) and "surface" (fixed or variable). Paired *t*-tests were used for post-hoc comparisons. Furthermore, for each type of stimulus, the relative delay between the EEG response and stimulation was assessed using one-sample *t*-tests against 0.

- Modulation of ongoing oscillations. Periodic sensory stimulation can also be expected to induce a periodic modulation of the magnitude of ongoing EEG oscillations (Elisabeth Colon et al., 2017; Liberati et al., 2019). In order to assess whether periodic 0.2 Hz cool and warm stimulation delivered using a fixed or variable surface induced a periodic modulation of ongoing EEG oscillations within different frequency bands, we estimated the signal envelopes within theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (30-70 Hz) frequency bands, as follows. First, all the unaveraged EEG epochs were band-pass filtered using a 4th order Butterworth filter to retain the EEG signal within theta, alpha, beta and gamma frequency bands. A Hilbert transform was then applied to the EEG epochs such as to estimate the envelope of the signal within these frequency bands. These envelopes were then studied in the same way as the original signals. The signal envelopes were averaged across trials and then averaged across each stimulation cycle, and FT was computed.

4.4.2. RESULTS

A - EXPERIMENT 1: TIME COURSE OF HEAT AND COOL PERCEPTION

Fourteen right-handed healthy volunteers took part to this experiment (9 women and 5 men, aged 24-35 years).

The time-courses of the percepts elicited by the different warm and cool stimuli and averaged across the stimulation cycles are shown in Appendix A (see Figure A.8).

The grand average time courses of the percepts elicited by the different warm and cool stimuli along the cycles are depicted in Figure 4.11a. The outcomes of the ANOVAs assessing the effects of stimulation temperature and surface on intensity ratings are summarized in Table 4.9. Subsequent post-hoc comparisons are illustrated in Figure 4.11 and the results are detailed hereunder.

- Average features. The intensity of the percept elicited by periodic warm stimulation was greater than the intensity of the percept elicited by periodic cool stimulation, regardless of the stimulation surface employed ("Mean peak" in Table 4.9). The latency of the sensation elicited by cool stimulation was significantly shorter than the latency of the sensation elicited by warm stimulation ("Mean latency" in Table 4.9). Furthermore, for cool stimulation, the average intensity of the percept was lower when stimuli were delivered using a fixed surface as compared to a variable surface (small-fixed versus small-variable; Figure 4.11b). Cool stimulation using a fixed surface also increased the latency of the maximum rating as compared to stimulation using a variable surface (small-fixed versus small-variable; Figure 4.11c).

- Temporal dynamics of heat and cool perception across stimulation cycles. Both for cool and warm stimulation, and regardless of whether stimulation was applied using a fixed versus a variable surface, the amplitude of the cyclic variations in ratings induced by the periodic stimulus tended to decrease along the stimulation cycles (Figure 4.11a). This habituation of perception appeared to be stronger for cool stimulation as compared to warm stimulation, especially when stimulation was delivered using a fixed surface.

- *Immediate changes in perception occurring after the first stimulation cycle.* As shown in Figure 4.11d, there was no marked change in the intensity of the percept elicited by warm stimulation between the first and the second stimulation cycle. In contrast, maximum ratings of the intensity of the percept elicited by cool stimulation tended to decrease from the first to the second stimulation cycle, especially for stimuli delivered

using a fixed surface. The ANOVA revealed significant main effect of temperature (cool versus warm) on the change in rating between the first and second cycle of stimulation, but no main effect of stimulation surface (fixed versus variable), and no interaction between the two factors ("δ Peak" in Table 4.9). The latency of the maximum rating of intensity of perception also differed between the first and the second stimulation cycle. The ANOVA showed a main effect of temperature, a main effect of stimulation surface, and an interaction between the two factors ('δ Latency' in Table 4.9). Post-hoc comparisons showed that all rating latencies increased in the second compared to the first cycle, and that this increase was larger for cool compared to warm stimulation when the stimulation surface was fixed (Figure 4.11e).

Table 4.9. ANOVAs for the features of the intensity ratings during periodic cool and warm stimulations.

		Temperature			Surface			Temperature*Surface		
		F	Prob>F	η_p^2	F	Prob>F	η_p^2	F	Prob>F	η_p^2
Mean	Mean peak (a.u.)	25.294	**0.000**	0.658	14.537	**0.000**	0.517	3.569	**0.043**	0.222
	Mean latency (s)	46.763	**0.000**	0.781	8.040	**0.013**	0.378	6.724	**0.005**	0.350
Early	δ Peak (%)	8.606	**0.011**	0.395	0.237	0.790	0.017	1.016	0.377	0.075
	δ Latency (s)	14.313	**0.002**	0.516	10.398	**0.000**	0.438	10.515	**0.000**	0.457
Global	Δ Peak (%)	6.058	**0.028**	0.314	10.107	**0.001**	0.428	0.558	0.579	0.043
	Δ Latency (s)	3.291	0.092	0.200	0.009	0.951	0.001	1.360	0.275	0.098

Note: Outcomes for the main effects and interactions from the repeated measures ANOVA performed for the average (two first rows) and dynamical (four last rows) features of the intensity ratings. Partial eta squared (η^2_P) are indicated for the effect sizes and the p-values smaller than the significance level of 0.05 are in bold. Table from Mulders et al. (2020)

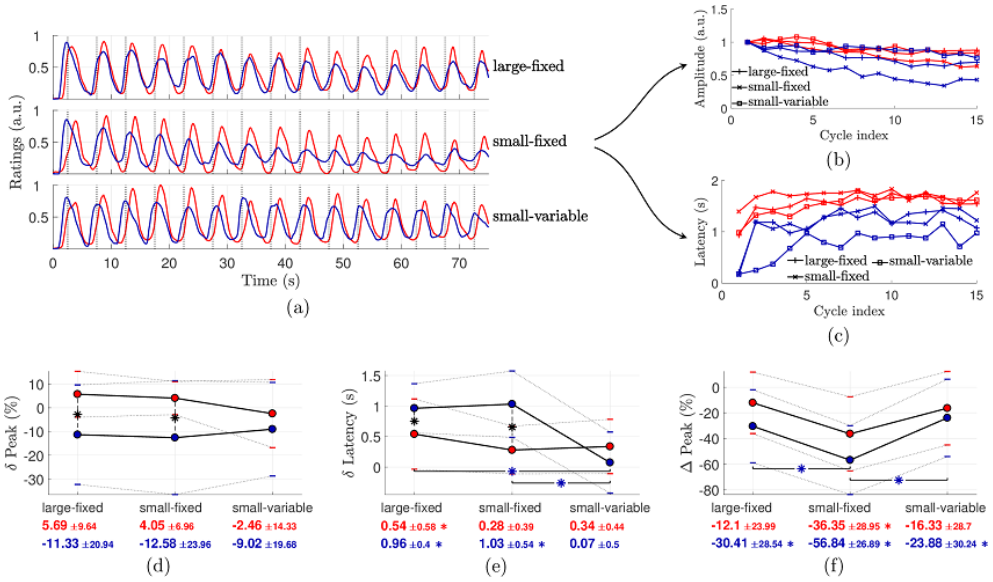


Figure 4.11. Intensity ratings dynamics during periodic cool and warm stimulations. (a) Group-level average intensity ratings along the cycles, in (light) red and (dark) blue for the warm and cool conditions respectively. The vertical dotted lines indicate the times of maximum temperature change (2.5 s after the beginning of each stimulation cycle). From each normalized individual time course, the rating peaks and their latencies within each cycle are extracted, their grand average being illustrated in (b) and (c). (d-e-f) Pairwise comparisons of (d) the early change in peak intensity rating, (e) the early change in peak latency and (f) the global change in peak intensity rating. The red (resp. blue) dots show the mean features for the warm (resp. cool) stimulation, the standard deviations being indicated with horizontal bars. These means and standard deviations are also reported below the plots with the corresponding color. Each asterisk in the plot indicates a significant difference according to paired samples *t*-tests with Holm-Bonferroni correction, in red, blue (horizontally) or black (vertically) respectively when the two compared conditions are warm, cool or different. An asterisk besides an x-axis tick label shows that the corresponding mean feature is significantly different from 0 based on one sample *t*-tests with Holm-Bonferroni correction. Figure adapted from Mulders et al. (2020)

- *Global changes in perception occurring after 15 stimulation cycles.* The ANOVA comparing the changes in intensity ratings between the first and the last stimulation cycle revealed significant effects of both temperature and surface ("Δ Peak" in

Table 4.9). Paired comparisons showed that, for cool stimulation, the global decrease in perception was stronger when stimulation was delivered using a fixed surface as compared to a variable surface (Figure 4.11f). The decrease in perception across the stimulation cycles was also greater for cool compared to warm stimulation, although the differences were not significant. The ANOVA comparing the changes in rating latencies did not show any significant effect ("Δ Latency" in Table 4.9).

B - EXPERIMENT 2: ELECTROENCEPHALOGRAPHIC RECORDINGS

Fifteen healthy right-handed healthy volunteers took part in this Experiment 2 (9 women and 6 men, aged 21-34 years)

- Frequency domain analysis. The EEG frequency spectra at electrode FCz obtained during warm and cool stimulation using a fixed or variable surface are shown in Figure 4.12a. FCz electrode was selected for illustration as the responses were expected to be close to maximal, at least for warm stimulations (Elisabeth Colon et al., 2017); however, frequency analysis was computed at each electrode. For cool stimulation, a significant but small response was observed at the frequency of stimulation (0.2 Hz) when stimulation was delivered using a variable surface, and no response was observed when stimulation was delivered using a fixed surface. For warm stimulation, a markedly greater response was observed, both when delivered using a fixed surface and when delivered using a variable surface. The increase was significant at the frequency of stimulation (0.2 Hz) and the three following harmonics (0.4, 0.6 and 0.8 Hz). The ANOVA conducted on the noise-subtracted amplitude at 0.2 Hz revealed main effects of temperature and surface (Table 4.10). Post-hoc comparisons showed that the periodic EEG response was greater for warm versus cool stimulation, and greater for stimulation using a variable versus fixed surface (Figure 4.12b).

Group-level average scalp topographies of the noise-subtracted amplitudes at 0.2 Hz are shown in Figure 4.12a. In all conditions in which stimulation elicited a significant periodic EEG response, its topography was maximal over fronto-central electrodes, and symmetrically distributed over the two hemispheres.

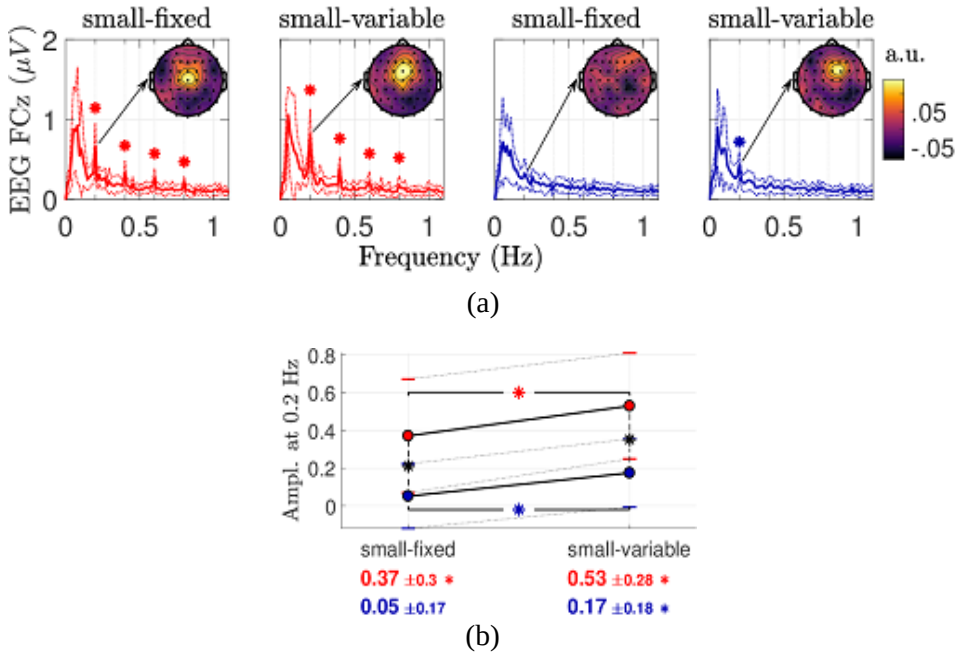


Figure 4.12. Frequency analysis of EEG responses to periodic warm and cool stimulations. (a) Group-level average (± standard deviation in dotted) Fourier transforms of the EEG signals at electrode FCz. A star indicates significance of the noise-subtracted peak at $\{k \cdot 0.2\}_{k=1}^{5}$ Hz (*t*-tests against 0). The scalp maps show the distributions of the noise-subtracted amplitudes at 0.2 Hz. (b) Pairwise comparisons of the noise-subtracted EEG amplitudes at 0.2 Hz at electrode FCz. The red (resp. blue) dots show the mean amplitudes for the warm (resp. cool) stimulation, the standard deviations being indicated with horizontal bars. These means and standard deviations are also reported below the plots with the corresponding color. Each asterisk in the plot indicates a significant difference according to paired samples *t*-tests, in red, blue (horizontally) or black (vertically) respectively when the two compared conditions are warm, cool or different. Figure adapted from Mulders et al. (2020)

Table 4.10. ANOVAs for the features of the EEG signals elicited by periodic cool and warm stimulations

	Temperature			Surface			Temperature*Surface		
	F	Prob>F	η_p^2	F	Prob>F	η_p^2	F	Prob>F	η_p^2
Ampl. at 0.2 Hz	36.883	**0.000**	0.725	13.272	**0.003**	0.487	0.144	0.710	0.010
Mean lat. (s)	22.196	**0.000**	0.613	0.141	0.713	0.010	1.383	0.259	0.090
AUC at 0.2 Hz	16.790	**0.001**	0.545	4.295	0.057	0.235	0.590	0.455	0.040

Note: Outcomes of the ANOVA performed to assess the main effects of the temperature and surface and their interaction on the EEG signals at FCz. Partial eta squared (η^2_P) are indicated for the effect sizes and the p-values smaller than the significance level of 0.05 are in bold. Table from Mulders et al. (2020)

- Time domain analysis. The EEG signals averaged across all stimulation cycles are depicted in Figure 4.13a. At electrode FCz, the EEG response elicited by warm stimulation consisted in a positive wave peaking approximately 1 second after the peak of heat stimulation. For cool stimulation the response also appeared to consist of a positive wave, but its amplitude was much smaller than for warm stimulation, especially when stimulation was delivered using a fixed surface. The peak latency of the response to cool stimulation was also much shorter than the peak latency of the response to heat stimulation (Figure 4.13b; Table 4.10).

- Modulation of ongoing oscillations. The results of the analysis of the envelopes within theta, alpha, beta and gamma frequency bands are provided in Appendix A (see Figures A.9, A.10, A.11 and A.12). Warm stimulation induced a periodic modulation (reduction) of the power of alpha- and beta-band oscillations, associated with a scalp topography maximal over the parietal area contralateral to the stimulated arm, compatible with previous observations (Elisabeth Colon et al., 2017). Both warm and cool stimulation was associated with a periodic 0.2 Hz modulation of power in the gamma-band, maximal at electrode Fz. All responses tended to decrease along the stimulation cycles.

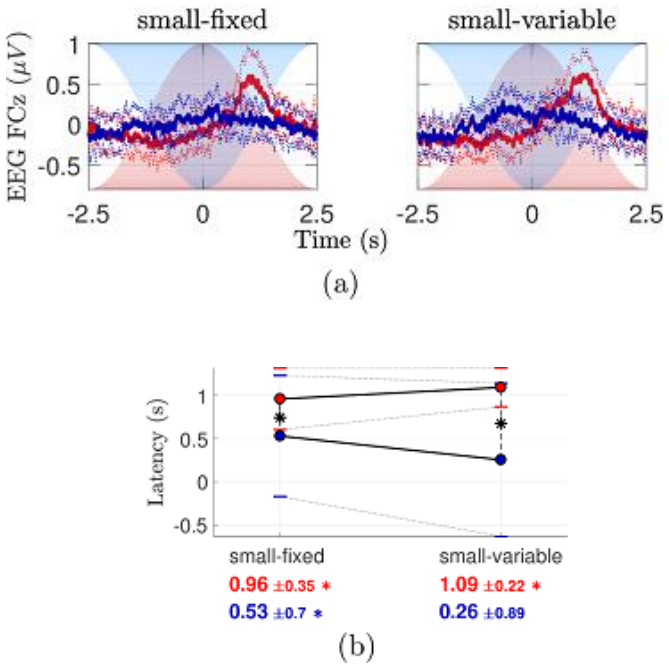


Figure 4.13. EEG averaged across warm/cool stimulation cycles. (a) Group-level average (± standard deviation in dotted) time courses of the EEG signals at electrode FCz averaged across stimulation cycles, in (light) red and (dark) blue for the warm and cool conditions respectively. The stimulation temperatures are shaded. (b) Pairwise comparisons of the latencies of the EEG peaks compared to the temperature peaks. The red (resp. blue) dots show the mean latencies for the warm (resp. cool) stimulation, the standard deviations being indicated with horizontal bars. These means and standard deviations are also reported below the plots with the corresponding color. Each asterisk in the plot indicates a significant difference across temperature according to paired samples *t*-tests. An asterisk besides an x-axis tick label shows that the corresponding mean latency is significantly different from 0 based on one sample *t*-tests with Holm-Bonferroni correction. Figure adapted from Mulders et al. (2020)

4.4.3. Discussion

The objective of this study was to compare the perception and EEG responses elicited by long-lasting warm and cool stimuli frequency-tagged by slowly and

periodically varying their intensity over time at a frequency of 0.2 Hz and applied either to the same patch of skin or to a varying patch of skin along the stimulation periods.

In previous studies using laser heat stimulation, it was shown that slowly and sinusoidally heating the skin between baseline and 50°C at a frequency of 0.2 Hz elicits a periodic EEG response mainly driven by the activation of unmyelinated C-fibers (Elisabeth Colon et al., 2017). Indeed, selectively blocking the conduction of Aδ-, thinly myelinated, fibers did not alter the elicited SSRs. In the present study, periodic contact heat stimulation at 0.2 Hz elicited a similar SSR, maximal at the scalp vertex and symmetrically distributed over the two hemispheres. Furthermore, such as in Colon et al., periodic contact heat stimulation at 0.2 Hz elicited a periodic modulation of ongoing oscillations, with a scalp topography maximal over parietal regions contralateral to the stimulated limb.

Innocuous periodic cooling of the skin at the same frequency also elicited a periodic EEG response. However, as compared to periodic heat stimulation, the magnitude of cool-evoked SSRs was markedly lower. This indicates that, at 0.2 Hz, the periodic activity generated by the activation of cool-sensitive afferents using contact cooling of the skin is not as strong as the periodic activity generated by the activation of heat-sensitive afferents.

Both heat- and cool-evoked responses tended to attenuate along the stimulation cycles. Possible explanations for this response attenuation are receptor fatigue or adaptation at peripheral level and /or habituation processes occurring at the level of the central nervous system (Greffrath et al., 2007). This response attenuation could explain why both heat-evoked and cool-evoked SSRs tend to be of smaller magnitude than the SSRs typically elicited by other types of stimuli, such as vibrotactile, visual or auditory stimuli (Elisabeth Colon et al., 2014; Norcia et al., 2015; Nozaradan, 2014). Assuming that habituation at central level would be similar for periodic heat and cool stimulation, the finding that attenuation of the EEG response and perception over time is stronger for cool as compared to heat stimulation suggests a significant contribution of peripheral mechanisms differentially affecting the responsiveness of heat versus

cool-sensitive afferents. Further supporting a contribution of peripheral mechanisms is the finding that the response attenuation was less pronounced when the stimulated skin area was varied along the stimulation cycles, i.e., when different free nerve endings were exposed to heat or cold across the stimulation cycles (Greffrath et al., 2007). Notably, when Colon et al. recorded heat-evoked SSRs using infrared laser stimulation, the laser beam was displaced between each stimulation cycle such that stimulation was never repeated at the same skin area over the entire duration of the periodic stimulus. Using such stimuli, no habituation of perception or SSRs was observed.

Another marked difference between the responses elicited by periodic heat and cool stimulation was a difference in latency. Regarding the time course of perception, the periodic variations of the intensity of heat perception were delayed by approximately 0.8 seconds as compared to the periodic variations of the intensity of cool perception. A similar delay of approximately 0.8 seconds was observed when comparing the time course of heat- and cool-evoked SSRs. These differences in response latency should be interpreted with caution, as they could be explained by at least three factors. First, these differences could be related to differences in peripheral conduction times, considering that the responses elicited by sinusoidal heating of the skin at 0.2 Hz are predominantly related to the activation of slowly-conducting unmyelinated C-fibers (Elisabeth Colon et al., 2017), whereas cool stimuli might be conveyed exclusively by faster-conducting thinly-myelinated Aδ-fibers (Dubin & Patapoutian, 2010; Schepers & Ringkamp, 2010). However, the observed latency differences could also be explained by differences in timings of peak discharge frequency and in relative activation thresholds. As mentioned in Section 1.2.1., C-fibers thermonociceptors can be either quickly adapting (QC) or slowly adapting (SC) (Meyer & Campbell, 1981; Wooten et al., 2014). SCs respond more gradually to the step increase in skin temperature, with a peak discharge approximately 2 seconds after stimulation onset, and then tend to maintain a tonic level of activity during the entire stimulus duration. In fact, it is for this reason that we had hypothesized that the responses elicited by slowly heating the skin using a 0.2 Hz sinusoidal pattern predominantly generates activity within SCs. The thermal activation threshold of SCs

has been shown to be around 46°C (Wooten et al., 2014). The response properties of cool-sensitive free nerve endings have not been characterized as extensively (Dubin & Patapoutian, 2010; Schepers & Ringkamp, 2010). Humans are able to detect transient decreases in skin temperature of as little as 0.2-0.5°C (Dyck et al., 1993), and to respond sharply at the onset of the stimulus (Darian-Smith et al., 1973). Therefore, when cooling the skin progressively from baseline to 14°C, activation of cool-sensitive afferents may be expected to occur earlier than the activation of SCs when the skin was progressively heated to 48°C, both because thermal activation threshold was reached earlier in the stimulation cycle for cool stimulation as compared to heat stimulation, and because cool-sensitive afferents may be expected to reach peak discharge rate earlier than SCs (Darian-Smith et al., 1973; Wooten et al., 2014).

Interestingly, the latency of the peak of cool perception across stimulation cycles was markedly affected by varying the stimulated skin area, with an important increase of latency occurring after the first stimulation cycle when the surface was kept fixed. This phenomenon could be explained by activity-dependent slowing (Serra et al., 1999) and/or peripheral adaptation of cool-sensitive receptors, leading to an increase in their threshold (Smith et al., 2008).

In summary, both sinusoidal contact heat stimulation and sinusoidal contact cold stimulation at 0.2 Hz elicit a sensation whose intensity varies periodically over time. Furthermore, both stimuli elicit a periodic EEG response at 0.2 Hz and its harmonics, although the EEG response elicited by cool stimulation is of much lower magnitude than the EEG response elicited by warm stimulation. The latencies of the perception and EEG responses elicited by cool stimulation were, on average, shorter than those elicited by warm stimulation. This latency difference was most pronounced during the first cycle of stimulation. Both the perception and the EEG responses to warm and cool stimulation tended to habituate over time. This habituation was stronger for cool stimulation as compared to warm stimulation. Response habituation was less pronounced when stimuli were delivered using a variable surface as compared to a fixed surface. Overall, SSRs to periodically modulated thermal stimuli display a rather low

SNR as compared to other sensory modalities. Therefore, the applicability of this technique to patients with DoC is questionable.

4.5. CONCLUSION

The studies of this chapter aimed to improve the neurophysiological approach at bedside of patients with DoC by three means : the use of an EEG multimodal assessment of the somatosensory pathways; the validation (in healthy subjects) of a highly transportable device, usable at bedside, to generate selective noxious-heat ERPs; and the characterization (in healthy subjects) of SSRs to periodic thermal stimulations, hypothesizing that they would display a high SNR, a significant advantage in patients with DoC. These studies encountered their objectives as so:

- EEG brain responses to noxious-heat, cool and vibrotactile stimuli are identifiable, at least in a subset of patients with DoC. The recorded responses were all in MCS patients while they were not observed in a small sample of UWS patients. Such recordings in eMCS patients was made difficult by large movement artifacts. All these observed responses displayed a very low SNR, suggesting that alternative means are required to assess the somatosensory pathways in patients with DoC.
- Thanks to a high-speed heating contact thermode, namely the TCSII, it has been shown possible to elicit brain responses to noxious-heat stimuli. These EEG brain responses resembles those observed with the CO_2-laser, i.e., the gold-standard for assessing the integrity of nociceptive pathways. It only requires an adjustment of the target temperature (i.e., a higher target temperature is required for the TCSII as compared to the temperature-controlled CO_2-laser). The TCSII is light and highly transportable, and even allows the generation of cool stimuli, making easier the data acquisition at the bedside.
- Steady-state responses to periodic thermal (warm and cool) stimuli were characterized using a temperature-controlled contact thermode (i.e., the TCSII). Both warm and cool stimuli elicited such brain responses, however with a higher SNR for warm condition, and when the stimulation site was varied during the stimulus.

Moreover, responses to periodic thermal stimuli tended to habituate quickly over time, resulting in SSRs of smaller amplitude than those observed in other sensory modalities. Those observations compromise the translation of this technique in patients with DoC.

These studies suffer from several limitations that should be accounted for a future experimental design:

- The population of patients with DoC is very heterogeneous (etiology, topography of brain lesions, age, level of consciousness) but also limited in number, making it difficult to generalize results. This also supports the need to develop a strong methodology to assess these patients with comparable means across research groups.
- Because of this heterogeneity, brain responses tend to vary in latencies, resulting in signal cancellation during grand averaging procedure. Therefore, results should always be analyzed first at a single-subject level.
- The absence of collaboration from the patient results in large movement artifacts. Therefore, more stimulations should be used in order to be very selective when discarding epochs for artifacts. Only keeping epochs of very good quality would help increasing the SNR and the quality of the EEG preprocessing.
- SSRs display a very low SNR in response to long-lasting thermal stimuli. Moreover, applying such long-lasting stimuli (75 seconds for each epoch) requires the subject's full collaboration, which is impossible in patients with DoC.
- In the exploratory study of this chapter, noxious-heat ERPs were elicited using a Nd: YAP laser. However, and as already discussed, the use of a validated temperature-controlled device able to generate such responses is highly recommended in patients with DoC. This device should also be transportable and allow its use in a clinical setting, such it is the case for the TCSII.

CHAPTER 4: NEUROPHYSIOLOGICAL ASSESSMENT
SUMMARY OF FINDINGS

A. EEG BRAIN RESPONSES TO LASER, COOL AND VIBROTACTILE STIMULATIONS ARE IDENTIFIABLE IN A SUBSET OF MCS PATIENTS AT A SINGLE-SUBJECT LEVEL. IN THIS EXPLORATORY STUDY, NO RESPONSES WERE IDENTIFIABLE IN UWS PATIENTS.

B. THE USE OF A HIGH-SPEED HEATING CONTACT-THERMODE (TCSII) ALLOWS TO RECORD ERPS TO NOXIOUS-HEAT STIMULI COMPARABLE TO THOSE OBTAINED WITH A CO_2-LASER DEVICE, BY ADJUSTING ITS TARGET TEMPERATURE.

C. WARM AND COOL PERIODIC STIMULI ELICIT SSRS IDENTIFIABLE IN HEALTHY SUBJECTS.

LIMITATIONS

A. SNR ARE VERY LOW IN PATIENTS WITH DOC USING THESE STIMULATION PARAMETERS FOR THE RECORDING OF TIME-LOCKED ERPS.

B. FUTURE RESEARCHES SHOULD FOCUS ON THE ANALYSIS OF TIME-LOCKED BUT NON-PHASE LOCKED RESPONSES IN THE EEG.

C. AMPLITUDES AND SNR OF WARM AND COOL-RELATED SSRS ARE LOW, COMPROMISING THE TRANSLATION OF THIS TECHNIQUE IN PATIENTS WITH DOC.

CHAPTER 5. FUTURE PERSPECTIVES AND CONCLUDING REMARKS

"Of pain you could only wish for one thing:
That it should stop.
Nothing in the world was so bad as physical pain.
In the face of pain there are no heroes."

- George Orwell

Addressing the question of pain perception in patients with DoC is also addressing the question of their quality of life (QoL). According to the EuroQol group, QoL can be described in five dimensions: mobility, self-care, usual activities, anxiety/depression and pain/discomfort (R. Brooks, 2013). Trying to assess (and, hopefully, to improve) the different QoL dimensions of patients with DoC is part of the medical responsibility. If the first three are easy to assess, anxiety/depression and pain/discomfort are not. However, it is not only a matter of medical responsibility: assessing and trying to improve QoL in these patients is also a moral duty towards them. Indeed, the presence of (chronic) physical pain (alone or with the occurrence of "psychological pain") is devastating and is cited as one of the major reasons for requesting euthanasia in 96% of cases in Belgium (Commission fédérale de Contrôle et d'Évaluation de l'Euthanasie, 2018). However, due to the frequent absence of advance directives, euthanasia is very rarely applied in patients with DoC. A comprehensive approach towards the continuation or discontinuation of treatments (including withdrawal of clinically assisted nutrition and hydration) is therefore necessary and has been the topic of vast debates in the last few years (e.g., Fritz, 2017; Lejeune, 2017; Wade, 2018). Current western bioethics relies on four major principles: autonomy (including self-determination), justice, beneficence and non-maleficence. The principle of

self-determination becomes a cornerstone of decision-making processes. For instance, in Belgium, two crucial laws were enacted in 2002 in order to increase this right to self-determination; the first describes the rights of the patients, including their rights to accept or to refute any medical treatments, and the second describes the conditions for decriminalizing the act of euthanasia (Loi relative à l'euthanasie, 2002; Loi relative aux droits des patients, 2002). In this societal context, and according to the deep-seated principle of self-determination, a comprehensive approach of the end-of-life decisions process in the specific case of patients with DoC was recently developed, aiming to give a voice to patients that are unable to communicate their wills (Lejeune et al., 2020). One of the methods discussed in this paper relies on the presumed wills of the patients, based on the reconstruction of their personal history made by their relatives. However, this approach can be subject to discussion; indeed, nobody knows what it is like to be in a DoC. Although it is impossible to know it, we can try imagining what we could feel and what we would fear in this case. Some will have the fear of being locked in their own body without being able to communicate, or the fear of remaining in a persistent state of disability and being a burden for their relatives. Others will fear to feel pain or to suffer, without being able to communicate about it, without any chance to be relieved. Hence, knowing that we will be carefully assessed and managed for pain may change our perspective when writing our advance directives.

Although we are still far from being able to ensure an adequate pain assessment and management, the approach proposed in this thesis is probably an option to pursue. Indeed, thanks to its complementary advantages, the combination of neurophysiological and behavioral assessment will hopefully lead to this objective. Next sections present the following steps in this direction.

Characterizing the relationships between the NCS-R and brain function status assessed using resting-state PET imaging

As discussed in Chapter 3, we are now able to better understand the meaning of a very low (NCS-R < 2) or a very high (NCS-R ≥ 5) NCS-R score. A very low score

indicates with a high sensitivity that the response is not related to a noxious stimulus, while a very high score suggests that the patients have the neural basis for experiencing pain. However, 74% of MCS patients of our database display a score below this threshold score of 5 and the relationship between brain function status in patients having intermediate scores (from 2 to 4) is unknown. This represents 59% of the patients included in our database (147/248). In the future, a retrospective study will be conducted focusing on patients having intermediate NCS-R scores. Patients will be categorized in three groups according to their behavior with regard to NCS-R items. The first group ("Reflexive behavior group") will include patients showing only reflexive behaviors (e.g., abnormal posturing, oral reflexive movement) or no reaction following nailbed pressure. The second group ("Conscious behavior group") will include patients showing at least one conscious behavior following nailbed pressure (e.g., localization to noxious stimulation, intelligible verbalization). Finally, the third group ("Behavior of uncertain significance") will include patients showing behaviors that we do not know if they are reflexive (e.g., grimace, groaning), and not showing any conscious behavior following nailbed pressure. We will then compare global and local metabolic patterns sampled using FDG-PET imaging. The insula and the ACC will be used as *a priori* regions of interest, because both regions have been shown to be consistently activated during pain experience in healthy volunteers. PET imaging data will be compared across the three groups, as well as a group of gender- and aged-matched healthy controls, using the same methodology as used in Section 3.2.

We expect that patients with only reflexive behaviors to noxious stimuli will exhibit decreased metabolism in the insula and the ACC as compared to patients showing conscious behaviors to noxious stimuli, regardless of the total NCS-R score.

Furthermore, in the group of patients showing "Behaviors of uncertain significance", we will assess the relationship between PET imaging data and each of these behaviors, with the aim of better understanding the significance of these behaviors, and whether they should be considered as purely reflexive or of higher-order.

EXTENDING AND REFINING THE NCS-R BY COMBINING AND CONTRASTING BEHAVIORAL RESPONSES TO CONTROLLED NOCICEPTIVE AND NON-NOCICEPTIVE SOMATIC STIMULI

In the current version of the NCS-R, noxious stimulation consists in applying pressure on the nailbed. However, nailbed pressure is a stimulus that is not specific for nociceptors, as it inevitably also activates low-threshold mechanoreceptors. In a future study, NCS-R scores will be compared using either a noxious stimulus that selectively activates skin nociceptors (noxious heat stimulation of the skin delivered using a temperature-controlled contact stimulator), an innocuous stimulus that selectively activates low-threshold mechanoreceptors (mechanical vibrotactile stimulus delivered using a force-controlled mechanical transducer), and an innocuous stimulus that nevertheless activates free nerve endings of the skin and is thus also conveyed to the brain via the STT (innocuous cooling of the skin using a temperature-controlled contact stimulator). For each stimulus modality, the behavior triggered by a range of stimulation intensities will be characterized, such as to build stimulus-response functions. Patients will be categorized in four groups based on their CRS-R assessment: UWS, MCS-, MCS+ and eMCS without functional communication. Assessing stimulus-response functions using a range of intensities will also allow us to examine whether different behaviors have different thresholds, and whether some behaviors are observed only for specific stimulation modalities independently of its intensity.

We hypothesize that, across patients, the behavioral responses to vibrotactile stimuli may vary differently than the behavioral responses to both noxious-heat and cool stimuli as vibrotactile inputs are conveyed to the brain through the DCML tract whereas heat and cool input is conveyed by the STT. Furthermore, we hypothesize that a certain degree of cortical integration (present in MCS patients) is required to react differentially to different types of stimuli, especially nociceptive heat versus innocuous cool inputs conveyed by the STT. Therefore, UWS patients could react in a more similar and reflexive fashion to all stimuli, whereas patients with residual cortical integration may

exhibit more conscious behaviors and react differently to nociceptive versus non-nociceptive stimuli.

CHARACTERIZING EEG RESPONSES TO NOCICEPTIVE AND NON-NOCICEPTIVE SOMATOSENSORY STIMULI IN PATIENTS WITH DOC

In the future, we aim to compare EEG responses to nociceptive and non-nociceptive stimuli conveyed by the STT or the DCML. We will test whether the ability to process nociceptive inputs, conveyed by the STT, is more resilient to brain damage as compared to the ability to process non-nociceptive inputs, conveyed by the DCML. As shown in Chapter 4, event-related brain potentials elicited by a small number of nociceptive heat or vibrotactile stimuli in patients with DoC display a poor SNR. This is probably mainly due to the extensive brain damage, the abnormal large-amplitude EEG, and numerous sources of movement artifacts, hampering their use in a clinical context. Thanks to the approaches developed in this thesis, we expect to acquire EEG responses to nociceptive and non-nociceptive somatosensory stimuli having a higher SNR. In addition, we aim to improve the blind-source separation methods in order to reduce contamination of the signals by artifacts. Time-frequency analysis of the recorded signals will also be used to extract stimulus-evoked activity that is not phase-locked to the onset of the stimulus. Moreover, as we validated the TCSII in healthy volunteers for the recording of noxious-heat EEG responses, we are now able to elicit and record EEG brain responses to both noxious-heat and cool stimuli with a single, highly transportable device that is compatible with bedside assessments. Using these approaches, we will record in patients with different levels of consciousness, brain-evoked responses to noxious heat, innocuous cold and vibrotactile stimuli. Importantly, patients with DoC exhibit important fluctuations in their level of wakefulness and awareness over time. Therefore, we will also compare stimulus-evoked EEG responses obtained during multiple sessions and relate these responses to the behavioral assessment of consciousness (multiple assessments using the CRS-R to reduce the misdiagnosis rate (Wannez et al., 2017)), as well as fluctuations of the

frequency content of their ongoing EEG, taken as a measure of "brain state". Finally, we will test whether the presence of EEG responses and heart-rate variability (Riganello et al., 2019) to noxious heat, innocuous cold and vibrotactile stimuli relates to the NCS-R scores obtained using these same stimuli.

DEVELOPING A NEUROPATHIC PAIN SCREENING TOOL FOR PATIENTS WITH DOC

Neuropathic pain is defined as *"a pain caused by a lesion or disease of the somatosensory nervous* system" (International Association for the Study of Pain, 2017). This type of pain can be characterized by ongoing pain, allodynia and/or hyperalgesia. Because patients with DoC cannot self-report pain symptoms, the occurrence of chronic neuropathic pain is probably underestimated, or even neglected.

Because at least a subset of patients with DoC are likely to be able to perceive pain, and considering that these patients often have lesions involving the somatosensory pathways (Adams et al., 2000; Jellinger, 2013), some patients with DoC are at high risk of developing neuropathic pain (NP). Unfortunately, available clinical screening tools for NP cannot be used in patients unable to communicate, and no means has yet been developed for other non-communicative patients (e.g., demented patients, infants, etc.). Existing tools rely, for instance, on subjective reports of the characteristics of pain symptoms or sensory testing.

In the future, we aim to elaborate a clinical screening tool that could be used to identify signs of NP in non-communicative patients, therefore offering new perspectives for their pain management. First, to create this Neuropathic pain Screening Tool for patients with DoC (NST-DoC), the "physical examination" part of the Standardized Evaluation of Pain (StEP) will serve as the basis, as it screens a vast majority of the clinical findings in neuropathic pain conditions (Scholz et al., 2009). However, because several items require a feedback from the patients, these items will be adapted to patients with DoC. To adapt them, we will use methodologies derived from animal models of NP (withdrawal behavior) (Yoon et al., 1994) or by measuring

muscle activity in response to a stimulus by means of electromyography. Second, we will validate the NST-DoC in a group of conscious patients presenting with NP due to a lesion of the central nervous system and a group of age- and gender-matched healthy subjects. Subjects will perform four assessments on two consecutive days with three different examiners. This will allow the assessment of intra- and inter-rater reliability, as well as concurrent validity with the Leeds assessment of neuropathic symptoms and signs (LANSS). The order of administration will be randomized across the sample of patients. The use of three different examiners will allow to remain blind regarding the results of all other behavioral assessments (Figure 5.1). Third, the scale will be tested in a group of patients with DoC diagnosed as MCS, to examine whether, in these patients, a subgroup at risk of suffering from neuropathic pain can be identified. MRI lesions of these patients versus the other patients will be compared, to examine whether differences can be observed (frequency of lesions involving somatosensory pathways).

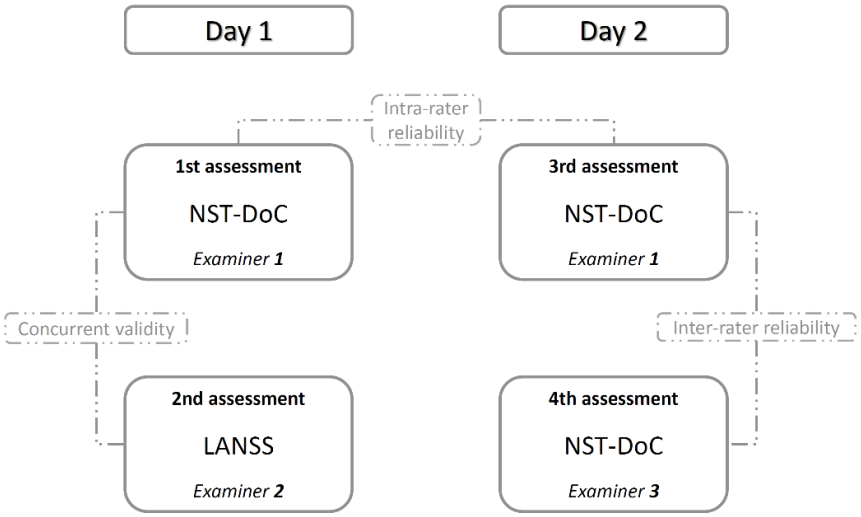


Figure 5.1. Example of assessment procedure for the validation, in conscious subjects with neuropathic pain, of the Neuropathic pain Screening Tool for patients with DoC (NST-DoC).

CONCLUDING REMARKS

Pain and consciousness are intimately related cognitive processes. Indeed, the complete disruption of consciousness imply the absence of conscious pain experience. Studying patients with DoC to assess their ability to perceive pain constitutes a unique pathological model: they offer us the opportunity to study how a damaged brain is able to generate a conscious experience of pain. In this thesis, we aimed to improve the understanding of existing tools and how they are related to the conscious experience of pain. We also tried to develop a methodology to investigate the processing of somatosensory inputs, specifically dedicated to the patients with DoC.

Understanding this complex relationship is a major challenge in neurosciences, and there is still a long way to go; but just because it is difficult does not mean it is impossible. Moreover, understanding this relationship is also a responsibility of medical and research teams, for the sake of these extremely vulnerable patients. This imply that each step allowed by these researches, even if contributing more to the fundamental of neurosciences, should always be thought to be, at least partially, also beneficial for the patients. Thinking about the clinical implications of these researches is however obvious when you once took care of these patients. Who can say s/he has never been shaken by the feeling of distress that may emanate from them, when they look (or they are) in pain? Who can say s/he has never felt helpless, wondering how to relieve them, or what lies behind their facial expressions? Is it always about pain? Or anxiety? Or psychological distress? Unfortunately, no tool does exist to date to assess it.

The concept of pain is probably universal, even if some languages do not have a strict translation for this concept (Wierzbicka, 2012) and that representations of pain vary across socio-economic classes, cultures or religion. Almost every human has experienced it at least once in his life, and almost anyone has already seen the expression of pain on another human being. When observing the expression of pain in someone else, brain areas involved in the affective process of pain are activated, resulting in a feeling of empathy for this person (Singer et al., 2004). These empathic processes happen every day in professional caregivers, when their patients are in pain and report

the burden of their suffering, the unbearableness of the pain. However, these empathic processes can be undermined when the patients are not able to communicate, to share directly their feelings with the caregivers. If we consider that showing empathy towards somebody is recognizing him/her as a human, the pitfall is that patients unable to express their pain (i.e., limiting therefore the empathy towards them) might be seen a little less than human beings.

As a warning signal of potential threat for our body, pain has always been intimately linked to survival. But if pain is so deeply related to survival, is the absence of it related to death? Do we need pain to feel alive? Do we define ourselves by our relationship to pain? "I feel pain, therefore I am"? Whether this statement is true or not, one essential purpose of assessing the ability to perceive pain of patients with a disorder of consciousness, is helping to restore their condition as human beings in their own rights.

REFERENCES

Adams, J. H., Graham, D. I., & Jennett, B. (2000). The neuropathology of the vegetative state after an acute brain insult. *Brain: A Journal of Neurology*, *123 (Pt 7)*, 1327-1338. https://doi.org/10.1093/brain/123.7.1327

Adler, J., Giabbiconi, C.-M., & Müller, M. M. (2009). Shift of attention to the body location of distracters is mediated by perceptual load in sustained somatosensory attention. *Biological Psychology*, *81*(2), 77-85. https://doi.org/10.1016/j.biopsycho.2009.02.001

Andersson, S. A., & Rydenhag, B. (1985). Cortical nociceptive systems. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *308*(1136), 347-359. https://doi.org/10.1098/rstb.1985.0035

Annen, J., Blandiaux, S., Lejeune, N., Bahri, M. A., Thibaut, A., Cho, W., Guger, C., Chatelle, C., & Laureys, S. (2018). BCI Performance and Brain Metabolism Profile in Severely Brain-Injured Patients Without Response to Command at Bedside. *Frontiers in Neuroscience*, *12*, 370. https://doi.org/10.3389/fnins.2018.00370

Apkarian, A. V., Bushnell, M. C., Treede, R.-D., & Zubieta, J.-K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain (London, England)*, *9*(4), 463-484. https://doi.org/10.1016/j.ejpain.2004.11.001

Arendt-Nielsen, L., & Chen, A. C. N. (2003). Lasers and other thermal stimulators for activation of skin nociceptors in humans. *Neurophysiologie Clinique = Clinical Neurophysiology*, *33*(6), 259-268.

Atherton, D. D., Facer, P., Roberts, K. M., Misra, V. P., Chizh, B. A., Bountra, C., & Anand, P. (2007). Use of the novel Contact Heat Evoked Potential Stimulator (CHEPS) for the assessment of small fibre neuropathy : Correlations with skin flare responses and intra-epidermal nerve fibre counts. *BMC Neurology*, *7*, 21. https://doi.org/10.1186/1471-2377-7-21

Aubinet, C., Larroque, S. K., Heine, L., Martial, C., Majerus, S., Laureys, S., & Di Perri, C. (2018). Clinical subcategorization of minimally conscious state according to resting functional connectivity. *Human Brain Mapping*, *39*(11), 4519-4532. https://doi.org/10.1002/hbm.24303

Bach, M., & Meigen, T. (1999). Do's and don'ts in Fourier analysis of steady-state potentials. *Documenta Ophthalmologica. Advances in Ophthalmology*, *99*(1), 69-82. https://doi.org/10.1023/a:1002648202420

Bagnato, S., Boccagni, C., Sant'Angelo, A., Alito, A., & Galardi, G. (2018). Pain assessment with the revised nociception coma scale and outcomes of patients with unresponsive wakefulness syndrome : Results from a pilot study. *Neurological Sciences*, *39*(6), 1073-1077. https://doi.org/10.1007/s10072-018-3330-5

Baier, B., zu Eulenburg, P., Geber, C., Rohde, F., Rolke, R., Maihöfner, C., Birklein, F., & Dieterich, M. (2014). Insula and sensory insular cortex and somatosensory control in patients with insular stroke. *European Journal of Pain (London, England)*, *18*(10), 1385-1393. https://doi.org/10.1002/j.1532-2149.2014.501.x

Baumgärtner, U., Greffrath, W., & Treede, R.-D. (2012). Contact heat and cold, mechanical, electrical and chemical stimuli to elicit small fiber-evoked potentials : Merits and limitations for basic science and clinical use. *Neurophysiologie Clinique = Clinical Neurophysiology*, *42*(5), 267-280. https://doi.org/10.1016/j.neucli.2012.06.002

Baumgärtner, Ulf, Cruccu, G., Iannetti, G. D., & Treede, R.-D. (2005). Laser guns and hot plates. *Pain*, *116*(1-2), 1-3. https://doi.org/10.1016/j.pain.2005.04.021

Baumgärtner, Ulf, Tiede, W., Treede, R.-D., & Craig, A. D. B. (2006). Laser-evoked potentials are graded and somatotopically organized anteroposteriorly in the operculoinsular cortex of anesthetized monkeys. *Journal of Neurophysiology*, *96*(5), 2802-2808. https://doi.org/10.1152/jn.00512.2006

Bekinschtein, T. A., Dehaene, S., Rohaut, B., Tadel, F., Cohen, L., & Naccache, L. (2009). Neural signature of the conscious processing of auditory regularities. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(5), 1672-1677. https://doi.org/10.1073/pnas.0809667106

Bingel, U., Lorenz, J., Glauche, V., Knab, R., Gläscher, J., Weiller, C., & Büchel, C. (2004). Somatotopic organization of human somatosensory cortices for pain : A single trial fMRI study. *NeuroImage*, *23*(1), 224-232. https://doi.org/10.1016/j.neuroimage.2004.05.021

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

Bodien, Y. G., Giacino, J. T., & Edlow, B. L. (2017). Functional MRI Motor Imagery Tasks to Detect Command Following in Traumatic Disorders of Consciousness. *Frontiers in Neurology*, *8*. https://doi.org/10.3389/fneur.2017.00688

Boly, M., Faymonville, M.-E., Schnakers, C., Peigneux, P., Lambermont, B., Phillips, C., Lancellotti, P., Luxen, A., Lamy, M., Moonen, G., Maquet, P., & Laureys, S. (2008). Perception of pain in the minimally conscious state with PET activation : An observational study. *The Lancet. Neurology*, *7*(11), 1013-1020. https://doi.org/10.1016/S1474-4422(08)70219-9

Bonin, E. A. C., Lejeune, N., Thibaut, A., Cassol, H., Antonopoulos, G., Wannez, S., Martial, C., Schnakers, C., Laureys, S., & Chatelle, C. (2019). Nociception Coma Scale Revised allows to identify patients with preserved neural basis for pain experience. *The Journal of Pain: Official Journal of the American Pain Society*. https://doi.org/10.1016/j.jpain.2019.11.004

Bragard, D., Chen, A. C., & Plaghki, L. (1996). Direct isolation of ultra-late (C-fibre) evoked brain potentials by CO2 laser stimulation of tiny cutaneous surface areas in man. *Neuroscience Letters*, *209*(2), 81-84. https://doi.org/10.1016/0304-3940(96)12604-5

Brock, J. A., Pianova, S., & Belmonte, C. (2001). Differences between nerve terminal impulses of polymodal nociceptors and cold sensory receptors of the guinea-pig cornea. *The Journal of Physiology*, *533*(Pt 2), 493-501. https://doi.org/10.1111/j.1469-7793.2001.0493a.x

Bromm, B., & Treede, R. D. (1984). Nerve fibre discharges, cerebral potentials and sensations induced by CO2 laser stimulation. *Human Neurobiology*, *3*(1), 33-40.

Brooks, J. C. W., Nurmikko, T. J., Bimson, W. E., Singh, K. D., & Roberts, N. (2002). fMRI of thermal pain : Effects of stimulus laterality and attention. *NeuroImage*, *15*(2), 293-301. https://doi.org/10.1006/nimg.2001.0974

Brooks, J., & Tracey, I. (2005). From nociception to pain perception : Imaging the spinal and supraspinal pathways. *Journal of anatomy*, *207*(1), 19-33.

Brooks, R. (2013). *The EuroQol Group after 25 years*. Springer Netherlands. https://doi.org/10.1007/978-94-007-5158-3

Bruno, M.-A., Majerus, S., Boly, M., Vanhaudenhuyse, A., Schnakers, C., Gosseries, O., Boveroux, P., Kirsch, M., Demertzi, A., Bernard, C., Hustinx, R., Moonen, G., & Laureys, S. (2012). Functional neuroanatomy underlying the clinical subcategorization of minimally conscious state patients. *Journal of Neurology*, *259*(6), 1087-1098. https://doi.org/10.1007/s00415-011-6303-7

Buckner, R. L., Andrews-Hanna, J. R., & Schacter, D. L. (2008). The brain's default network : Anatomy, function, and relevance to disease. *Annals of the New York Academy of Sciences*, *1124*, 1-38. https://doi.org/10.1196/annals.1440.011

Burgess, P. R., & Perl, E. R. (1967). Myelinated afferent fibres responding specifically to noxious stimulation of the skin. *The Journal of Physiology*, *190*(3), 541-562. https://doi.org/10.1113/jphysiol.1967.sp008227

Bushnell, M. C., & Apkarian, A. V. (2006). Representation of pain in the brain. *Wall and Melzack's Textbook of Pain, 5th Edition*, 107-124.

Bushnell, M. C., Duncan, G. H., Hofbauer, R. K., Ha, B., Chen, J. I., & Carrier, B. (1999). Pain perception : Is there a role for primary somatosensory cortex? *Proceedings of the National Academy of Sciences of the United States of America*, *96*(14), 7705-7709. https://doi.org/10.1073/pnas.96.14.7705

Capilla, A., Pazo-Alvarez, P., Darriba, A., Campo, P., & Gross, J. (2011). Steady-state visual evoked potentials can be explained by temporal superposition of transient event-related responses. *PloS One*, *6*(1), e14543. https://doi.org/10.1371/journal.pone.0014543

Carmon, A., Mor, J., & Goldberg, J. (1976). Evoked cerebral responses to noxious thermal stimuli in humans. *Experimental Brain Research*, *25*(1), 103-107. https://doi.org/10.1007/bf00237330

Cavanna, A. E. (2007). The precuneus and consciousness. *CNS Spectrums*, *12*(7), 545-552. https://doi.org/10.1017/s1092852900021295

Charlesworth, G., Soryal, I., Smith, S., & Sisodiya, S. M. (2009). Acute, localised paroxysmal pain as the initial manifestation of focal seizures : A case report and a brief review of the literature. *Pain*, *141*(3), 300-305. https://doi.org/10.1016/j.pain.2008.11.005

Chatelle, C., De Val, M.-D., Catano, A., Chaskis, C., Seeldrayers, P., Laureys, S., Biston, P., & Schnakers, C. (2016). Is the Nociception Coma Scale-Revised a Useful Clinical Tool for Managing Pain in Patients With Disorders of Consciousness? *The Clinical Journal of Pain*, *32*(4), 321-326. https://doi.org/10.1097/AJP.0000000000000259

Chatelle, C., Hauger, S. L., Martial, C., Becker, F., Eifert, B., Boering, D., Giacino, J. T., Laureys, S., Løvstad, M., & Maurer-Karattup, P. (2018). Assessment of Nociception and Pain in Participants in an Unresponsive or Minimally Conscious State After Acquired Brain Injury : The Relation Between the Coma Recovery Scale-Revised and the Nociception Coma Scale-Revised. *Archives of Physical Medicine and Rehabilitation*, *99*(9), 1755-1762. https://doi.org/10.1016/j.apmr.2018.03.009

Chatelle, C., Majerus, S., Whyte, J., Laureys, S., & Schnakers, C. (2012). A sensitive scale to assess nociceptive pain in patients with disorders of consciousness. *Journal of Neurology, Neurosurgery, and Psychiatry*, *83*(12), 1233-1237. https://doi.org/10.1136/jnnp-2012-302987

Chatelle, C., Thibaut, A., Bruno, M.-A., Boly, M., Bernard, C., Hustinx, R., Schnakers, C., & Laureys, S. (2014). Nociception coma scale-revised scores correlate with

metabolism in the anterior cingulate cortex. *Neurorehabilitation and Neural Repair*, *28*(2), 149-152. https://doi.org/10.1177/1545968313503220

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: A Journal of Neurology*, *140*(8), 2120-2132. https://doi.org/10.1093/brain/awx163

Chennu, S., Finoia, P., Kamau, E., Allanson, J., Williams, G. B., Monti, M. M., Noreika, V., Arnatkeviciute, A., Canales-Johnson, A., Olivares, F., Cabezas-Soto, D., Menon, D. K., Pickard, J. D., Owen, A. M., & Bekinschtein, T. A. (2014). Spectral signatures of reorganised brain networks in disorders of consciousness. *PLoS Computational Biology*, *10*(10), e1003887. https://doi.org/10.1371/journal.pcbi.1003887

Coghill, R. C., Sang, C. N., Maisog, J. M., & Iadarola, M. J. (1999). Pain intensity processing within the human brain : A bilateral, distributed mechanism. *Journal of Neurophysiology*, *82*(4), 1934-1943. https://doi.org/10.1152/jn.1999.82.4.1934

Colon, E., Legrain, V., & Mouraux, A. (2012). Steady-state evoked potentials to study the processing of tactile and nociceptive somatosensory input in the human brain. *Neurophysiologie Clinique = Clinical Neurophysiology*, *42*(5), 315-323. https://doi.org/10.1016/j.neucli.2012.05.005

Colon, Elisabeth, Legrain, V., & Mouraux, A. (2014). EEG frequency tagging to dissociate the cortical responses to nociceptive and nonnociceptive stimuli. *Journal of Cognitive Neuroscience*, *26*(10), 2262-2274. https://doi.org/10.1162/jocn_a_00648

Colon, Elisabeth, Liberati, G., & Mouraux, A. (2017). EEG frequency tagging using ultra-slow periodic heat stimulation of the skin reveals cortical activity specifically related to C fiber thermonociceptors. *NeuroImage*, *146*, 266-274. https://doi.org/10.1016/j.neuroimage.2016.11.045

Colon, Elisabeth, Nozaradan, S., Legrain, V., & Mouraux, A. (2012). Steady-state evoked potentials to tag specific components of nociceptive cortical processing. *NeuroImage*, *60*(1), 571-581. https://doi.org/10.1016/j.neuroimage.2011.12.015

Commission fédérale de Contrôle et d'Évaluation de l'Euthanasie. (2018). *Huitième rapport aux Chambres législatives, années 2016-2017*.

Constant, I., & Sabourdin, N. (2015). Monitoring depth of anesthesia : From consciousness to nociception. A window on subcortical brain activity. *Pediatric anesthesia*, *25*(1), 73-82.

Craig, A. D., Chen, K., Bandy, D., & Reiman, E. M. (2000). Thermosensory activation of insular cortex. *Nature Neuroscience*, *3*(2), 184-190. https://doi.org/10.1038/72131

Crick, F., & Koch, C. (1990). Towards a neurobiological theory of consciousness. *Seminars in the neurosciences*, *2*, 263-275.

Cruccu, G., Aminoff, M. J., Curio, G., Guerit, J. M., Kakigi, R., Mauguiere, F., Rossini, P. M., Treede, R.-D., & Garcia-Larrea, L. (2008). Recommendations for the clinical use of somatosensory-evoked potentials. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *119*(8), 1705-1719. https://doi.org/10.1016/j.clinph.2008.03.016

Cruccu, G., Sommer, C., Anand, P., Attal, N., Baron, R., Garcia-Larrea, L., Haanpaa, M., Jensen, T. S., Serra, J., & Treede, R.-D. (2010). EFNS guidelines on neuropathic pain assessment : Revised 2009. *European Journal of Neurology*, *17*(8), 1010-1018. https://doi.org/10.1111/j.1468-1331.2010.02969.x

Cruse, D., Chennu, S., Chatelle, C., Bekinschtein, T. A., Fernández-Espejo, D., Pickard, J. D., Laureys, S., & Owen, A. M. (2011). Bedside detection of awareness in the vegetative state : A cohort study. *The Lancet*, *378*(9809), 2088-2094. https://doi.org/10.1016/S0140-6736(11)61224-5

Darian-Smith, I., Johnson, K. O., & Dykes, R. (1973). « Cold » fiber population innervating palmar and digital skin of the monkey : Responses to cooling pulses. *Journal of Neurophysiology*, *36*(2), 325-346. https://doi.org/10.1152/jn.1973.36.2.325

Dawson, G. D. (1947). CEREBRAL RESPONSES TO ELECTRICAL STIMULATION OF PERIPHERAL NERVE IN MAN. *Journal of Neurology, Neurosurgery, and Psychiatry*, *10*(3), 134-140. https://doi.org/10.1136/jnnp.10.3.134

De Keyser, R., van den Broeke, E. N., Courtin, A., Dufour, A., & Mouraux, A. (2018). Event-related brain potentials elicited by high-speed cooling of the skin : A robust and non-painful method to assess the spinothalamic system in humans. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *129*(5), 1011-1019. https://doi.org/10.1016/j.clinph.2018.02.123

De Salvo, S., Naro, A., Bonanno, L., Russo, M., Muscarà, N., Bramanti, P., & Marino, S. (2015). Assessment of nociceptive system in vegetative and minimally conscious state by using laser evoked potentials. *Brain Injury*, *29*(12), 1467-1474. https://doi.org/10.3109/02699052.2015.1071430

de Tommaso, M., Navarro, J., Lanzillotti, C., Ricci, K., Buonocunto, F., Livrea, P., & Lancioni, G. E. (2015). Cortical responses to salient nociceptive and not

nociceptive stimuli in vegetative and minimal conscious state. *Frontiers in Human Neuroscience*, *9*, 17. https://doi.org/10.3389/fnhum.2015.00017

de Tommaso, M., Navarro, J., Ricci, K., Lorenzo, M., Lanzillotti, C., Colonna, F., Resta, M., Lancioni, G., & Livrea, P. (2013). Pain in prolonged disorders of consciousness : Laser evoked potentials findings in patients with vegetative and minimally conscious states. *Brain Injury*, *27*(7-8), 962-972. https://doi.org/10.3109/02699052.2013.775507

Derbyshire, S. W., Jones, A. K., Gyulai, F., Clark, S., Townsend, D., & Firestone, L. L. (1997). Pain processing during three levels of noxious stimulation produces differential patterns of central activity. *Pain*, *73*(3), 431-445. https://doi.org/10.1016/s0304-3959(97)00138-3

DeVolder, A. G., Goffinet, A. M., Bol, A., Michel, C., de Barsy, T., & Laterre, C. (1990). Brain glucose metabolism in postanoxic syndrome. Positron emission tomographic study. *Archives of Neurology*, *47*(2), 197-204. https://doi.org/10.1001/archneur.1990.00530020103022

Di Perri, C., Bahri, M. A., Amico, E., Thibaut, A., Heine, L., Antonopoulos, G., Charland-Verville, V., Wannez, S., Gomez, F., Hustinx, R., Tshibanda, L., Demertzi, A., Soddu, A., & Laureys, S. (2016). Neural correlates of consciousness in patients who have emerged from a minimally conscious state : A cross-sectional multimodal imaging study. *The Lancet. Neurology*, *15*(8), 830-842. https://doi.org/10.1016/S1474-4422(16)00111-3

Dowman, R., Rissacher, D., & Schuckers, S. (2008). EEG indices of tonic pain-related activity in the somatosensory cortices. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *119*(5), 1201-1212. https://doi.org/10.1016/j.clinph.2008.01.019

Downar, J., Crawley, A. P., Mikulis, D. J., & Davis, K. D. (2000). A multimodal cortical network for the detection of changes in the sensory environment. *Nature Neuroscience*, *3*(3), 277-283. https://doi.org/10.1038/72991

Dubin, A. E., & Patapoutian, A. (2010). Nociceptors : The sensors of the pain pathway. *The Journal of Clinical Investigation*, *120*(11), 3760-3772. https://doi.org/10.1172/JCI42843

Duclaux, R., Franzen, O., Chatt, A. B., Kenshalo, D. R., & Stowell, H. (1974). Responses recorded from human scalp evoked by cutaneous thermal stimulation. *Brain Research*, *78*(2), 279-290. https://doi.org/10.1016/0006-8993(74)90552-6

Dufour, A., Guergova, S., Pebayle, T., & Touzalin-Chretien, P. (2011). On the selective activation of unmyelinated C-fibers using sinusoidal electrical stimulation : An

ERP study. *Clinical Neurophysiology*, *122*(5), 1042-1047. https://doi.org/10.1016/j.clinph.2011.01.046

Dyck, P. J., Zimmerman, I., Gillen, D. A., Johnson, D., Karnes, J. L., & O'Brien, P. C. (1993). Cool, warm, and heat-pain detection thresholds : Testing methods and inferences about anatomic distribution of receptors. *Neurology*, *43*(8), 1500-1508. https://doi.org/10.1212/wnl.43.8.1500

Eccleston, C., & Crombez, G. (1999). Pain demands attention : A cognitive-affective model of the interruptive function of pain. *Psychological Bulletin*, *125*(3), 356-366. https://doi.org/10.1037/0033-2909.125.3.356

Erlanger, J., & Gasser, H. S. (1937). *Electrical signs of nervous activity*. Univ. Penn. Press.

Evrard, H. C. (2019). The Organization of the Primate Insular Cortex. *Frontiers in neuroanatomy*, *13*, 43.

Faugeras, F., Rohaut, B., Weiss, N., Bekinschtein, T., Galanaud, D., Puybasset, L., Bolgert, F., Sergent, C., Cohen, L., Dehaene, S., & Naccache, L. (2012). Event related potentials elicited by violations of auditory regularities in patients with impaired consciousness. *Neuropsychologia*, *50*(3), 403-418. https://doi.org/10.1016/j.neuropsychologia.2011.12.015

Feinstein, J. S., Khalsa, S. S., Salomons, T. V., Prkachin, K. M., Frey-Law, L. A., Lee, J. E., Tranel, D., & Rudrauf, D. (2016). Preserved emotional awareness of pain in a patient with extensive bilateral damage to the insula, anterior cingulate, and amygdala. *Brain Structure & Function*, *221*(3), 1499-1511. https://doi.org/10.1007/s00429-014-0986-3

Fischer, C., Dailler, F., & Morlet, D. (2008). Novelty P3 elicited by the subject's own name in comatose patients. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *119*(10), 2224-2230. https://doi.org/10.1016/j.clinph.2008.03.035

Fischer, C., Luaute, J., & Morlet, D. (2010). Event-related potentials (MMN and novelty P3) in permanent vegetative or minimally conscious states. *Clinical Neurophysiology*, *121*(7), 1032-1042. https://doi.org/10.1016/j.clinph.2010.02.005

Flor, H., Nikolajsen, L., & Staehelin Jensen, T. (2006). Phantom limb pain : A case of maladaptive CNS plasticity? *Nature Reviews. Neuroscience*, *7*(11), 873-881. https://doi.org/10.1038/nrn1991

Forgacs, P. B., Conte, M. M., Fridman, E. A., Voss, H. U., Victor, J. D., & Schiff, N. D. (2014). Preservation of electroencephalographic organization in patients with impaired consciousness and imaging-based evidence of command-following. *Annals of neurology*, *76*(6), 869-879.

Formisano, R., Contrada, M., Aloisi, M., Ferri, G., Schiattone, S., Iosa, M., & Buzzi, M. G. (2019). Nociception Coma Scale with personalized painful stimulation versus standard stimulus in non-communicative patients with disorders of consciousness. *Neuropsychological Rehabilitation*, 1-12. https://doi.org/10.1080/09602011.2019.1614464

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(27), 9673-9678. https://doi.org/10.1073/pnas.0504136102

Fritz, Z. (2017). Can 'Best Interests' derail the trolley ? Examining withdrawal of clinically assisted nutrition and hydration in patients in the permanent vegetative state. *Journal of medical ethics*, *43*(7), 450-454.

Galambos, R. (1982). Tactile and auditory stimuli repeated at high rates (30-50 per sec) produce similar event related potentials. *Annals of the New York Academy of Sciences*, *388*, 722-728. https://doi.org/10.1111/j.1749-6632.1982.tb50841.x

Ganesh, S., Guernon, A., Chalcraft, L., Harton, B., Smith, B., & Louise-Bender Pape, T. (2013). Medical comorbidities in disorders of consciousness patients and their association with functional outcomes. *Archives of Physical Medicine and Rehabilitation*, *94*(10), 1899-1907. https://doi.org/10.1016/j.apmr.2012.12.026

Garcia-Larrea, L. (2012). The posterior insular-opercular region and the search of a primary cortex for pain. *Neurophysiologie Clinique = Clinical Neurophysiology*, *42*(5), 299-313. https://doi.org/10.1016/j.neucli.2012.06.001

Garcia-Larrea, L., Frot, M., & Valeriani, M. (2003). Brain generators of laser-evoked potentials : From dipoles to functional significance. *Neurophysiologie Clinique = Clinical Neurophysiology*, *33*(6), 279-292.

Garcia-Larrea, Luis. (2004). Somatosensory volleys and cortical evoked potentials : « first come, first served »? *Pain*, *112*(1-2), 5-7. https://doi.org/10.1016/j.pain.2004.09.003

Garcia-Larrea, Luis, & Peyron, R. (2013). Pain matrices and neuropathic pain matrices : A review. *Pain*, *154 Suppl 1*, S29-43. https://doi.org/10.1016/j.pain.2013.09.001

Gardner, E. P., & Johnson, K. O. (2014). The Somatosensory System : Receptors and Central Pathways. In *Principles of Neural Science* (Fifth Edition). McGraw-Hill Education. neurology.mhmedical.com/content.aspx?aid=1101679414

Gélinas, C., Boitor, M., Puntillo, K. A., Arbour, C., Topolovec-Vranic, J., Cusimano, M. D., Choinière, M., & Streiner, D. L. (2019). Behaviors Indicative of Pain in Brain-Injured Adult Patients With Different Levels of Consciousness in the

Intensive Care Unit. *Journal of Pain and Symptom Management*, *57*(4), 761-773. https://doi.org/10.1016/j.jpainsymman.2018.12.333

Gélinas, C., Puntillo, K. A., Levin, P., & Azoulay, E. (2017). The Behavior Pain Assessment Tool for critically ill adults : A validation study in 28 countries. *Pain*, *158*(5), 811-821. https://doi.org/10.1097/j.pain.0000000000000834

Giabbiconi, C.-M., Trujillo-Barreto, N. J., Gruber, T., & Müller, M. M. (2007). Sustained spatial attention to vibration is mediated in primary somatosensory cortex. *NeuroImage*, *35*(1), 255-262. https://doi.org/10.1016/j.neuroimage.2006.11.022

Giacino, J. T., Ashwal, S., Childs, N., Cranford, R., Jennett, B., Katz, D. I., Kelly, J. P., Rosenberg, J. H., Whyte, J., Zafonte, R. D., & Zasler, N. D. (2002). The minimally conscious state : Definition and diagnostic criteria. *Neurology*, *58*(3), 349-353. https://doi.org/10.1212/wnl.58.3.349

Giacino, J. T., Kalmar, K., & Whyte, J. (2004). The JFK Coma Recovery Scale-Revised : Measurement characteristics and diagnostic utility. *Archives of Physical Medicine and Rehabilitation*, *85*(12), 2020-2029.

Giacino, J. T., Katz, D. I., Schiff, N. D., Whyte, J., Ashman, E. J., Ashwal, S., Barbano, R., Hammond, F. M., Laureys, S., Ling, G. S. F., Nakase-Richardson, R., Seel, R. T., Yablon, S., Getchius, T. S. D., Gronseth, G. S., & Armstrong, M. J. (2018). 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*(10), 450-460. https://doi.org/10.1212/WNL.0000000000005926

Gosseries, O., Zasler, N. D., & Laureys, S. (2014). Recent advances in disorders of consciousness : Focus on the diagnosis. *Brain Injury*, *28*(9), 1141-1150. https://doi.org/10.3109/02699052.2014.920522

Greffrath, W., Baumgärtner, U., & Treede, R.-D. (2007). Peripheral and central components of habituation of heat pain perception and evoked potentials in humans. *Pain*, *132*(3), 301-311. https://doi.org/10.1016/j.pain.2007.04.026

Guenther, T., Schönknecht, P., Becker, G., Olbrich, S., Sander, C., Hesse, S., Meyer, P. M., Luthardt, J., Hegerl, U., & Sabri, O. (2011). Impact of EEG-vigilance on brain glucose uptake measured with [(18)F]FDG and PET in patients with depressive episode or mild cognitive impairment. *NeuroImage*, *56*(1), 93-101. https://doi.org/10.1016/j.neuroimage.2011.01.059

Haanpää, M., Attal, N., Backonja, M., Baron, R., Bennett, M., Bouhassira, D., Cruccu, G., Hansson, P., Haythornthwaite, J. A., Iannetti, G. D., Jensen, T. S., Kauppila,

T., Nurmikko, T. J., Rice, A. S. C., Rowbotham, M., Serra, J., Sommer, C., Smith, B. H., & Treede, R.-D. (2011). NeuPSIG guidelines on neuropathic pain assessment. *Pain*, *152*(1), 14-27. https://doi.org/10.1016/j.pain.2010.07.031

Hämäläinen, H., Kekoni, J., Sams, M., Reinikainen, K., & Näätänen, R. (1990). Human somatosensory evoked potentials to mechanical pulses and vibration : Contributions of SI and SII somatosensory cortices to P50 and P100 components. *Electroencephalography and Clinical Neurophysiology*, *75*(2), 13-21. https://doi.org/10.1016/0013-4694(90)90148-d

Hardy, J. D., & Muschenheim, C. (1934). THE RADIATION OF HEAT FROM THE HUMAN BODY. IV. THE EMISSION, REFLECTION, AND TRANSMISSION OF INFRA-RED RADIATION BY THE HUMAN SKIN. *The Journal of Clinical Investigation*, *13*(5), 817-831. https://doi.org/10.1172/JCI100624

Hari, R. (1980). Evoked potentials elicited by long vibrotactile stimuli in the human EEG. *Pflugers Archiv: European Journal of Physiology*, *384*(2), 167-170. https://doi.org/10.1007/bf00584434

Harrison, J. L. K., & Davis, K. D. (1999). Cold-evoked pain varies with skin type and cooling rate : A psychophysical study in humans. *PAIN*, *83*(2), 123-135. https://doi.org/10.1016/S0304-3959(99)00099-8

Heine, L., Tillmann, B., Hauet, M., Juliat, A., Dubois, A., Laureys, S., Kandel, M., Plailly, J., Luauté, J., & Perrin, F. (2017). Effects of preference and sensory modality on behavioural reaction in patients with disorders of consciousness. *Brain Injury*, *31*(10), 1307-1311. https://doi.org/10.1080/02699052.2017.1306108

Hensel, H., & Zotterman, Y. (1951). Action potentials of cold fibres and intracutaneous temperature gradient. *Journal of Neurophysiology*, *14*(5), 377-385. https://doi.org/10.1152/jn.1951.14.5.377

Herrmann, C. S. (2001). Human EEG responses to 1-100 Hz flicker : Resonance phenomena in visual cortex and their potential correlation to cognitive phenomena. *Experimental Brain Research*, *137*(3-4), 346-353. https://doi.org/10.1007/s002210100682

Ho, H.-N., & Jones, L. A. (2006). Contribution of thermal cues to material discrimination and localization. *Perception & Psychophysics*, *68*(1), 118-128. https://doi.org/10.3758/BF03193662

Hofbauer, R. K., Rainville, P., Duncan, G. H., & Bushnell, M. C. (2001). Cortical representation of the sensory dimension of pain. *Journal of Neurophysiology*, *86*(1), 402-411. https://doi.org/10.1152/jn.2001.86.1.402

Hofbauer, Robert K., Fiset, P., Plourde, G., Backman, S. B., & Bushnell, M. C. (2004). Dose-dependent effects of propofol on the central processing of thermal pain.

Anesthesiology, *100*(2), 386-394. https://doi.org/10.1097/00000542-200402000-00031

Hu, L., Mouraux, A., Hu, Y., & Iannetti, G. D. (2010). A novel approach for enhancing the signal-to-noise ratio and detecting automatically event-related potentials (ERPs) in single trials. *NeuroImage*, *50*(1), 99-111. https://doi.org/10.1016/j.neuroimage.2009.12.010

Hu, L., Valentini, E., Zhang, Z. G., Liang, M., & Iannetti, G. D. (2014). The primary somatosensory cortex contributes to the latest part of the cortical response elicited by nociceptive somatosensory stimuli in humans. *NeuroImage*, *84*, 383-393. https://doi.org/10.1016/j.neuroimage.2013.08.057

Hüllemann, P., Nerdal, A., Binder, A., Helfert, S., Reimer, M., & Baron, R. (2016). Cold-evoked potentials—Ready for clinical use? *European Journal of Pain (London, England)*, *20*(10), 1730-1740. https://doi.org/10.1002/ejp.896

Iannetti, G., Baumgärtner, U., Tracey, I., Treede, R.-D., & Magerl, W. (2013). Pinprick-evoked brain potentials : A novel tool to assess central sensitization of nociceptive pathways in humans. *Journal of neurophysiology*, *110*(5), 1107-1116.

Iannetti, G. D., Hughes, N. P., Lee, M. C., & Mouraux, A. (2008). Determinants of laser-evoked EEG responses : Pain perception or stimulus saliency? *Journal of Neurophysiology*, *100*(2), 815-828. https://doi.org/10.1152/jn.00097.2008

Iannetti, G. D., & Mouraux, A. (2010). From the neuromatrix to the pain matrix (and back). *Experimental Brain Research*, *205*(1), 1-12. https://doi.org/10.1007/s00221-010-2340-1

Iannetti, G. D., Zambreanu, L., Cruccu, G., & Tracey, I. (2005). Operculoinsular cortex encodes pain intensity at the earliest stages of cortical processing as indicated by amplitude of laser-evoked potentials in humans. *Neuroscience*, *131*(1), 199-208. https://doi.org/10.1016/j.neuroscience.2004.10.035

Ingvar, M. (1999). Pain and functional imaging. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *354*(1387), 1347-1358. https://doi.org/10.1098/rstb.1999.0483

International Association for the Study of Pain. (2017). *IASP Taxonomy. Pain terms. Neuropathic pain*. www.iasp-pain.org/Taxonomy#Neuropathicpain

International Council of Nurses. (2012). *The ICN code of ethics for nurses*. International Council of Nurses.

Jamal, G. A., Hansen, S., Weir, A. I., & Ballantyne, J. P. (1989). Cerebral cortical potentials to pure non-painful temperature stimulation : An objective technique for the assessment of small fibre pathway in man. *Journal of Neurology, Neurosurgery, and Psychiatry*, *52*(1), 99-105. https://doi.org/10.1136/jnnp.52.1.99

Jankovski, A., Plaghki, L., & Mouraux, A. (2013). Reliable EEG responses to the selective activation of C-fibre afferents using a temperature-controlled infrared laser stimulator in conjunction with an adaptive staircase algorithm. *Pain*, *154*(9), 1578-1587. https://doi.org/10.1016/j.pain.2013.04.032

Jellinger, K. A. (2013). Neuropathology of prolonged unresponsive wakefulness syndrome after blunt head injury : Review of 100 post-mortem cases. *Brain Injury*, *27*(7-8), 917-923. https://doi.org/10.3109/02699052.2013.793395

Jennett, B., & Plum, F. (1972). Persistent vegetative state after brain damage. A syndrome in search of a name. *Lancet (London, England)*, *1*(7753), 734-737. https://doi.org/10.1016/s0140-6736(72)90242-5

Jones, A. K., Friston, K., & Frackowiak, R. S. (1992). Localization of responses to pain in human cerebral cortex. *Science (New York, N.Y.)*, *255*(5041), 215-216. https://doi.org/10.1126/science.1553549

Julius, D., & Basbaum, A. I. (2001). Molecular mechanisms of nociception. *Nature*, *413*(6852), 203-210. https://doi.org/10.1038/35093019

Kakigi, R., Shibasaki, H., & Ikeda, A. (1989). Pain-related somatosensory evoked potentials following CO2 laser stimulation in man. *Electroencephalography and Clinical Neurophysiology*, *74*(2), 139-146. https://doi.org/10.1016/0168-5597(89)90019-1

Kalmar, K., & Giacino, J. T. (2005). The JFK Coma Recovery Scale—Revised. *Neuropsychological Rehabilitation*, *15*(3-4), 454-460. https://doi.org/10.1080/09602010443000425

Kassubek, J., Juengling, F. D., Els, T., Spreer, J., Herpers, M., Krause, T., Moser, E., & Lücking, C. H. (2003). Activation of a residual cortical network during painful stimulation in long-term postanoxic vegetative state : A 15O-H2O PET study. *Journal of the Neurological Sciences*, *212*(1-2), 85-91. https://doi.org/10.1016/s0022-510x(03)00106-0

Katz, N. (2002). The impact of pain management on quality of life. *Journal of Pain and Symptom Management*, *24*(1 Suppl), S38-47. https://doi.org/10.1016/s0885-3924(02)00411-6

Kekoni, J., Hämäläinen, H., Saarinen, M., Gröhn, J., Reinikainen, K., Lehtokoski, A., & Näätänen, R. (1997). Rate effect and mismatch responses in the somatosensory system : ERP-recordings in humans. *Biological Psychology*, *46*(2), 125-142. https://doi.org/10.1016/S0301-0511(97)05249-6

Kenshalo, D., & Bergen, D. (1975). A device to measure cutaneous temperature sensitivity in humans and subhuman species. *Journal of applied physiology*, *39*(6), 1038–1040.

Kleinböhl, D., Trojan, J., Konrad, C., & Hölzl, R. (2006). Sensitization and habituation of AMH and C-fiber related percepts of repetitive radiant heat stimulation. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *117*(1), 118-130. https://doi.org/10.1016/j.clinph.2005.08.023

Koenig, T., & Melie-García, L. (2010). A method to determine the presence of averaged event-related fields using randomization tests. *Brain topography*, *23*(3), 233-242.

Kotchoubey, B. (2017). Evoked and event-related potentials in disorders of consciousness : A quantitative review. *Consciousness and Cognition*, *54*, 155-167. https://doi.org/10.1016/j.concog.2017.05.002

Krzanowski, W. J., & Hand, D. J. (2009). *Roc curves for continuous data*. CRC Press.

Laureys, S., Lemaire, C., Maquet, P., Phillips, C., & Franck, G. (1999). Cerebral metabolism during vegetative state and after recovery to consciousness. *Journal of Neurology, Neurosurgery, and Psychiatry*, *67*(1), 121. https://doi.org/10.1136/jnnp.67.1.121

Laureys, S., Perrin, F., Faymonville, M.-E., Schnakers, C., Boly, M., Bartsch, V., Majerus, S., Moonen, G., & Maquet, P. (2004). Cerebral processing in the minimally conscious state. *Neurology*, *63*(5), 916-918. https://doi.org/10.1212/01.wnl.0000137421.30792.9b

Laureys, Steven. (2005). Death, unconsciousness and the brain. *Nature Reviews Neuroscience*, *6*(11), 899-909. https://doi.org/10.1038/nrn1789

Laureys, Steven. (2007). Eyes open, brain shut. *Scientific American*, *296*(5), 84-89. https://doi.org/10.1038/scientificamerican0507-84

Laureys, Steven, Celesia, G. G., Cohadon, F., Lavrijsen, J., León-Carrión, J., Sannita, W. G., Sazbon, L., Schmutzhard, E., von Wild, K. R., Zeman, A., Dolce, G., & European Task Force on Disorders of Consciousness. (2010). Unresponsive wakefulness syndrome : A new name for the vegetative state or apallic syndrome. *BMC Medicine*, *8*, 68. https://doi.org/10.1186/1741-7015-8-68

Laureys, Steven, Faymonville, M. E., Peigneux, P., Damas, P., Lambermont, B., Del Fiore, G., Degueldre, C., Aerts, J., Luxen, A., Franck, G., Lamy, M., Moonen, G., & Maquet, P. (2002). Cortical processing of noxious somatosensory stimuli in the persistent vegetative state. *NeuroImage*, *17*(2), 732-741.

Laureys, Steven, Owen, A. M., & Schiff, N. D. (2004). Brain function in coma, vegetative state, and related disorders. *The Lancet. Neurology*, *3*(9), 537-546. https://doi.org/10.1016/S1474-4422(04)00852-X

Lechinger, J., Bothe, K., Pichler, G., Michitsch, G., Donis, J., Klimesch, W., & Schabus, M. (2013). CRS-R score in disorders of consciousness is strongly related to spectral EEG at rest. *Journal of Neurology*, *260*(9), 2348-2356. https://doi.org/10.1007/s00415-013-6982-3

Lee, M. C., Mouraux, A., & Iannetti, G. D. (2009). Characterizing the cortical activity through which pain emerges from nociception. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *29*(24), 7909-7916. https://doi.org/10.1523/JNEUROSCI.0014-09.2009

Legrain, V., Iannetti, G. D., Plaghki, L., & Mouraux, A. (2011). The pain matrix reloaded : A salience detection system for the body. *Progress in Neurobiology*, *93*(1), 111-124. https://doi.org/10.1016/j.pneurobio.2010.10.005

Lejeune, N. (2017). Withdrawal of clinically assisted nutrition and hydration decisions in patients with prolonged disorders of consciousness : Best interests of the patients and advance directives are the keys. *Journal of medical ethics*, *43*(7), 457-458.

Lejeune, N., van Erp, W. S., Rohaut, B., Sanz, L., Laureys, S., & Chatelle, C. (2020). États de conscience altérée : Soins palliatifs et décisions de fin de vie. In *Manuel de Soins Palliatifs* (5ème édition). Dunod.

Lenoir, C., Huang, G., Vandermeeren, Y., Hatem, S. M., & Mouraux, A. (2017). Human primary somatosensory cortex is differentially involved in vibrotaction and nociception. *Journal of Neurophysiology*, *118*(1), 317-330. https://doi.org/10.1152/jn.00615.2016

Leone, C., Dufour, A., Di Stefano, G., Fasolino, A., Di Lionardo, A., La Cesa, S., Galosi, E., Valeriani, M., Nolano, M., Cruccu, G., & Truini, A. (2019). Cooling the skin for assessing small-fibre function. *Pain*, *160*(9), 1967-1975. https://doi.org/10.1097/j.pain.0000000000001584

Levy, D. E., Sidtis, J. J., Rottenberg, D. A., Jarden, J. O., Strother, S. C., Dhawan, V., Ginos, J. Z., Tramo, M. J., Evans, A. C., & Plum, F. (1987). Differences in cerebral blood flow and glucose utilization in vegetative versus locked-in patients. *Annals of Neurology*, *22*(6), 673-682. https://doi.org/10.1002/ana.410220602

Lewis, T., & Pochin, E. (1937). The double pain response of the human skin to a single stimulus. *Clin Sci*, *3*(67-76), 50.

Liang, M., Mouraux, A., Hu, L., & Iannetti, G. D. (2013). Primary sensory cortices contain distinguishable spatial patterns of activity for each sense. *Nature Communications*, *4*, 1979. https://doi.org/10.1038/ncomms2979

Liberati, G., Algoet, M., Santos, S. F., Ribeiro-Vaz, J. G., Raftopoulos, C., & Mouraux, A. (2019). Tonic thermonociceptive stimulation selectively modulates ongoing neural oscillations in the human posterior insula : Evidence from intracerebral EEG. *NeuroImage*, *188*, 70-83. https://doi.org/10.1016/j.neuroimage.2018.11.059

Loi relative à l'euthanasie, (2002). http://www.ejustice.just.fgov.be/cgi_loi/change_lg.pl?language=fr&la=F&cn=2002052837&table_name=loi

Loi relative aux droits des patients, (2002). http://www.ejustice.just.fgov.be/cgi_loi/change_lg.pl?language=fr&la=F&cn=2002082245&table_name=loi

Luauté, J., Morlet, D., & Mattout, J. (2015). BCI in patients with disorders of consciousness : Clinical perspectives. *Annals of Physical and Rehabilitation Medicine*, *58*(1), 29-34. https://doi.org/10.1016/j.rehab.2014.09.015

Maihöfner, C., Kaltenhäuser, M., Neundörfer, B., & Lang, E. (2002). Temporo-spatial analysis of cortical activation by phasic innocuous and noxious cold stimuli—A magnetoencephalographic study. *Pain*, *100*(3), 281-290. https://doi.org/10.1016/s0304-3959(02)00276-2

Mancini, F., Pepe, A., Bernacchia, A., Di Stefano, G., Mouraux, A., & Iannetti, G. D. (2018). Characterizing the Short-Term Habituation of Event-Related Evoked Potentials. *ENeuro*, *5*(5). https://doi.org/10.1523/ENEURO.0014-18.2018

Marchandise, E., Mouraux, A., Plaghki, L., & Henrotte, F. (2014). Finite element analysis of thermal laser skin stimulation for a finer characterization of the nociceptive system. *Journal of Neuroscience Methods*, *223*, 1-10. https://doi.org/10.1016/j.jneumeth.2013.11.010

Maris, E., & Oostenveld, R. (2007). Nonparametric statistical testing of EEG-and MEG-data. *Journal of neuroscience methods*, *164*(1), 177-190.

Markl, A., Yu, T., Vogel, D., Müller, F., Kotchoubey, B., & Lang, S. (2013). Brain processing of pain in patients with unresponsive wakefulness syndrome. *Brain and Behavior*, *3*(2), 95-103. https://doi.org/10.1002/brb3.110

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*. https://doi.org/10.1016/j.rehab.2019.10.004

Mason, M. F., Norton, M. I., Van Horn, J. D., Wegner, D. M., Grafton, S. T., & Macrae, C. N. (2007). Wandering minds : The default network and stimulus-independent thought. *Science (New York, N.Y.)*, *315*(5810), 393-395. https://doi.org/10.1126/science.1131295

McCormack, K. (1999). Signal transduction in neuropathic pain, with special emphasis on the analgesic role of opioids-Part II: Moving basic science towards a new pharmacotherapy. *Pain Reviews*, *6*(2).

Melzack, R., & Wall, P. D. (1965). Pain mechanisms : A new theory. *Science (New York, N.Y.)*, *150*(3699), 971-979. https://doi.org/10.1126/science.150.3699.971

Melzack, Ronald, & Katz, J. (2013). Pain. *Wiley Interdisciplinary Reviews. Cognitive Science*, *4*(1), 1-15. https://doi.org/10.1002/wcs.1201

Merskey, H. (1994). Part III Pain Terms, A Current List with Definitions and Notes on usage. *Classification of chronic pain-descriptions of chronic pain syndromes and definitions of pain terms*, 207-214.

Meyer, R. A., & Campbell, J. N. (1981). Evidence for two distinct classes of unmyelinated nociceptive afferents in monkey. *Brain Research*, *224*(1), 149-152. https://doi.org/10.1016/0006-8993(81)91124-0

Meyer, R. A., Walker, R. E., & Mountcastle, V. B. (1976). A laser stimulator for the study of cutaneous thermal and pain sensations. *IEEE Transactions on Bio-Medical Engineering*, *23*(1), 54-60. https://doi.org/10.1109/tbme.1976.324616

Mokkink, L. B., Terwee, C. B., Knol, D. L., Stratford, P. W., Alonso, J., Patrick, D. L., Bouter, L. M., & de Vet, H. C. (2010). The COSMIN checklist for evaluating the methodological quality of studies on measurement properties : A clarification of its content. *BMC Medical Research Methodology*, *10*(1), 22. https://doi.org/10.1186/1471-2288-10-22

Monti, M. M., Laureys, S., & Owen, A. M. (2010). The vegetative state. *BMJ (Clinical Research Ed.)*, *341*, c3765. https://doi.org/10.1136/bmj.c3765

Monti, M. M., Vanhaudenhuyse, A., Coleman, M. R., Boly, M., Pickard, J. D., Tshibanda, L., Owen, A. M., & Laureys, S. (2010). Willful modulation of brain activity in disorders of consciousness. *The New England Journal of Medicine*, *362*(7), 579-589. https://doi.org/10.1056/NEJMoa0905370

Mountcastle, V. B. (2005). *The sensory hand : Neural mechanisms of somatic sensation*. Harvard University Press.

Mouraux, A., Guérit, J. M., & Plaghki, L. (2003). Non-phase locked electroencephalogram (EEG) responses to CO2 laser skin stimulations may reflect central interactions between A partial partial differential- and C-fibre afferent volleys. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *114*(4), 710-722. https://doi.org/10.1016/s1388-2457(03)00027-0

Mouraux, A., & Iannetti, G. D. (2008). Across-trial averaging of event-related EEG responses and beyond. *Magnetic Resonance Imaging*, *26*(7), 1041-1054. https://doi.org/10.1016/j.mri.2008.01.011

Mouraux, A., & Iannetti, G. D. (2009). Nociceptive laser-evoked brain potentials do not reflect nociceptive-specific neural activity. *Journal of Neurophysiology*, *101*(6), 3258-3269. https://doi.org/10.1152/jn.91181.2008

Mouraux, A., Iannetti, G. D., & Plaghki, L. (2010). Low intensity intra-epidermal electrical stimulation can activate Aδ-nociceptors selectively. *Pain*, *150*(1), 199-207. https://doi.org/10.1016/j.pain.2010.04.026

Mouraux, André. (2015). The cortical processing of pain. In C. Chatelle & S. Laureys (Éd.), *Assessing Pain and Communication in Disorders of Consciousness* (1st éd., p. 12-42). Routledge.

Mouraux, André, Diukova, A., Lee, M. C., Wise, R. G., & Iannetti, G. D. (2011). A multisensory investigation of the functional significance of the « pain matrix ». *NeuroImage*, *54*(3), 2237-2249. https://doi.org/10.1016/j.neuroimage.2010.09.084

Mouraux, André, & Iannetti, G. D. (2018). The search for pain biomarkers in the human brain. *Brain: A Journal of Neurology*, *141*(12), 3290-3307. https://doi.org/10.1093/brain/awy281

Mouraux, André, Iannetti, G. D., Colon, E., Nozaradan, S., Legrain, V., & Plaghki, L. (2011). Nociceptive steady-state evoked potentials elicited by rapid periodic thermal stimulation of cutaneous nociceptors. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *31*(16), 6079-6087. https://doi.org/10.1523/JNEUROSCI.3977-10.2011

Münte, T. F., Jöbges, E. M., Wieringa, B. M., Klein, S., Schubert, M., Johannes, S., & Dengler, R. (1996). Human evoked potentials to long duration vibratory stimuli : Role of muscle afferents. *Neuroscience Letters*, *216*(3), 163-166. https://doi.org/10.1016/0304-3940(96)13036-6

Nahra, H., & Plaghki, L. (2003). The effects of A-fiber pressure block on perception and neurophysiological correlates of brief non-painful and painful CO2 laser stimuli in humans. *European Journal of Pain (London, England)*, *7*(2), 189-199. https://doi.org/10.1016/S1090-3801(02)00099-X

Nakayama, N., Okumura, A., Shinoda, J., Nakashima, T., & 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. *Journal of Neurology, Neurosurgery, and Psychiatry*, *77*(7), 856-862. https://doi.org/10.1136/jnnp.2005.080523

Nangini, C., Ross, B., Tam, F., & Graham, S. J. (2006). Magnetoencephalographic study of vibrotactile evoked transient and steady-state responses in human somatosensory cortex. *NeuroImage*, *33*(1), 252-262. https://doi.org/10.1016/j.neuroimage.2006.05.045

Naro, A., Russo, M., Leo, A., Rifici, C., Pollicino, P., Bramanti, P., & Calabrò, R. S. (2015). Cortical Responsiveness to Nociceptive Stimuli in Patients with

Chronic Disorders of Consciousness : Do C-Fiber Laser Evoked Potentials Have a Role? *PloS One*, *10*(12), e0144713. https://doi.org/10.1371/journal.pone.0144713

Norcia, A. M., Appelbaum, L. G., Ales, J. M., Cottereau, B. R., & Rossion, B. (2015). The steady-state visual evoked potential in vision research : A review. *Journal of Vision*, *15*(6), 4. https://doi.org/10.1167/15.6.4

Novotny, G. E., & Gommert-Novotny, E. (1988). Intraepidermal nerves in human digital skin. *Cell and Tissue Research*, *254*(1), 111-117. https://doi.org/10.1007/bf00220023

Nozaradan, S. (2014). Exploring how musical rhythm entrains brain activity with electroencephalogram frequency-tagging. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1658), 20130393. https://doi.org/10.1098/rstb.2013.0393

Opsommer, E., Masquelier, E., & Plaghki, L. (1999). Determination of nerve conduction velocity of C-fibres in humans from thermal thresholds to contact heat (thermode) and from evoked brain potentials to radiant heat (CO2 laser). *Neurophysiologie Clinique = Clinical Neurophysiology*, *29*(5), 411-422. https://doi.org/10.1016/S0987-7053(00)87265-2

Ostrowsky, K., Magnin, M., Ryvlin, P., Isnard, J., Guenot, M., & Mauguière, F. (2002). Representation of pain and somatic sensation in the human insula : A study of responses to direct electrical cortical stimulation. *Cerebral Cortex (New York, N.Y.: 1991)*, *12*(4), 376-385. https://doi.org/10.1093/cercor/12.4.376

Owen, A. M., Coleman, M. R., Boly, M., Davis, M. H., Laureys, S., & Pickard, J. D. (2006). Detecting awareness in the vegetative state. *Science (New York, N.Y.)*, *313*(5792), 1402. https://doi.org/10.1126/science.1130197

Perchet, C., Godinho, F., Mazza, S., Frot, M., Legrain, V., Magnin, M., & Garcia-Larrea, L. (2008). Evoked potentials to nociceptive stimuli delivered by CO2 or Nd:YAP lasers. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *119*(11), 2615-2622. https://doi.org/10.1016/j.clinph.2008.06.021

Peyron, R., Frot, M., Schneider, F., Garcia-Larrea, L., Mertens, P., Barral, F. G., Sindou, M., Laurent, B., & Mauguière, F. (2002). Role of operculoinsular cortices in human pain processing : Converging evidence from PET, fMRI, dipole modeling, and intracerebral recordings of evoked potentials. *NeuroImage*, *17*(3), 1336-1346. https://doi.org/10.1006/nimg.2002.1315

Peyron, R., Laurent, B., & García-Larrea, L. (2000). Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiologie Clinique* =

Clinical Neurophysiology, *30*(5), 263-288. https://doi.org/10.1016/s0987-7053(00)00227-6

Pfurtscheller, G., & Da Silva, F. L. (1999). Event-related EEG/MEG synchronization and desynchronization : Basic principles. *Clinical neurophysiology*, *110*(11), 1842-1857.

Phillips, C. L., Bruno, M.-A., Maquet, P., Boly, M., Noirhomme, Q., Schnakers, C., Vanhaudenhuyse, A., Bonjean, M., Hustinx, R., Moonen, G., Luxen, A., & Laureys, S. (2011). « Relevance vector machine » consciousness classifier applied to cerebral metabolism of vegetative and locked-in patients. *NeuroImage*, *56*(2), 797-808. https://doi.org/10.1016/j.neuroimage.2010.05.083

Plaghki, L., & Mouraux, A. (2003). How do we selectively activate skin nociceptors with a high power infrared laser? Physiology and biophysics of laser stimulation. *Neurophysiologie Clinique = Clinical Neurophysiology*, *33*(6), 269-277.

Plaghki, Léon, & Mouraux, A. (2002). Brain responses to signals ascending through C-fibers. *International Congress Series*, *1232*, 181.

Plaghki, Léon, & Mouraux, A. (2005). EEG and laser stimulation as tools for pain research. *Current Opinion in Investigational Drugs (London, England: 2000)*, *6*(1), 58-64.

Ploner, M., Schmitz, F., Freund, H. J., & Schnitzler, A. (1999). Parallel activation of primary and secondary somatosensory cortices in human pain processing. *Journal of Neurophysiology*, *81*(6), 3100-3104. https://doi.org/10.1152/jn.1999.81.6.3100

Posner, J. B., Plum, F., Saper, C. B., & Schiff, N. (2007). *Plum and Posner's Diagnosis of Stupor and Coma*. Oxford University Press, USA.

Poulsen, I., Brix, P., Andersen, S., Westergaard, L., & Guldager, R. (2016). Pain Assessment Scale for Patients With Disorders of Consciousness : A Preliminary Validation Study. *The Journal of Neuroscience Nursing: Journal of the American Association of Neuroscience Nurses*, *48*(3), 124-131. https://doi.org/10.1097/JNN.0000000000000206

Rainville, P., Duncan, G. H., Price, D. D., Carrier, B., & Bushnell, M. C. (1997). Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science (New York, N.Y.)*, *277*(5328), 968-971. https://doi.org/10.1126/science.277.5328.968

Regan, D. (1966). Some characteristics of average steady-state and transient responses evoked by modulated light. *Electroencephalography and Clinical Neurophysiology*, *20*(3), 238-248. https://doi.org/10.1016/0013-4694(66)90088-5

Regan, D. (1989). *Human brain electrophysiology : Evoked potentials and evoked magnetic fields in science and medicine*. Elsevier.

Reilly, D. M., Ferdinando, D., Johnston, C., Shaw, C., Buchanan, K. D., & Green, M. R. (1997). The epidermal nerve fibre network : Characterization of nerve fibres in human skin by confocal microscopy and assessment of racial variations. *The British Journal of Dermatology*, *137*(2), 163-170. https://doi.org/10.1046/j.1365-2133.1997.18001893.x

Riganello, F., Chatelle, C., Schnakers, C., & Laureys, S. (2019). Heart Rate Variability as an Indicator of Nociceptive Pain in Disorders of Consciousness? *Journal of Pain and Symptom Management*, *57*(1), 47-56. https://doi.org/10.1016/j.jpainsymman.2018.09.016

Sanders, R. D., Tononi, G., Laureys, S., & Sleigh, J. W. (2012). Unresponsiveness ≠ unconsciousness. *Anesthesiology*, *116*(4), 946-959. https://doi.org/10.1097/ALN.0b013e318249d0a7

Schepers, R. J., & Ringkamp, M. (2010). Thermoreceptors and thermosensitive afferents. *Neuroscience and Biobehavioral Reviews*, *34*(2), 177-184. https://doi.org/10.1016/j.neubiorev.2009.10.003

Schiff, N. D. (2015). Cognitive Motor Dissociation Following Severe Brain Injuries. *JAMA Neurology*, *72*(12), 1413-1415. https://doi.org/10.1001/jamaneurol.2015.2899

Schnakers, C., Bessou, H., Rubi-Fessen, I., Hartmann, A., Fink, G. R., Meister, I., Giacino, J. T., Laureys, S., & Majerus, S. (2015). Impact of aphasia on consciousness assessment : A cross-sectional study. *Neurorehabilitation and Neural Repair*, *29*(1), 41-47. https://doi.org/10.1177/1545968314528067

Schnakers, C., Chatelle, C., Vanhaudenhuyse, A., Majerus, S., Ledoux, D., Boly, M., Bruno, M.-A., Boveroux, P., Demertzi, A., Moonen, G., & Laureys, S. (2010). The Nociception Coma Scale : A new tool to assess nociception in disorders of consciousness. *Pain*, *148*(2), 215-219. https://doi.org/10.1016/j.pain.2009.09.028

Schnakers, C., Vanhaudenhuyse, A., Giacino, J., Ventura, M., Boly, M., Majerus, S., Moonen, G., & Laureys, S. (2009). Diagnostic accuracy of the vegetative and minimally conscious state : Clinical consensus versus standardized neurobehavioral assessment. *BMC Neurology*, *9*, 35. https://doi.org/10.1186/1471-2377-9-35

Scholz, J., Mannion, R. J., Hord, D. E., Griffin, R. S., Rawal, B., Zheng, H., Scoffings, D., Phillips, A., Guo, J., Laing, R. J., & others. (2009). A novel tool for the assessment of pain : Validation in low back pain. *PLoS medicine*, *6*(4), e1000047.

Seel, R. T., Sherer, M., Whyte, J., Katz, D. I., Giacino, J. T., Rosenbaum, A. M., Hammond, F. M., Kalmar, K., Pape, T. L.-B., Zafonte, R., Biester, R. C., Kaelin, D., Kean, J., & Zasler, N. (2010). Assessment Scales for Disorders of

Consciousness : Evidence-Based Recommendations for Clinical Practice and Research. *Archives of Physical Medicine and Rehabilitation*, *91*(12), 1795-1813. https://doi.org/10.1016/j.apmr.2010.07.218

Serra, J., Campero, M., Ochoa, J., & Bostock, H. (1999). Activity-dependent slowing of conduction differentiates functional subtypes of C fibres innervating human skin. *The Journal of Physiology*, *515 (Pt 3)*, 799-811. https://doi.org/10.1111/j.1469-7793.1999.799ab.x

Severens, M., Farquhar, J., Desain, P., Duysens, J., & Gielen, C. (2010). Transient and steady-state responses to mechanical stimulation of different fingers reveal interactions based on lateral inhibition. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *121*(12), 2090-2096. https://doi.org/10.1016/j.clinph.2010.05.016

Shackman, A. J., Salomons, T. V., Slagter, H. A., Fox, A. S., Winter, J. J., & Davidson, R. J. (2011). The integration of negative affect, pain and cognitive control in the cingulate cortex. *Nature Reviews. Neuroscience*, *12*(3), 154-167. https://doi.org/10.1038/nrn2994

Shaffer, J. P. (1995). Multiple Hypothesis Testing. *Annual Review of Psychology*, *46*(1), 561-584. https://doi.org/10.1146/annurev.ps.46.020195.003021

SIFEC. (2009). *Manuel d'utilisation du Laser Stimulation Device*.

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R. J., & Frith, C. D. (2004). Empathy for pain involves the affective but not sensory components of pain. *Science (New York, N.Y.)*, *303*(5661), 1157-1162. https://doi.org/10.1126/science.1093535

Smith, B. W., Tooley, E. M., Montague, E. Q., Robinson, A. E., Cosper, C. J., & Mullins, P. G. (2008). Habituation and sensitization to heat and cold pain in women with fibromyalgia and healthy controls. *Pain*, *140*(3), 420-428. https://doi.org/10.1016/j.pain.2008.09.018

Snyder, A. Z. (1992). Steady-state vibration evoked potentials : Descriptions of technique and characterization of responses. *Electroencephalography and Clinical Neurophysiology*, *84*(3), 257-268. https://doi.org/10.1016/0168-5597(92)90007-x

Soddu, A., Vanhaudenhuyse, A., Demertzi, A., Bruno, M.-A., Tshibanda, L., Di, H., Mélanie, B., Papa, M., Laureys, S., & Noirhomme, Q. (2011). Resting state activity in patients with disorders of consciousness. *Functional Neurology*, *26*(1), 37-43.

Stender, J., Gosseries, O., Bruno, M.-A., Charland-Verville, V., Vanhaudenhuyse, A., Demertzi, A., Chatelle, C., Thonnard, M., Thibaut, A., Heine, L., Soddu, A., Boly, M., Schnakers, C., Gjedde, A., & Laureys, S. (2014). Diagnostic precision

of PET imaging and functional MRI in disorders of consciousness : A clinical validation study. *Lancet (London, England)*, *384*(9942), 514-522. https://doi.org/10.1016/S0140-6736(14)60042-8

Stender, J., Kupers, R., Rodell, A., Thibaut, A., Chatelle, C., Bruno, M.-A., Gejl, M., Bernard, C., Hustinx, R., Laureys, S., & Gjedde, A. (2015). Quantitative rates of brain glucose metabolism distinguish minimally conscious from vegetative state patients. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *35*(1), 58-65. https://doi.org/10.1038/jcbfm.2014.169

Stender, J., Mortensen, K. N., Thibaut, A., Darkner, S., Laureys, S., Gjedde, A., & Kupers, R. (2016). The Minimal Energetic Requirement of Sustained Awareness after Brain Injury. *Current Biology: CB*, *26*(11), 1494-1499. https://doi.org/10.1016/j.cub.2016.04.024

Suraseranivongse, S., Yuvapoositanont, P., Srisakkrapikoop, P., Pommul, R., Phaka, W., & Itthimathin, P. (2015). A Comparison of Pain Scales in Patients with Disorders of Consciousness Following Craniotomy. *Journal of the Medical Association of Thailand = Chotmaihet Thangphaet*, *98*(7), 684-692.

Symonds, L. L., Gordon, N. S., Bixby, J. C., & Mande, M. M. (2006). Right-lateralized pain processing in the human cortex : An FMRI study. *Journal of Neurophysiology*, *95*(6), 3823-3830. https://doi.org/10.1152/jn.01162.2005

Taylor, C. M., Aird, V. H., Tate, R. L., & Lammi, M. H. (2007). Sequence of recovery during the course of emergence from the minimally conscious state. *Archives of Physical Medicine and Rehabilitation*, *88*(4), 521-525. https://doi.org/10.1016/j.apmr.2007.01.013

Teasdale, G., & Jennett, B. (1974). Assessment of coma and impaired consciousness. A practical scale. *Lancet (London, England)*, *2*(7872), 81-84. https://doi.org/10.1016/s0140-6736(74)91639-0

Thibaut, A., Chatelle, C., Wannez, S., Deltombe, T., Stender, J., Schnakers, C., Laureys, S., & Gosseries, O. (2015). Spasticity in disorders of consciousness : A behavioral study. *European Journal of Physical and Rehabilitation Medicine*, *51*(4), 389-397.

Tiest, W. M. B. (2010). Tactual perception of material properties. *Vision Research*, *50*(24), 2775-2782. https://doi.org/10.1016/j.visres.2010.10.005

Tobimatsu, S., Zhang, Y. M., & Kato, M. (1999). Steady-state vibration somatosensory evoked potentials : Physiological characteristics and tuning function. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical*

Neurophysiology, *110*(11), 1953-1958. https://doi.org/10.1016/s1388-2457(99)00146-7

Tölle, T. R., Kaufmann, T., Siessmeier, T., Lautenbacher, S., Berthele, A., Munz, F., Zieglgänsberger, W., Willoch, F., Schwaiger, M., Conrad, B., & Bartenstein, P. (1999). Region-specific encoding of sensory and affective components of pain in the human brain : A positron emission tomography correlation analysis. *Annals of Neurology*, *45*(1), 40-47. https://doi.org/10.1002/1531-8249(199901)45:1<40::aid-art8>3.0.co;2-l

Tommasino, C., Grana, C., Lucignani, G., Torri, G., & Fazio, F. (1995). Regional cerebral metabolism of glucose in comatose and vegetative state patients. *Journal of Neurosurgical Anesthesiology*, *7*(2), 109-116. https://doi.org/10.1097/00008506-199504000-00006

Tommaso, M. de, Navarro, J., Ricci, K., Lorenzo, M., Lanzillotti, C., Colonna, F., Resta, M., Lancioni, G., & Livrea, P. (2013). Pain in prolonged disorders of consciousness : Laser evoked potentials findings in patients with vegetative and minimally conscious states. *Brain Injury*, *27*(7-8), 962-972. https://doi.org/10.3109/02699052.2013.775507

Tracey, I., & Mantyh, P. W. (2007). The cerebral signature for pain perception and its modulation. *Neuron*, *55*(3), 377-391. https://doi.org/10.1016/j.neuron.2007.07.012

Treede, R. D., Kenshalo, D. R., Gracely, R. H., & Jones, A. K. (1999). The cortical representation of pain. *Pain*, *79*(2-3), 105-111. https://doi.org/10.1016/s0304-3959(98)00184-5

Treede, R. D., Kief, S., Hölzer, T., & Bromm, B. (1988). Late somatosensory evoked cerebral potentials in response to cutaneous heat stimuli. *Electroencephalography and Clinical Neurophysiology*, *70*(5), 429-441. https://doi.org/10.1016/0013-4694(88)90020-x

Treede, R. D., Meier, W., Kunze, K., & Bromm, B. (1988). Ultralate cerebral potentials as correlates of delayed pain perception : Observation in a case of neurosyphilis. *Journal of Neurology, Neurosurgery, and Psychiatry*, *51*(10), 1330-1333. https://doi.org/10.1136/jnnp.51.10.1330

Treede, R. D., Meyer, R. A., Raja, S. N., & Campbell, J. N. (1995). Evidence for two different heat transduction mechanisms in nociceptive primary afferents innervating monkey skin. *The Journal of Physiology*, *483*(Pt 3), 747-758.

Treede, R.-D. (2007). Das somatosensorische System—(Kapitel 14). *Physiologie des Menschen: mit Pathophysiologie*, 296-323.

Treede, R.-D., Lorenz, J., & Baumgärtner, U. (2003). Clinical usefulness of laser-evoked potentials. *Neurophysiologie Clinique/Clinical Neurophysiology*, *33*(6), 303-314. https://doi.org/10.1016/j.neucli.2003.10.009

Truini, A., Galeotti, F., Romaniello, A., Virtuoso, M., Iannetti, G. D., & Cruccu, G. (2005). Laser-evoked potentials : Normative values. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *116*(4), 821-826. https://doi.org/10.1016/j.clinph.2004.10.004

Valentini, E., Hu, L., Chakrabarti, B., Hu, Y., Aglioti, S. M., & Iannetti, G. D. (2012). The primary somatosensory cortex largely contributes to the early part of the cortical response elicited by nociceptive stimuli. *NeuroImage*, *59*(2), 1571-1581. https://doi.org/10.1016/j.neuroimage.2011.08.069

Valeriani, M., Restuccia, D., Barba, C., Le Pera, D., Tonali, P., & Mauguière, F. (2000). Sources of cortical responses to painful CO(2) laser skin stimulation of the hand and foot in the human brain. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *111*(6), 1103-1112. https://doi.org/10.1016/s1388-2457(00)00273-x

Valeriani, Massimiliano, Restuccia, D., Le Pera, D., De Armas, L., Maiese, T., & Tonali, P. (2002). Attention-related modifications of ultra-late CO(2) laser evoked potentials to human trigeminal nerve stimulation. *Neuroscience Letters*, *329*(3), 329-333. https://doi.org/10.1016/s0304-3940(02)00671-7

van den Broeke, E. N., & Mouraux, A. (2014). High-frequency electrical stimulation of the human skin induces heterotopical mechanical hyperalgesia, heat hyperalgesia, and enhanced responses to nonnociceptive vibrotactile input. *Journal of Neurophysiology*, *111*(8), 1564-1573. https://doi.org/10.1152/jn.00651.2013

Vanhaudenhuyse, A., Demertzi, A., Schabus, M., Noirhomme, Q., Bredart, S., Boly, M., Phillips, C., Soddu, A., Luxen, A., Moonen, G., & Laureys, S. (2010). Two Distinct Neuronal Networks Mediate the Awareness of Environment and of Self. *Journal of Cognitive Neuroscience*, *23*(3), 570-578. https://doi.org/10.1162/jocn.2010.21488

Vanhaudenhuyse, A., Noirhomme, Q., Tshibanda, L. J.-F., Bruno, M.-A., Boveroux, P., Schnakers, C., Soddu, A., Perlbarg, V., Ledoux, D., Brichant, J.-F., Moonen, G., Maquet, P., Greicius, M. D., Laureys, S., & Boly, M. (2010). Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients. *Brain*, *133*(1), 161-171. https://doi.org/10.1093/brain/awp313

Vink, P., Eskes, A. M., Lindeboom, R., van den Munckhof, P., & Vermeulen, H. (2014). Nurses Assessing Pain with the Nociception Coma Scale : Interrater Reliability

and Validity. *Pain Management Nursing*, *15*(4), 881-887. https://doi.org/10.1016/j.pmn.2014.01.004

Vink, P., Lucas, C., Maaskant, J. M., van Erp, W. S., Lindeboom, R., & Vermeulen, H. (2017). Clinimetric properties of the Nociception Coma Scale (-Revised) : A systematic review. *European Journal of Pain (London, England)*, *21*(9), 1463-1474. https://doi.org/10.1002/ejp.1063

Vogt, B. A., Derbyshire, S., & Jones, A. K. (1996). Pain processing in four regions of human cingulate cortex localized with co-registered PET and MR imaging. *The European Journal of Neuroscience*, *8*(7), 1461-1473. https://doi.org/10.1111/j.1460-9568.1996.tb01608.x

Wade, D. T. (2018). Using best interests meetings for people in a prolonged disorder of consciousness to improve clinical and ethical management. *Journal of medical ethics*, *44*(5), 336-342.

Wang, F., Di, H., Hu, X., Jing, S., Thibaut, A., Di Perri, C., Huang, W., Nie, Y., Schnakers, C., & Laureys, S. (2015). Cerebral response to subject's own name showed high prognostic value in traumatic vegetative state. *BMC Medicine*, *13*, 83. https://doi.org/10.1186/s12916-015-0330-7

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. (2018). Prevalence of coma-recovery scale-revised signs of consciousness in patients in minimally conscious state. *Neuropsychological Rehabilitation*, *28*(8), 1350-1359. https://doi.org/10.1080/09602011.2017.1310656

Wannez, S., Heine, L., Thonnard, M., Gosseries, O., Laureys, S., & Coma Science Group collaborators. (2017). The repetition of behavioral assessments in diagnosis of disorders of consciousness. *Annals of Neurology*, *81*(6), 883-889. https://doi.org/10.1002/ana.24962

Whyte, J., Nordenbo, A. M., Kalmar, K., Merges, B., Bagiella, E., Chang, H., Yablon, S., Cho, S., Hammond, F., Khademi, A., & Giacino, J. (2013). Medical complications during inpatient rehabilitation among patients with traumatic disorders of consciousness. *Archives of Physical Medicine and Rehabilitation*, *94*(10), 1877-1883. https://doi.org/10.1016/j.apmr.2012.12.027

Wierzbicka, A. (2012). Is Pain a Human Universal? A Cross-Linguistic and Cross-Cultural Perspective on Pain: *Emotion Review*. https://doi.org/10.1177/1754073912439761

Wijdicks, E. F. M., Varelas, P. N., Gronseth, G. S., & Greer, D. M. (2010). Evidence-based guideline update : Determining brain death in adults. *Neurology*, *74*(23), 1911. https://doi.org/10.1212/WNL.0b013e3181e242a8

Williams, A. C. de C., & Craig, K. D. (2016). Updating the definition of pain. *PAIN*, *157*(11), 2420. https://doi.org/10.1097/j.pain.0000000000000613

Willis, W. D. (1985). Nociceptive pathways : Anatomy and physiology of nociceptive ascending pathways. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *308*(1136), 253-268.

Wooten, M., Weng, H.-J., Hartke, T. V., Borzan, J., Klein, A. H., Turnquist, B., Dong, X., Meyer, R. A., & Ringkamp, M. (2014). Three functionally distinct classes of C-fibre nociceptors in primates. *Nature Communications*, *5*, 4122. https://doi.org/10.1038/ncomms5122

Yoon, C., Wook, Y. Y., Sik, N. H., Ho, K. S., & Mo, C. J. (1994). Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. *Pain*, *59*(3), 369-376.

Youell, P. D., Wise, R. G., Bentley, D. E., Dickinson, M. R., King, T. A., Tracey, I., & Jones, A. K. P. (2004). Lateralisation of nociceptive processing in the human brain : A functional magnetic resonance imaging study. *NeuroImage*, *23*(3), 1068-1077. https://doi.org/10.1016/j.neuroimage.2004.07.004

Zeman, A. (2001). Consciousness. *Brain: A Journal of Neurology*, *124*(Pt 7), 1263-1289. https://doi.org/10.1093/brain/124.7.1263

Zhang, Z. G., Hu, L., Hung, Y. S., Mouraux, A., & Iannetti, G. D. (2012). Gamma-band oscillations in the primary somatosensory cortex—A direct and obligatory correlate of subjective pain intensity. *Journal of Neuroscience*, *32*(22), 7429-7438.

zu Eulenburg, P., Baumgärtner, U., Treede, R.-D., & Dieterich, M. (2013). Interoceptive and multimodal functions of the operculo-insular cortex : Tactile, nociceptive and vestibular representations. *NeuroImage*, *83*, 75-86. https://doi.org/10.1016/j.neuroimage.2013.06.057

APPENDIX A. SUPPLEMENTARY MATERIAL

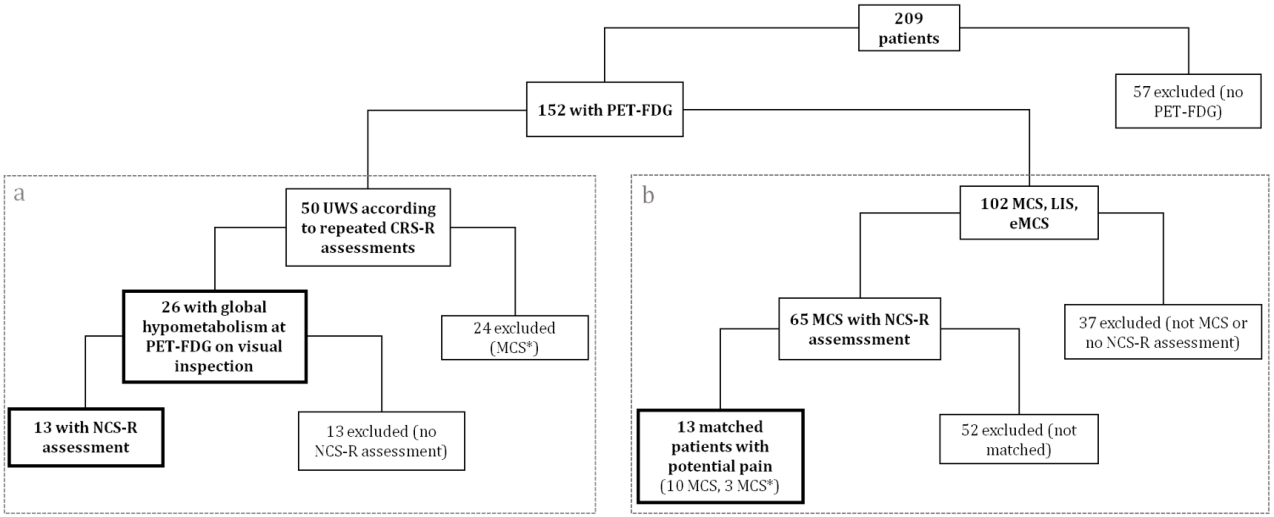


Figure A.1. Flow chart representing the selection of patients in (a) UWS with the CRS-R assessment and with a global hypometabolism at PET-FDG, (b) MCS with potential pain defined based on threshold (LIS = Locked-in syndrome, eMCS = emergence from minimally conscious state, UWS = Unresponsive wakefulness syndrome/vegetative state, MCS* = UWS with atypical cortical metabolism preservation). Figure adapted from Bonin et al. (2019)

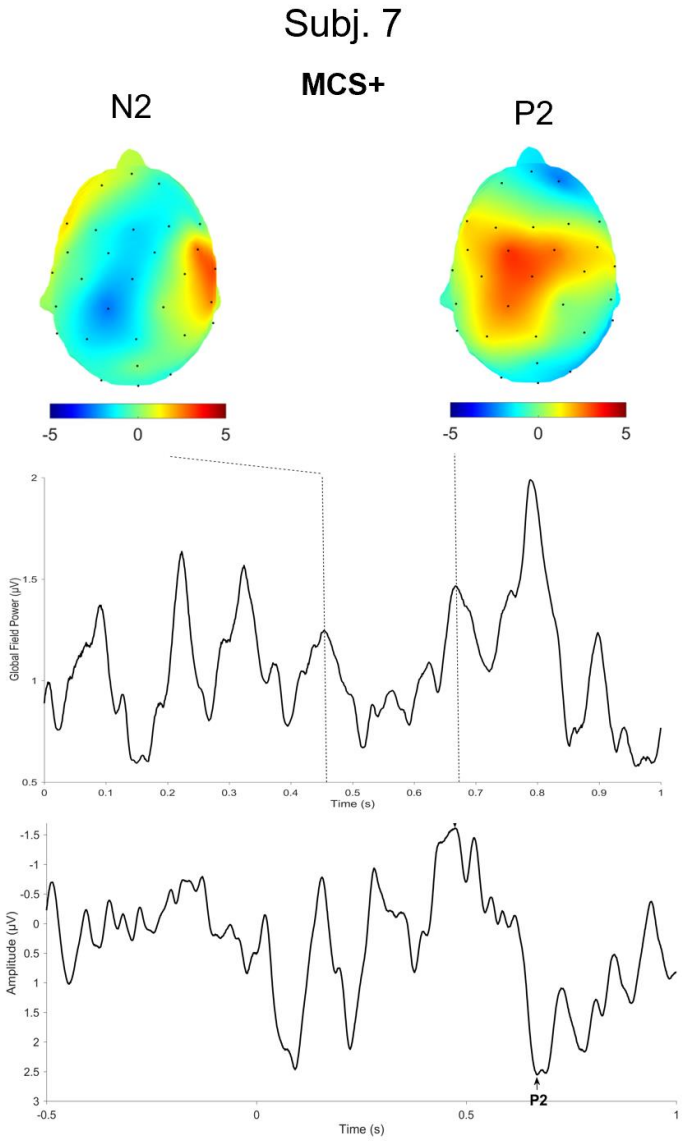


Figure A.2. Global field power (GFP) and corresponding topographic maps of nociceptive event-related brain potentials (**LEPs**). The last row corresponds to the LEPs recorded in one MCS patient (Subj. 7), using Cz versus an average reference. Despite its low SNR, an N2-P2 complex is identifiable.

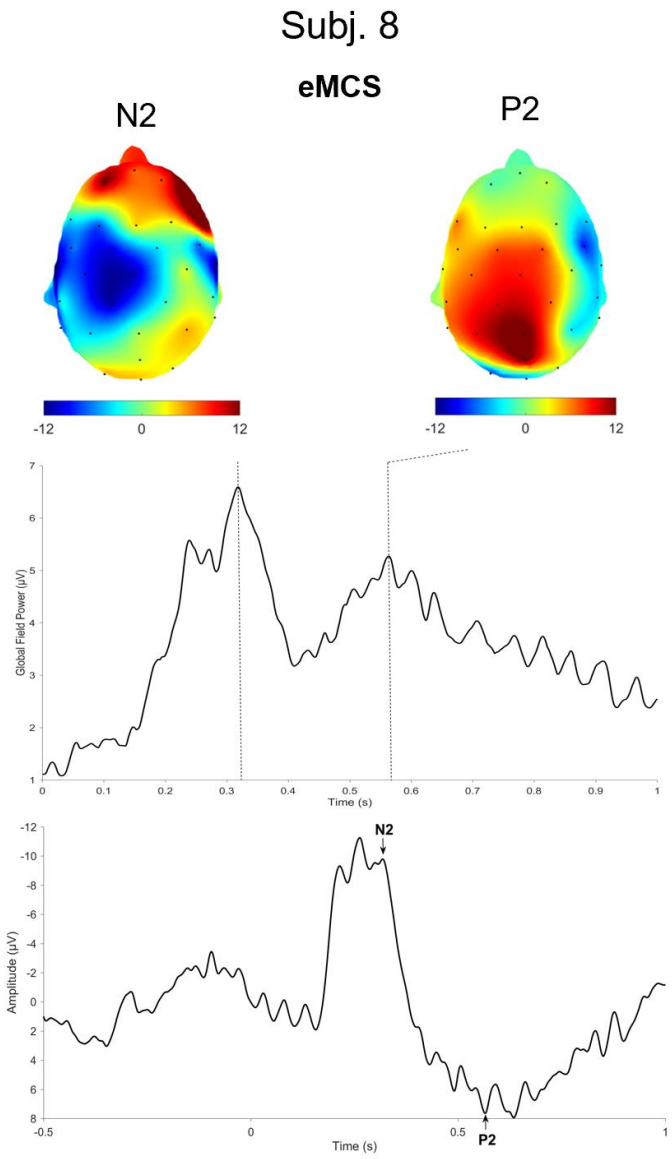


Figure A.3. Global field power (GFP) and corresponding topographic maps of nociceptive event-related brain potentials (**LEPs**). The last row corresponds to the LEPs recorded in one eMCS patient (Subj. 8), using Cz versus an average reference. Despite its low SNR, an N2-P2 complex is identifiable.

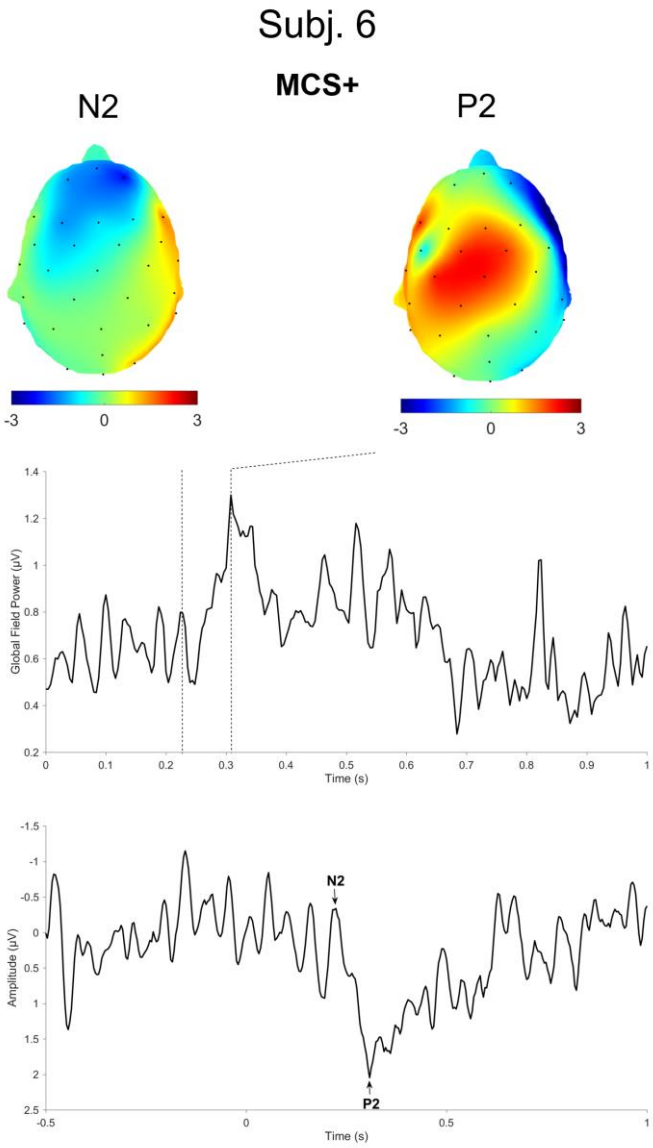


Figure A.4. Global field power (GFP) and corresponding topographic maps of cool event-related brain potentials (**CEPs**). The last row corresponds to the CEPs recorded in one MCS patient (Subj. 6), using Cz versus an average reference. Despite its very low SNR, an N2-P2 complex is identifiable.

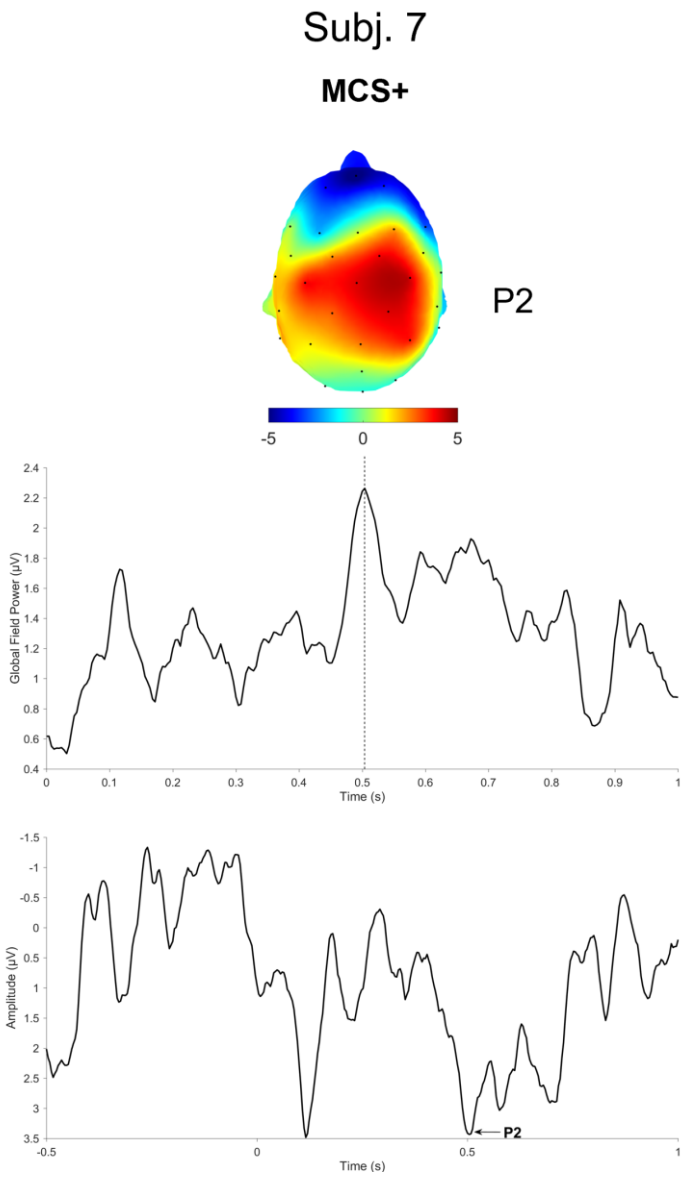


Figure A.5. Global field power (GFP) and corresponding topographic maps of cool event-related brain potentials (**CEPs**). Note that only the P2 wave topographic map is represented as N2 wave was not identifiable. The last row corresponds to the CEPs recorded in one MCS patient (Subj. 7), using Cz versus an average reference. Despite its very low SNR, an N2-P2 complex is identifiable.

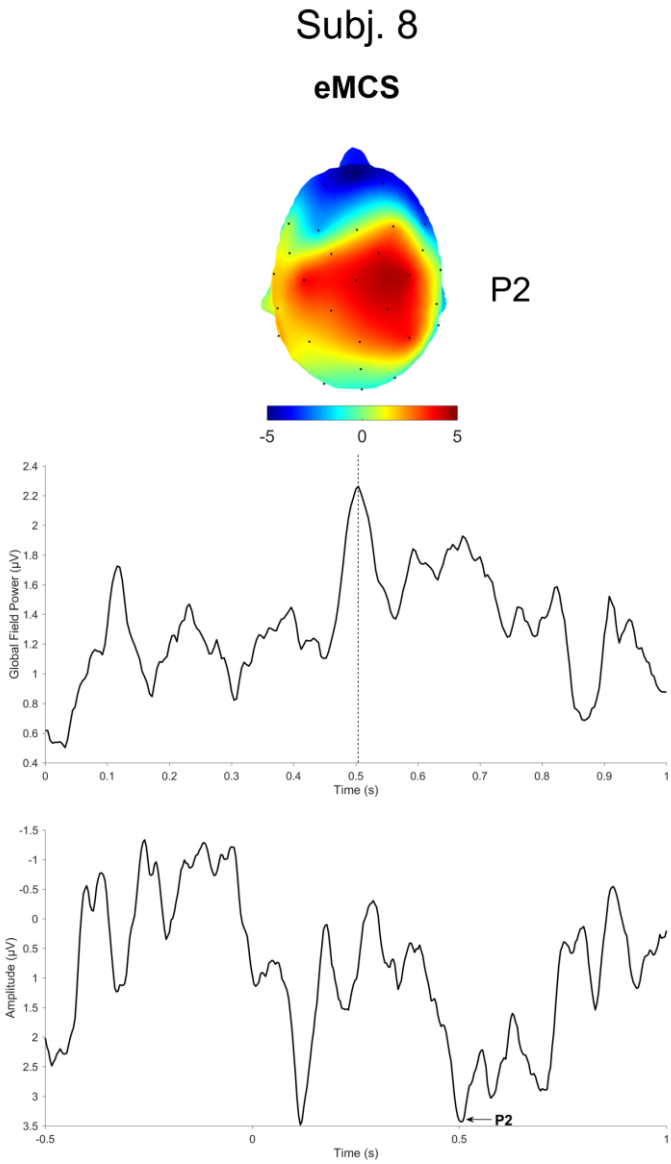


Figure A.6. Global field power (GFP) and corresponding topographic maps of cool event-related brain potentials (**CEPs**). Note that only the P2 wave topographic map is represented as N2 wave was not identifiable. The last row corresponds to the CEPs recorded in one eMCS patient (Subj. 8), using Cz versus an average reference. Despite its very low SNR, an N2-P2 complex is identifiable.

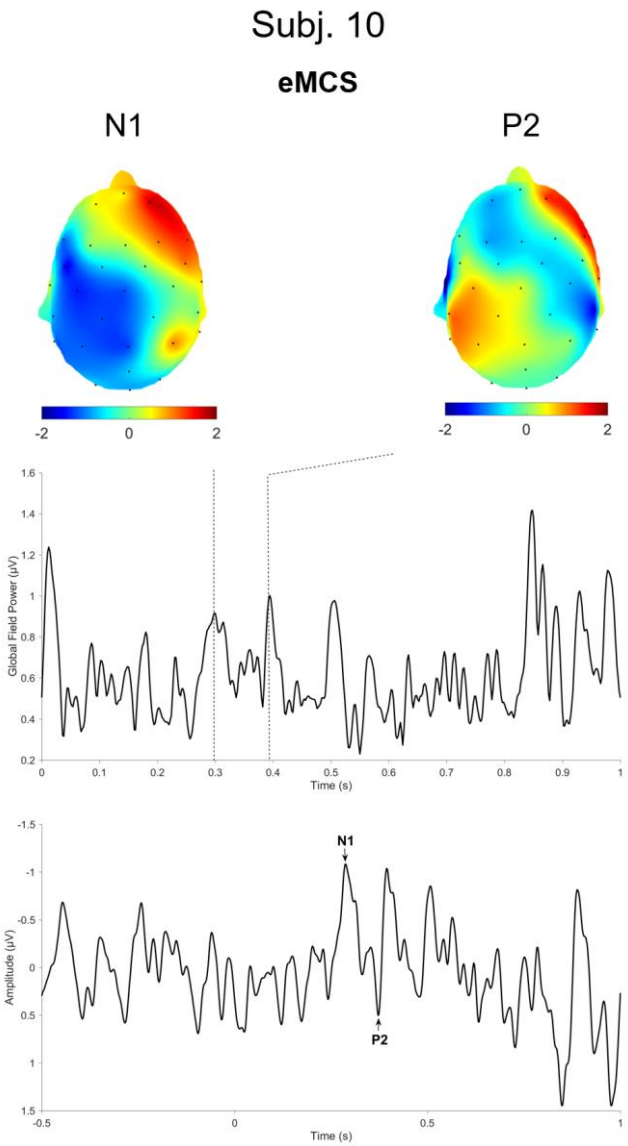


Figure A.7. GFP and corresponding topographic maps of vibrotactile event-related brain potentials (**Vibro-EPs**). The last row corresponds to the Vibro-EPs recorded in one eMCS patient (Subj. 10), using Cz versus an average reference. A P2 wave appears to be identifiable despite the very low SNR.

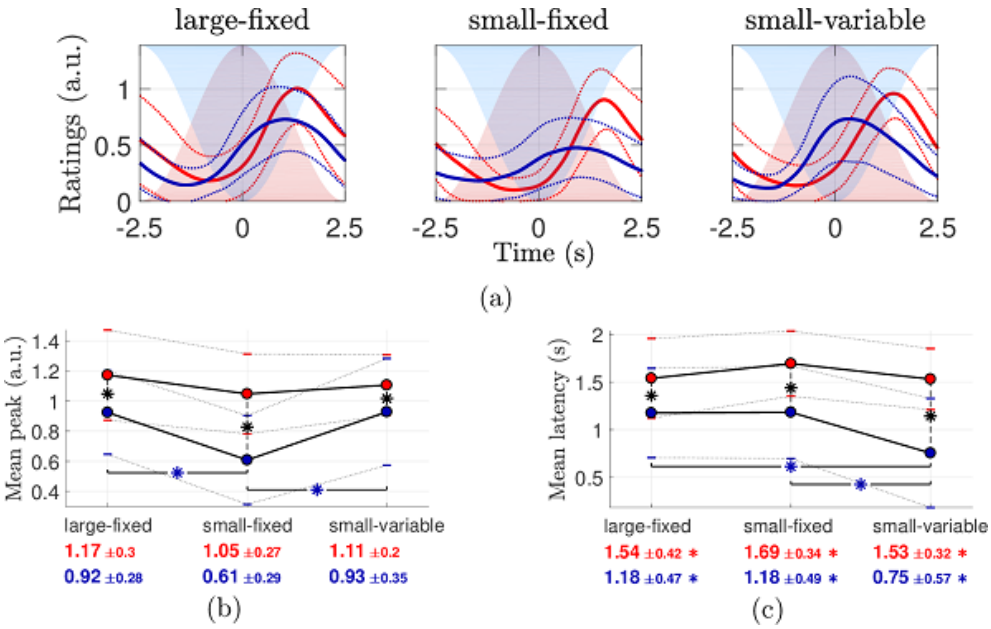


Figure A.8. Intensity ratings averaged across stimulation cycles. (a) Group-level average (± standard deviation in dotted) time courses of the intensity ratings averaged across stimulation cycles, in (light) red and (dark) blue for the warm and cool conditions respectively. The stimulation temperatures are shaded. (b-c) Pairwise comparisons of (b) the mean peak intensity rating and (c) the mean peak intensity latency. The red (resp. blue) dots show the mean features for the warm (resp. cool) stimulation, the standard deviations being indicated with horizontal bars. These means and standard deviations are also reported below the plots with the corresponding color. Each asterisk in the plot indicates a significant difference according to paired samples *t*-tests with Holm-Bonferroni correction, in red, blue (horizontally) or black (vertically) respectively when the two compared conditions are warm, cool or different. In (c), an asterisk besides an x-axis tick label shows that the corresponding mean feature is significantly different from 0 based on one sample *t*-tests with Holm-Bonferroni correction. Figure from Mulders et al. (2020)

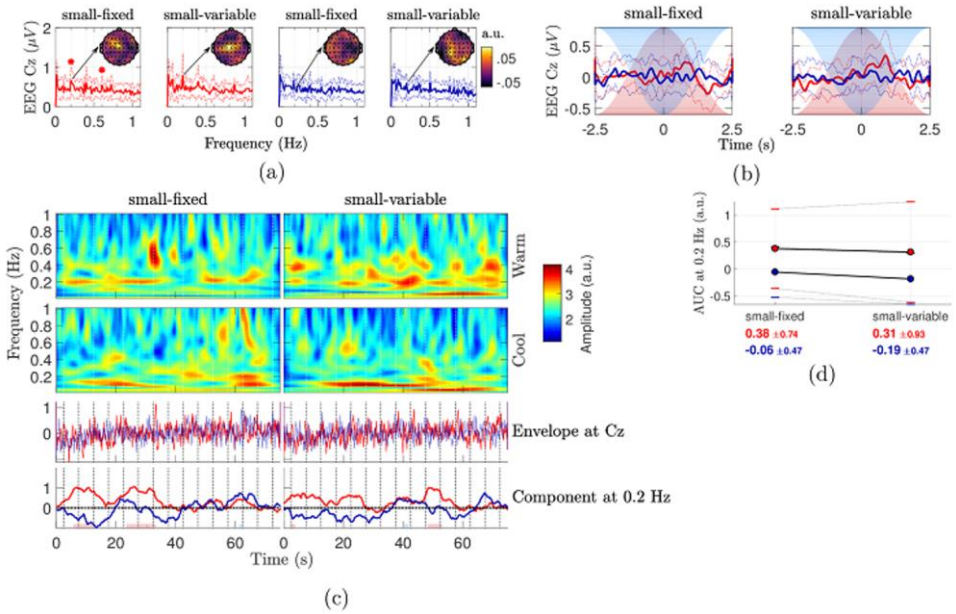


Figure A.9. Signal envelopes in the theta frequency band (4-8 Hz). (a) Fourier transforms of the envelopes averaged over the epochs (grand mean ± standard deviation) at electrode Cz. A star indicates significativity of the noise-subtracted peak at $\{k \cdot 0.2\}_{k=1}^{5}$ Hz (*t*-tests against 0). The scalp maps show the distribution of the noise-subtracted amplitudes at 0.2 Hz. (b) Envelopes averaged across stimulation periods (grand mean ± standard deviation). (c) Grand mean Continuous Wavelet Transform (CWT) of the average signals at Cz and (d) pairwise comparisons of the Area Under the Curve (AUC) of the stimulus-evoked envelope components at 0.2 Hz. The red (resp. blue) dots show the mean AUCs for the warm (resp. cool) stimulation, the standard deviations being indicated with horizontal bars. Each asterisk in the plot indicates a significant difference according to paired samples *t*-tests, in red, blue or black respectively when the two compared conditions are warm cool or different. Figure adapted from Mulders et al. (2020)

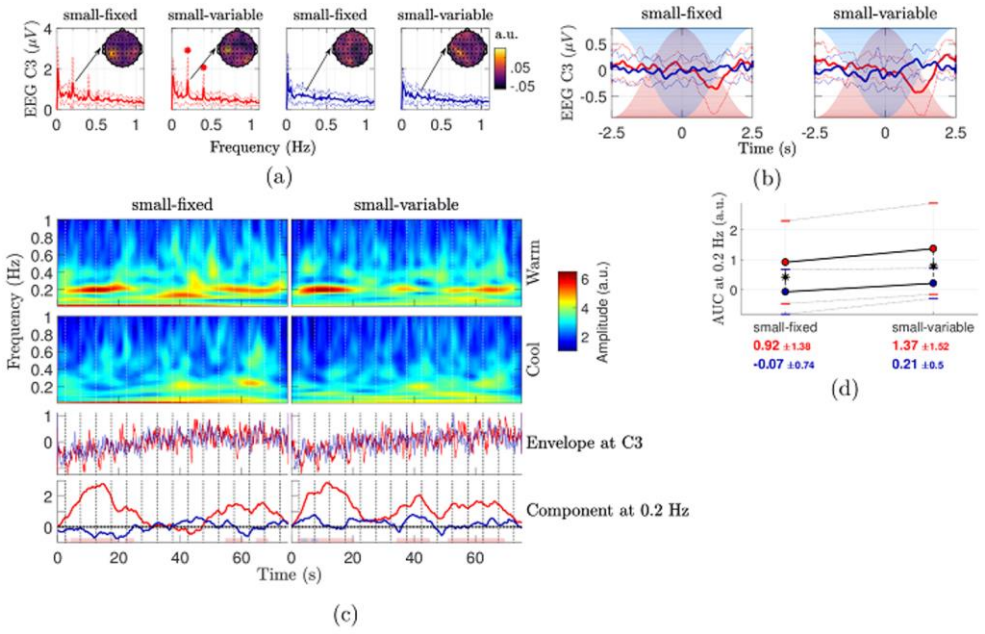


Figure A.10. Signal envelopes in the alpha frequency band (8-12 Hz). (a) Fourier transforms of the envelopes averaged over the epochs (grand mean ± standard deviation) at electrode C3. A star indicates significativity of the noise-subtracted peak at $\{k \cdot 0.2\}_{k=1}^{5}$ Hz (*t*-tests against 0). The scalp maps show the distribution of the noise-subtracted amplitudes at 0.2 Hz. (b) Envelopes averaged across stimulation periods (grand mean ± standard deviation). (c) Grand mean Continuous Wavelet Transform (CWT) of the average signals at C3 and (d) pairwise comparisons of the Area Under the Curve (AUC) of the stimulus-evoked envelope components at 0.2 Hz. The red (resp. blue) dots show the mean AUCs for the warm (resp. cool) stimulation, the standard deviations being indicated with horizontal bars. Each asterisk in the plot indicates a significant difference according to paired samples *t*-tests, in red, blue or black respectively when the two compared conditions are warm cool or different. Figure adapted from Mulders et al. (2020)

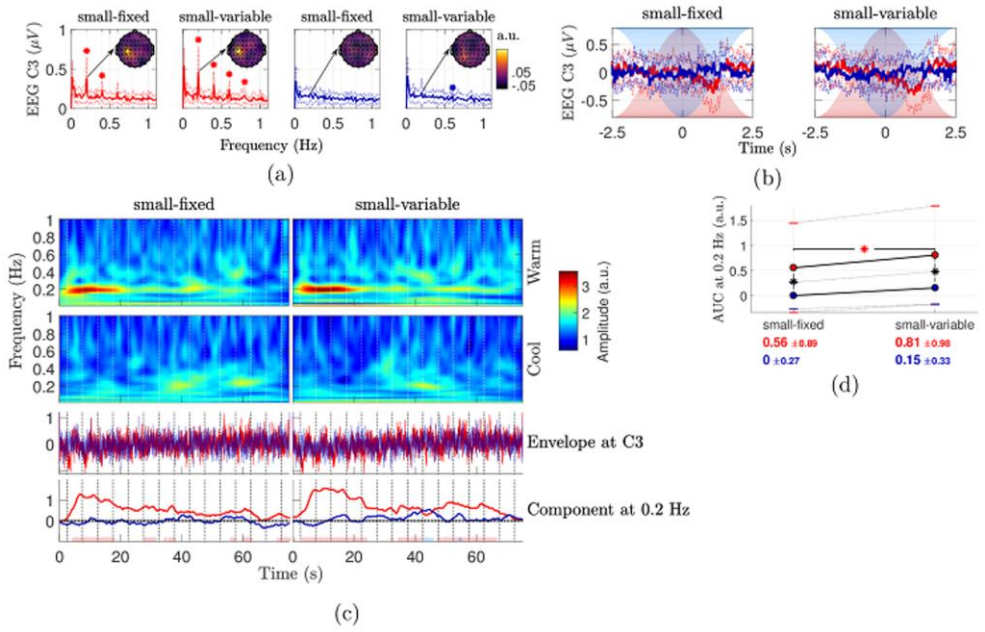


Figure A.11. Signal envelopes in the beta frequency band (12-30 Hz). (a) Fourier transforms of the envelopes averaged over the epochs (grand mean ± standard deviation) at electrode C3. A star indicates significativity of the noise-subtracted peak at $\{k \cdot 0.2\}_{k=1}^{5}$ Hz (*t*-tests against 0). The scalp maps show the distribution of the noise-subtracted amplitudes at 0.2 Hz. (b) Envelopes averaged across stimulation periods (grand mean ± standard deviation). (c) Grand mean Continuous Wavelet Transform (CWT) of the average signals at C3 and (d) pairwise comparisons of the Area Under the Curve (AUC) of the stimulus-evoked envelope components at 0.2 Hz. The red (resp. blue) dots show the mean AUCs for the warm (resp. cool) stimulation, the standard deviations being indicated with horizontal bars. Each asterisk in the plot indicates a significant difference according to paired samples *t*-tests, in red, blue or black respectively when the two compared conditions are warm cool or different. Figure adapted from Mulders et al. (2020)

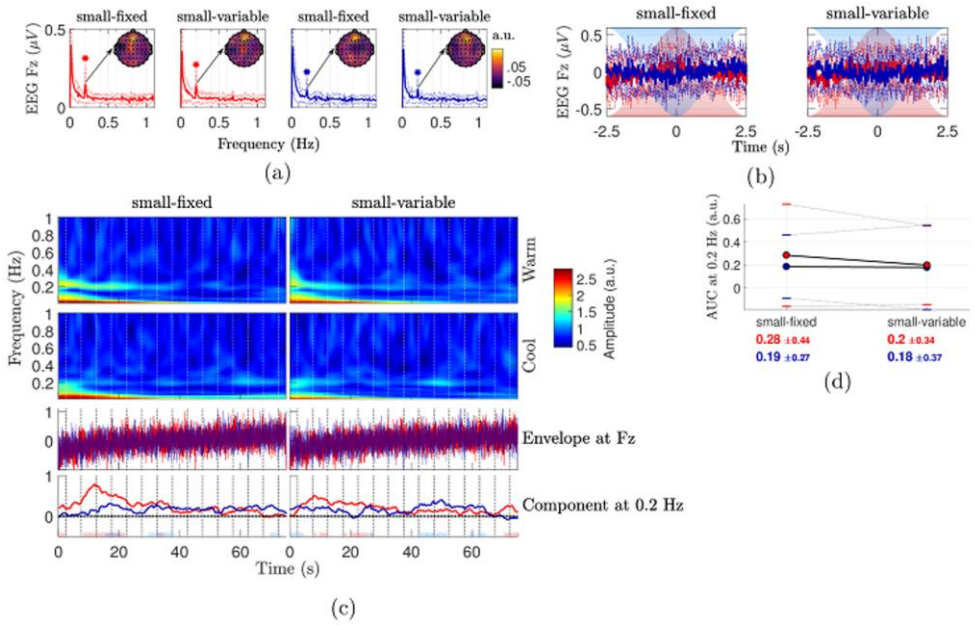


Figure A.12. Signal envelopes in the gamma frequency band (30-70 Hz). (a) Fourier transforms of the envelopes averaged over the epochs (grand mean ± standard deviation) at electrode Fz. A star indicates significativity of the noise-subtracted peak at $\{k \cdot 0.2\}_{k=1}^{5}$ Hz (*t*-tests against 0). The scalp maps show the distribution of the noise-subtracted amplitudes at 0.2 Hz. (b) Envelopes averaged across stimulation periods (grand mean ± standard deviation). (c) Grand mean Continuous Wavelet Transform (CWT) of the average signals at Fz and (d) pairwise comparisons of the Area Under the Curve (AUC) of the stimulus-evoked envelope components at 0.2 Hz. The red (resp. blue) dots show the mean AUCs for the warm (resp. cool) stimulation, the standard deviations being indicated with horizontal bars. Each asterisk in the plot indicates a significant difference according to paired samples *t*-tests, in red, blue or black respectively when the two compared conditions are warm cool or different. Figure adapted from Mulders et al. (2020)

APPENDIX B. LIST OF PUBLICATIONS

This thesis is based on the following publications:

ARTICLES

Can the Nociception Coma Scale-Revised be used in patients with a tracheostomy?

Lejeune, N., Thibaut, A., Martens, G., Martial, C., Wannez, S., Laureys, S., & Chatelle, C. (2019). *Archives of physical medicine and rehabilitation.* doi: 10.1016/j.apmr.2019.09.020.

Nociception Coma Scale Revised allows to identify patients with preserved neural basis for pain experience.

Bonin, E. A., Lejeune, N., Thibaut, A., Cassol, H., Antonopoulos, G., Wannez, S., Martial, C., Schnakers, C., Laureys, S. & Chatelle, C. (2019). *The Journal of Pain.* doi: 10.1016/j.jpain.2019.11.004

Investiguer la relation entre douleur et conscience: une approche lésionnelle au moyen de l'électroencéphalographie.

Lejeune, N., Mouraux, A. (2019). *Douleur et Analgésie 32(1), 69-70.*

Withdrawal of clinically assisted nutrition and hydration decisions in patients with prolonged disorders of consciousness: best interests of the patients and advance directives are the keys.

Lejeune, N. (2017). *Journal of medical ethics 43, 457–458.*

Dynamics of the perception and EEG signals triggered by tonic warm and cool stimulation.

Mulders, D., De Bodt, C., Lejeune, N., Courtin, A., Liberati, G., Verleysen, M. & Mouraux, A. (2020). *PLOS One, in press.*

BOOK CHAPTERS

Etats de conscience altérée : soins palliatifs et décisions de fin de vie.

Lejeune, N., Van Erp, W.S., Rohaut, B., Sanz, L.R.D., Laureys, S., Chatelle, C. (2020). *In Jacquemin, D. (Ed.), Manuel de soins palliatifs (5ème édition). Dunod. in press.*

POSTERS

Cold evoked potentials in patients with disorders of consciousness: a new bedside approach to probe spino-thalamic pathways in non-communicative patients.

Lejeune, N., Chatelle, C., Laureys, S. & Mouraux, A. *22th Congress of the Association for the Scientific Study of Consciousness (Krakow, Poland, June 2018)*

Investigating the influence of a tracheostomy on the evaluation with the Nociception Coma Scale – Revised.

Lejeune, N., Thibaut, A., Martens, G., Martial, C., Wannez, S., Laureys, S., Chatelle, C. *13th World Congress on Brain Injury (Toronto, Canada, March 2019) Morressier. https://doi.org/10.26226/morressier.5c7e3e2229d813000cb41d95*