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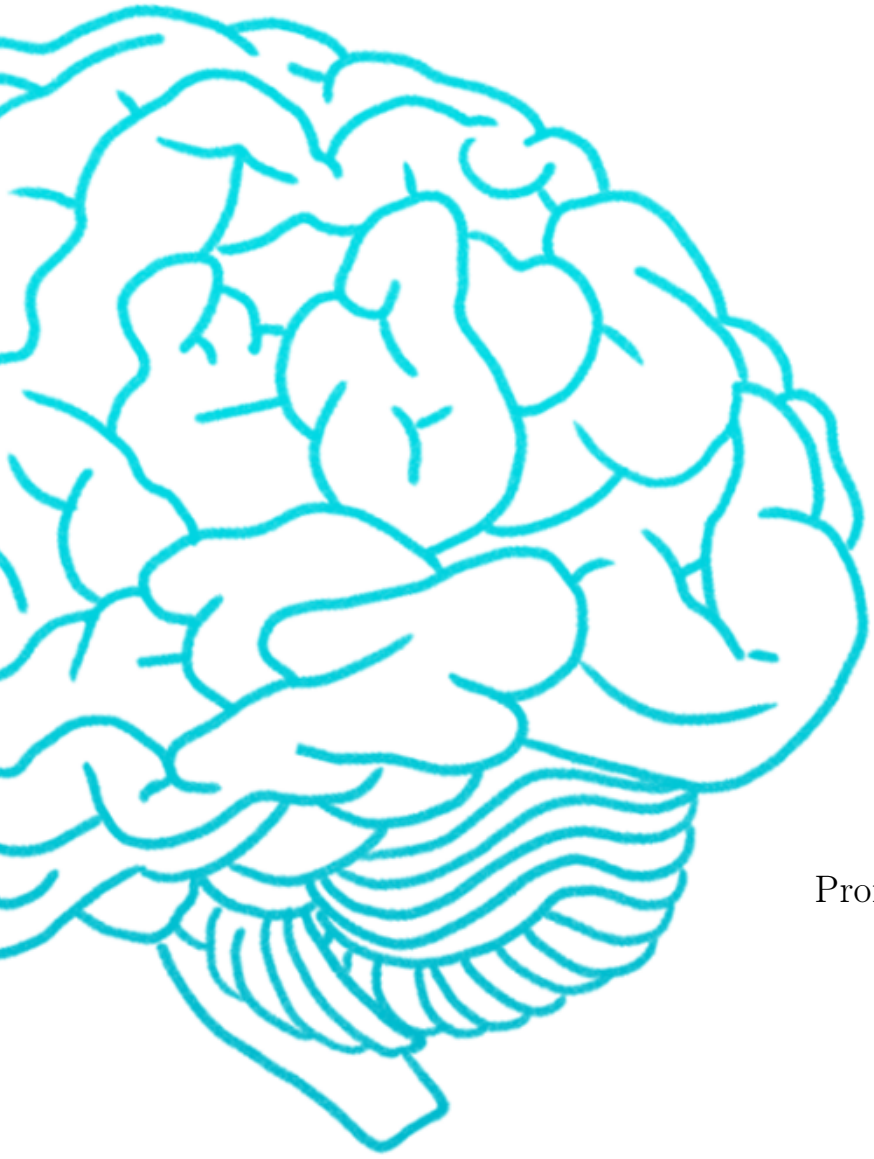
Pain and nociception assessment and management in patients with severe brain injuries



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Disclosure

I, Estelle Bonin, declare that this thesis entitled *Pain and nociception assessment and management in patients with severe brain injuries* and the data presented in it are original and my own work.

No part of this work has previously been submitted for a degree at this or any other university.

References to the work of others have been clearly acknowledged. Quotations from the work of others have been clearly indicated, and attributed to them.

In cases where others have contributed to part of this work, such contribution has been clearly acknowledged and distinguished from my own work.

All authors and co-authors of the articles presented in this work report having no conflicts of interest.

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Scientific publications

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Bonin, E.A.C., Gosseries, O., Laureys, S. (2021) Module: Clinical Assessment, Session: Low Awareness States. EBrain (https://learning.ebrain.net/enrol/index.php?id=1548).

Bonin, E.A.C., Martens, G., Cassol, H., Chatelle, C., Laureys, S., Thibaut, A. (2020) PET Imaging in Altered States of Consciousness: Coma, Sleep, and Hypnosis. PET and SPECT in Neurology 2d edition. Publisher: Springer Nature

Other Publications

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Annen, J., Mertel, I., Xu, R., Chatelle, C., Lesenfants, D., Ortner, R., **Bonin, E.A.C.**, Guger, C., Laureys, S.,Müller, F. (2020) Auditory and somatosensory p3 are complementary for the assessment of patients with disorders of consciousness. Brain Sci; 10(10), 748. https://doi.org/10.3390/brainsci10100748

Thibaut, A., Panda, R.M.E., Annen, J., Sanz, L.R.D., Naccache, L., Martial, C., Chatelle, C., Aubinet, C., **Bonin, E.A.C.**, Barra, A., Briand, M.M., Cecconi, B., Wannez, S., Stender, J., Laureys, S., Gosseries, O. (2021). Preservation of Brain Activity in Unresponsive Patients Identifies MCS Star. Annals of Neurology (90). https://doi.org/10.1002/ana.26095

Cassol, H., **Bonin, E.A.C.**, Bastin, C., Puttaert, N., Charland-Verville, V., Laureys, S., Martial, C. (2020). Near-Death Experience Memories Include More Episodic Components Than Flashbulb Memories. Front. Psychol. 11:888. https://doi.org/10.3389/fpsyg.2020.00888

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Abbreviations

AAN	American Academy of Neurology
ACC	Anterior Cingulate Cortex
ACE	Angiotensin Converting Enzyme inhibitors
BCI	Brain Computer Interface
BH	Benjamini-Hochberg method
BOLD	Blood Oxygen Level Dependent
CCP	Covert Cortical Processing
CMD	Cognitive Motor Dissociation
CMRglc	Cerebral Metabolic Rate of glucose
CTRL	Healthy Control
DMN	Default Mode Network
DoC	Disorders of Consciousness
DTI	Diffusion Tensor Imaging
EAN	European Academy of Neurology
EEG	Electroencephalography
EMCS	Emergence from the Minimally Conscious State
F	Female
FDG-PET	Fluorodeoxyglucose-Positron Emission Tomography
FN/FP	False Negative/False Positive
FOUR	The Full Outline of Unresponsiveness
fMRI	functional Magnetic Resonance Imaging
GCS	Glasgow Coma Scale
HMD	Higher-order Motor Dissociation
HS	Healthy Subject
IBIA	International Brain Injury Association
ID	Identification

IQR	Interquartile Range
INS	Insula
LL/UL	Lower Limb/Upper Limb
LIS	Locked-In Syndrome
LoC	Level of Consciousness
M	Male
MAS	Modified Ashworth Scale
MCS	Minimally Conscious State
MCS*	UWS patient with atypical cortical metabolism preservation
MNI	Montreal Neurological Institute
NA	Not Applicable
NCS-R	Nociception Coma Scale-Revised
NP	Non Pharmacological treatment
NS	No Significance
P	Pharmacological treatment
PCC	Posterior Cingulate Cortex
PPI	Psycho-physiological Interaction
PT	Physiotherapy
PVS	Persistent or Permanent Vegetative State
REM	Rapid Eye Movement
S1	Primary Somatosensory Cortex
S2	Secondary Somatosensory Cortex
SAH	Subarachnoid Hemorrhage
SD	Standard Deviation
SECONDs	Simplified Evaluation of CONsciousness Disorders
SMO	Spastic Muscle Overactivity
SUV	Standardized Uptake Values
TBI	Traumatic Brain Injury
TN/TP	True Negative/True Positive
UWS/VS	Unresponsive Wakefulness Syndrome/Vegetative State
VAS	Visual Analogue Scale
WHO	World Health Organization

Abstract

English version

Severely brain injured patients such as patient with disorders of consciousness (DoC) or in locked-in syndrome (LIS) might remain unable or demonstrate difficulties to functionnally communicate about potential pain. Therefore, the detection of clinical signs of pain by the medical team becomes essential for their well-being and their management. In this context, the aim of this work was to improve guidelines regarding pain assessment and management in patients who survived a severe brain injury but remain in DoC or LIS. To this end, this work has been divided into two parts.

In the first part of this thesis, the goal was to improve pain assessment in patients with DoC and LIS. To this end, we first conducted a retrospective study on patient with DoC aiming to establish a conservative Nociception Coma Scale-Revised (NCS-R) cut-off score for conscious perception of pain based on Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET) data. This work allows us to establish a NCS-R cut-off score of 5 that can be used in clinical settings to detect potentially painful patients as well as patients with covert consciousness. Then we performed a survey focusing on patients in LIS in order to investigate and better characterize pain in this specific population of patients. This survey highlight that a lot of patients in LIS experienced pain but half of them do not communicate about it, despite the fact that it can have a negative impact on their quality of life. This alarming finding highlight the importance of good detection of behavioral and physiological signs of pain by medical teams.

In the second part of this work, the aim was to investigate potential sources of pain and therapeutic options to reduce pain in this population of patients. We conducted another retrospective cross-sectional study aiming to investigate the relationship between the presence of pain and the presence of spastic muscle overactivity (SMO) in patients with DoC. The aim was to better characterize the spastic profiles most likely to induce pain in order to propose an adequate management. We found a high prevalence of SMO during physical therapy and a positive correlation between the presence of SMO and the presence of pain. We also showed that physical therapy involving the upper limbs are the most likely to cause pain in patients with DoC. Then we performed a randomized double-blind placebo-controlled crossover trial carried out in patients with DoC to study the effects of analgesic treatment on NCS-R scores during

physical therapy sessions (i.e., stretching). The aim was to determine if physical therapy was potentially painful and if the NCS-R can be used clinically to detect changes in behavior induced by pain medication and thus adapt the management of patients on a daily basis. We found that physical therapy sessions could induce pain in patients with DoC and highlighted the importance of the facial expression subscales in detecting pain. This study did not allow us to find an effect of pain medication on the NCS-R score suggesting either a lack of efficiency of the medication or a lack a sensitivity of the NCS-R to detect subtles behavior changes. Further studies are still needed to demonstrate the usefulness of the NCS-R in monitoring pain treatments. This work also highlighted that despite the possible side effects on attention, pharmacological treatments are the most widely used to manage pain in patients with DoC or in LIS. Hence, the development of novel non-pharmacological treatments is warranted to avoid these side effects and promote quality of life of severely brain injured patients who suffer from communication impairments.

Version Française

Les patients souffrant de lésions cérébrales graves, tel que les patients en état de conscience altérée (ECA) ou ayant un syndrome d'enfermement (LIS), peuvent être incapables de communiquer de manière fonctionnelle au sujet d'une douleur potentielle ou éprouver des difficultés à le faire. Dès lors, la détection des signes cliniques de la douleur par l'équipe médicale devient essentielle pour leur bien-être et leur prise en charge. Dans ce contexte, l'objectif de ce travail était d'améliorer les directives concernant l'évaluation et la prise en charge de la douleur chez les patients qui ont survécu à une lésion cérébrale grave mais qui restent en ECA ou en LIS. À cette fin, ce travail a été divisé en deux parties.

Dans la première partie de cette thèse, l'objectif était d'améliorer l'évaluation de la douleur chez les patients en ECA et en LIS. Pour ce faire, nous avons tout d'abord mené une étude rétrospective sur des patients en ECA visant à établir un score seuil conservateur de l'échelle de nociception du coma-révisée (NCS-R) associé à la perception consciente de la douleur et s'appuyant sur des données de tomographie par émission de positons au fluorodésoxyglucose (FDG-PET). Ce travail nous a permis d'établir un score seuil de NCS-R de 5 pouvant être utilisé en clinique pour détecter les patients potentiellement douloureux ainsi que les patients ayant une conscience latente. Nous avons ensuite réalisé une enquête à destination des patients en LIS afin d'étudier et de mieux caractériser la douleur dans cette population spécifique. Cette enquête a mis en évidence que de nombreux patients en LIS ressentent de la douleur mais que la moitié d'entre eux ne communiquent pas à ce sujet, malgré le fait que cela peut avoir un impact négatif sur leur qualité de vie. Ce constat alarmant souligne l'importance d'une bonne détection des signes comportementaux et physiologiques de la douleur par les équipes médicales.

Dans la deuxième partie de ce travail, l'objectif était d'étudier les sources potentielles de douleur chez cette population spécifique de patients et les options thérapeutiques permettant de la soulager. Nous avons mené une étude transversale rétrospective visant à étudier la relation entre la présence de la douleur et la présence d'une hyperactivité musculaire spastique (SMO) chez les patients en ECA. L'objectif était de mieux caractériser les profils spastiques les plus susceptibles d'induire la douleur afin de proposer une prise en charge adéquate. Nous avons trouvé une forte prévalence de SMO pendant la kinésithérapie et une corrélation positive entre la présence de la SMO et la présence de la douleur. Nous avons également montré que les mobilisations impliquant les membres supérieurs sont les plus susceptibles de provoquer des douleurs chez les patients en ECA. Nous avons ensuite réalisé un essai croisé randomisé en double aveugle placebo contrôle chez des patients en ECA pour étudier les effets d'un traitement analgésique sur les scores de NCS-R pendant les séances de kinésithérapie (étirements). L'objectif était de déterminer si la kinésithérapie était potentiellement douloureuse et si la NCS-R pouvait être utilisée cliniquement pour détecter les changements de comportement induits par les analgésiques et ainsi adapter la prise en charge des patients au quotidien. Nous avons constaté que les séances de kinésithérapie pouvaient induire de la douleur chez les patients en ECA et avons souligné l'importance de la sous-échelle expression faciale dans la détection de la douleur. Cette étude ne nous a pas permis de montrer l'effet de la prise d'analgésiques sur les scores de NCS-R, suggérant soit un manque d'efficacité des médicaments, soit un manque de sensibilité de la NCS-R pour détecter ces changements de comportement. D'autres études sont encore nécessaires pour démontrer l'utilité de la NCS-R dans le suivi des traitements de la douleur. Ce travail a également mis en évidence le fait que, malgré les effets secondaires possibles sur l'attention, les traitements pharmacologiques sont les plus utilisés pour gérer la douleur chez les patients en ECA ou en LIS. Par conséquent, le développement de nouveaux traitements non pharmacologiques est justifié pour éviter ces effets secondaires et promouvoir la qualité de vie des patients atteints de lésions cérébrales graves qui souffrent de troubles de la communication.

Contents

Chapter 1

Introduction

"Tout le monde peut maîtriser une douleur excepté celui qui l'a." - William Shakespeare, 1859.

This quote is especially true for patient with severe brain injuries, such as patients with disorders of consciousness (DoC) or suffering from a Locked-in Syndrome (LIS). Indeed, due to their condition, these patients cannot or can hardly communicate about potential pain. Thus, the detection of pain by clinical teams becomes essential for their well-being and their management.

In the first part of this chapter, we will first define consciousness and those altered states that can occur following a severe brain injury. We will then describe the behavioral and neuroimaging tools available for the diagnosis of these patients. In the second part of this chapter, we will define pain and nociception. Finally, we will dive in the neural correlates underlying these two phenomena as well as the existing tools allowing pain and nociception assessment in this sensitive population.

Based on the following publications:

Bonin, E.A.C., Gosseries, O., Laureys, S. (2021) Module: Clinical Assessment, Session: Low Awareness States. EBrain (`https://learning.ebrain.net/enrol/index.php?id=1548`).

Bonin, E.A.C., Gosseries, O., Laureys, S. (2021) Module: Clinical Assessment, Session: Locked-in Syndrom. EBrain.(`https://learning.ebrain.net/enrol/index.php?id=1562`)

Bonin, E.A.C., Martens, G., Cassol, H., Chatelle, C., Laureys, S., Thibaut, A. (2020) PET Imaging in Altered States of Consciousness: Coma, Sleep, and Hypnosis. PET and SPECT in Neurology 2d edition. Publisher: Springer Nature.

1.1 Assessment of the level of consciousness of severely brain injured patients

1.1.1 Defining disorders of consciousness (DoC)

Consciousness is a multidimensional concept that is defined differently according to the field of research (i.e., philosophy, psychology or medicine). In the context of brain injury, Plum and Posner have defined consciousness in 1983 as a *"state of full awareness of the self and one's relationship to the environment"*. In clinical neuroscience, consciousness can be described on the basis of two components: arousal and awareness (also called content of consciousness, Posner et al. [2007]). Arousal reflects the levels of alert/vigilance and is characterized by the presence of eye-opening. It is mediated by the brainstem, the hypothalamus, and the basal forebrain. Awareness represents all cognitive and affective functions moderated by the cerebral cortex (Laureys et al. [2004]). It can be divided into two entities depending on the networks involved: Awareness of the self refers to stimulus-independent thoughts, mental imagery, inner speech, daydreaming or mind-wandering. It is mediated by the internal network of consciousness involving the precuneus, the thalami and the anterior cingulate gyrus. Awareness of the environment is defined as the conscious perception of one's environment via the mediation of external sensory stimuli (i.e., visual, auditory, somesthetic or olfactory perception). It is processed by the external network of consciousness involving lateral fronto-parietal cortex. In ordinary state of consciousness, it seems that the activity of these two networks is anti-correlated meaning that the more one is activated, the less is the other. In healthy subjects, a switch between the two networks occurs every 20 seconds, but in patients with severe brain damage, the interaction between these two networks is altered (Vanhaudenhuyse et al. [2011]).

There are physiological, pharmacological and pathological states of consciousness where arousal and awareness can correlate or not. On the one hand, in non-pathological states (i.e., normal consciousness, sleep, general anesthesia) these two components are positively correlated, except during rapid eye movement sleep (REM) or lucid dreaming in which the subject presents no arousal but reports a subjective experience. On the other hand, in pathological states, such as post-coma disorders, these two components do not correlate (see **Figure 1.1**) (Laureys et al. [2004]). Such states of consciousness are due to severe brain injuries, which may be traumatic (e.g., car accident) or non traumatic (e.g., stroke or anoxia). Progress in intensive care medicine have increased the number of survivors among these severely brain injured patients. These patients will experience a transient period of coma and then either evolve into brain death or recover arousal and/or awareness and progress in different states of altered consciousness. The American Academy of Neurology (AAN) suggests that a patient with DoC within 28 days post-injury

should be considered in "acute DoC" while a patient in DoC since more than 28 days post-injury should be considered in "prolonged DoC" (Giacino et al. [2018]).

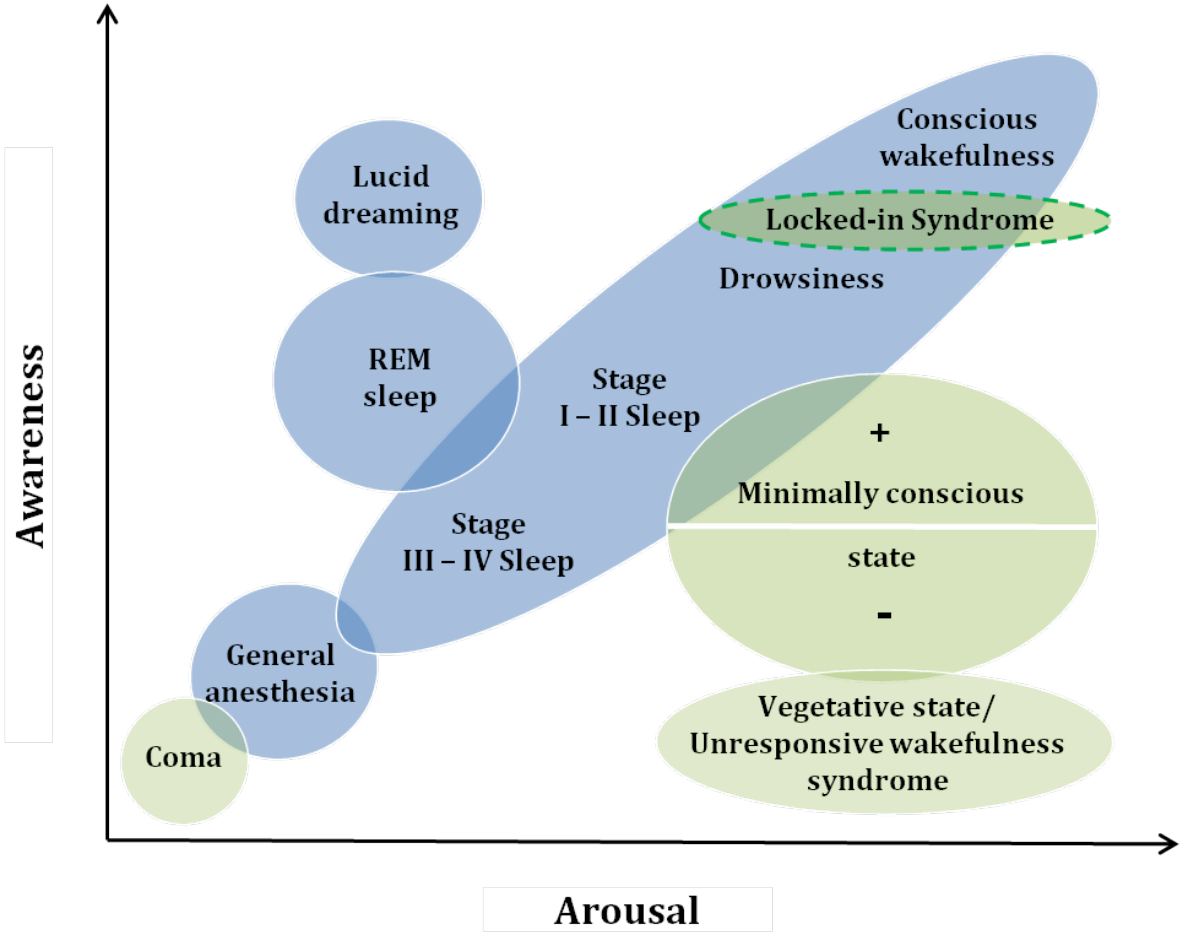


Figure 1.1: Variation in the level of arousal and awareness according to the individual's non-pathological (in blue) or pathological state (in green) (based on Laureys et al. [2004]).

In the 1960s, the development of resuscitation techniques and mechanical ventilation led to a redefinition of death. *Brain death* is now defined as the complete and permanent cessation of vital functions (i.e., respiration, circulation, consciousness and brainstem reflexes) of the body as a whole (Wijdicks [2015]). This loss of function is only considered permanent if it cannot resume spontaneously or be restored through medical intervention. Brain death may be due to a massive traumatic brain injury or a permanent cessation of the brain oxygenation (i.e., intracranial hemorrhage or anoxia) (Greer et al. [2020]). I[2020]). In order to determine whether a patient is brain dead or not, it is important to exclude all confounding factors that may interfere with the diagnosis such as residual sedation, use of neuromuscular blockers or treatment with hypothermia (for more details refer to Greer et al. [2020]). To be considered brain dead, a patient must have the following criteria: irreversible coma, no spontaneous respiration, no brainstem reflexes, absence of motor response at rest and following tactile or painful stimulation,

hypothermia (< 36°C), systolic blood pressure of at least 100mm Hg. A repeat evaluation in 6 hours is advised, but the time period is considered arbitrary (Gosseries et al. [2011]).

Coma is a state where the patient shows no signs of arousal and no signs of awareness of self or environment. The patient is unable to open the eyes at rest and following tactile or painful stimulation. The sleep-wake cycle is absent (Teasdale and Jennett [1974]). Reflex responses can be observed following a painful stimulus as the neural structures involved in such motor responses are different from those involved in consciousness (Posner et al. [2007]). Coma may be caused by diffuse cortical injury (e.g., after cardiac arrest), white matter injury resulting from diffuse neuronal or axonal damage (e.g., after a motor vehicle accident), focal but extensive brainstem injury (e.g., after a stroke or hemorrhage) (Zasler et al. [2007]). To be clearly distinguished from syncope, concussion or other transient states of unconsciousness, coma must persist for at least one hour. Recovery from coma can vary from a few hours to four weeks.

Patients in *Vegetative State/Unresponsive Wakefulness Syndrome* (VS/UWS) show signs of arousal characterized by the presence of sleep-wake cycles and eye-opening periods. However, these patients show no signs of awareness of self or environment and are unable to interact with others. There is no evidence of language comprehension or expression. Hypothalamic and brainstem autonomic functions (i.e., respiration, cardiovascular regulation or thermoregulation) are sufficiently preserved to allow survival with medical and nursing care (The Multi-Society Task Force on PVS [1994], Laureys et al. [2010]). [2010]). In 1994, the Multi-Society Task Force suggested that if a patient is in a VS/UWS for more than a month he or she should be considered in a "persistent VS" while a patient who is in a VS for more than 3 months (after a non-TBI) or more than a year (after a TBI) will be considered in a "permanent VS". Unfortunately, these two terms share the same abbreviation PVS, which leads to confusion (The Multi-Society Task Force on PVS [1994]). To circumvent this issue, the AAN do not recommend using these two terms but rather to refer to "chronic VS/UWS" and specify the time since injury (Giacino et al. [2018]). Since 2010, the term VS/UWS is preferred to VS as it is more descriptive and less pejorative in the eyes of families, clinicians and the general audience (Laureys et al. [2010]).

Minimally conscious state patients are divided in two subcategories depending on their language abilities. MCS minus (MCS-) patients will show signs of arousal as well as some signs of awareness related to non-reflexive behavior such as visual pursuit or fixation, pain localization, objects localization and manipulation or contingent behavior to emotional stimuli (e.g., smiling to mother's face and not to other faces) (Giacino et al. [2002], Bruno et al. [2011b]). MCS plus (MCS+) patients will show the behaviors mentioned above as well as a preservation of language abilities such as command following, intelligible verbalization or intentional communication (Thibaut et al. [2020]). Finally, a patient is considered to have emerged from the MCS (EMCS) when he or she regains functional communication or functional use of objects (Di Perri et al. [2016]).

In recent years, the use of neuroimaging techniques such as functional MRI (fMRI), positron emission tomography (PET) and electroencephalography (EEG) has also made it possible to clarify the diagnosis of patients with "atypical" brain activity patterns, which has led to the emergence of new terminologies. *Minimally conscious state** (MCS*) refers to patients behaviorally diagnosed in VS/UWS but with a brain activity at rest or during a passive or active paradigm compatible with MCS (Thibaut et al. [2021]). *Covert cortical processing* (also called covert consciousness; CCP or higher-order cortex motor dissociation; HMD) correspond to a patient whose behavioral assessments suggest a diagnosis of coma, or VS/UWS or MCS- but showing preservation of brain activity using a passive task (fMRI or EEG) (Edlow et al. [2017]). *Cognitive motor dissociation* (CMD) refers to a patient whose behavioral assessments suggest a diagnosis of coma, VS/UWS or MCS- but who shows preservation of brain activity on a passive or active task (fMRI or EEG). For example, this could be a patient with an VS/UWS who responds to a passive (at least MCS-) or active (at least MCS+) paradigm, or a patient in a MCS- who responds to an active paradigm (Schiff [2015]).

1.1.2 The case of the Locked-in Syndrome (LIS)

Locked-in syndrome (LIS) is often mixed up with coma and VS/UWS states; however it is not considered as a DoC. The most common cause of LIS is vascular pathology (i.e., after a ischemic or hemorrhagic stroke) following TBI (i.e., brainstem lesions), masses in the ventral pons, infection and demyelination (M Das et al. [2022]). This results in the paralysis of all four limbs, head and face (i.e., quadriplegia or quadriparesis), impaired verbalization/vocalization (i.e., aphonia or hypophonia), impaired breathing (i.e., apnea, hyperpnea) and impaired coordination (i.e., ataxia). Most of the time, the anatomy of the responsible lesion in the brainstem is such that patients in LIS are only able to use vertical eye movements and blinking to communicate their awareness (M Das et al. [2022], Laureys et al. [2005]). If the lesion of a patient in LIS are localized in the brainstem only, cognitive abilities will be preserved. On the other hand, some patients may also suffer from cortical lesions, which will impact their cognitive functions such as attention, learning, motor imagery, recognition of negative facial expressions, pathological laughter and crying, hallucinations and delusions (Leonard et al. [2019]). There are several categories of LIS according to the degree of motor or verbal impairment. A classical patient in LIS has quadriparesis but is still able to perform eye and eyelid movements. An incomplete patient in LIS is able to move partially meaning that he or she can perform small movements of the head, upper or lower limbs. Finally, a complete patient in LIS has quadriparesis and an inability to move the eyes or eyelids (Bauer et al., Surdyke et al.).

The first possible contact with patients in LIS is through the use of a communication code using eyelid blinks or vertical eye movements (e.g., one blink to say "yes", two blinks to say "no"). There are also other methods to try such as the establishment of a letter-spelling communication such as the vowel and consonants eye-communication method or the alphabetic code (Lugo et al. [2015]). To signal the target letter, the patient can use eye or head movements according to his or her abilities. Indeed, the majority of patients in LIS will be able to recover head movement, which may allow them to use a letter board or computer keyboard with their mouth or head. Some patients (60-70%) will also recover partial motor function of their hands and feet, which may allow them to use a mouse or an electric wheelchair. New technologies, such as EEG-based brain-computer interfaces, now allow patients without any voluntary muscle control to communicate via brain signals (Annen et al. [2020]).

1.1.3 Clinical assessments: behavioral and neuroimaging tools

Diagnosis and prognosis begin in the intensive care unit and have an impact on the patient's future. A study conducted among healthcare professionals showed that for 66% of them it was acceptable to stop the care of a patient in VS/UWS compared to 28% for MCS. Additionally, when asked if they wanted to stay alive if they found themselves in this situation, 33% said yes in a case of MCS versus 18% in case of VS/UWS. This study shows how important it is to make a precise diagnosis in the acute phase in order to make the most appropriate therapeutic decisions (Demertzi et al.). Prognosis plays a key role in therapeutic decision making. Indeed, a patient with a good chance of recovery will be transferred to a rehabilitation center offering more adapted care and treatment. Numerous studies have shown that patients diagnosed in MCS have a better chance of recovering consciousness compared to patients in VS/UWS (for a review refer to (Estraneo et al. [2018, 2021])). The etiology also plays an important role in the prognosis, patients with TBI have a higher chance of recovery than non-TBI patients (Whyte and Nakase-Richardson, Estraneo et al. [2021]). A recent study reported a mortality rate of 42.6% in patients in VS/UWS and 16% in patients in MCS within 24 months after onset. Older age and low Coma Recovery Scale-Revised total score (for patients in VS/UWS) as well as female sex and absence of alpha rhythm on EEG (for patients in MCS) were associated with a higher mortality rate (Estraneo et al. [2021]). These results show that demographic factors, as well as multimodal behavioral and neurophysiological assessments, can enable physicians to identify patients with a higher risk of mortality and thus guide future treatment decisions.

Behavioral assessment

In a clinical setting, it is not possible to assess self-awareness in DoC patients since it is a subjective experience, and given the fact that these patients are unable to communicate. Clinicians will therefore

aim to detect signs of environment-related awareness (i.e., patient's reaction to external stimuli). For this purpose, standardized behavioral scales are favored at the patient's bedside. The Glasgow Coma Scale (GCS) is the most widely used scale in neurology and intensive care units to assess acute brain injured patients, compare the efficiency of treatments, or estimate prognosis (Teasdale and Jennett [1974]). It assesses arousal (i.e., eye opening presence or absence), verbal response, and motor response, with a total score ranging from 3 to 15. However, this scale is not sensitive enough to distinguish VS/UWS and MCS patients. The Full Outline of Unresponsiveness (FOUR) assesses the level of consciousness based on eye and motor responses, but also includes respiratory and brainstem reflex changes, which makes it useful in the assessment of acute DoC as well as in detecting LIS (Schnakers et al. [2006]). The main asset of these scales is their very short administration time. However, they offer a less sensitive and reliable diagnosis of the state of consciousness than the Coma Recovery Scale-Revised (CRS-R), thus increasing t he risk of misdiagnosis (Seel et al. [2010]).

Indeed, the CRS-R is currently the gold standard in the diagnosis of acute and prolonged DoC (Giacino et al. [2004], Seel et al. [2010]). It is composed of 23 items organized in six categories corresponding to different cognitive functions: visual function, auditory function, motor function, oromotor/verbal function, communication and arousal. Each category includes behaviors ranging from reflex response (i.e., auditory startle) to higher level cognitive behavior (i.e., response to command). Each item of this scale is linked to a specific diagnosis (e.g., visual tracking corresponds to the diagnosis MCS-, functional communication corresponds to the diagnosis EMCS) (Giacino et al. [2004]). However, misdiagnosis is common among DoC patients. Indeed, patients may have motor, visual or auditory disabilities which prevents them from achieving certain items. A recent study shows that 38% of patients diagnosed with VS/UWS by a clinical consensus were actually MCS when diagnose with repeated CRS-R (Wang et al. [2020]). Moreover, the level of vigilance of severely brain damaged patients can fluctuate during the day, increasing diagnostic errors (Piarulli et al. [2016]). To avoid this, repeated assessments are recommended. Indeed, a 2017 study showed that the repeated use of the CRS-R significantly reduced the rate of diagnostic error (36% misdiagnosis with a single assessment versus 5% misdiagnosis with five assessments) (Wannez et al. [2017]). Lack of training of clinical teams in the use of the CRS-R can also lead to errors in assessments.

It is strongly recommended to have appropriate training on the administration of the CRS-R and to follow the guidelines recommended by the European Academy of Neurology (EAN) regarding clinical examination (Kondziella et al. [2020], Giacino et al. [2018]):

1. If the patient does not open his or her eyes spontaneously, it is recommended that the examiner open the eyelids during visual tasks in order to diagnose LIS patients or MCS patients with ptosis. In order not to miss a diagnosis of LIS, it is also essential to test for the presence of vertical and

horizontal eye movements. In addition, resistance to eye opening has been considered a sign of awareness since 2018 (van Ommen et al. [2018]).

2. For visual pursuit/fixation, it is more appropriate to use a mirror rather than an object.
3. It is recommended to note the presence of spontaneous motor behavior that may reflect covert consciousness.
4. The CRS-R, used repeatedly, is the most appropriate scale for assessing acute and chronic patients. In acute setting, it is preferable to use the FOUR, which is quicker to administer than the CRS-R and which, unlike the GCS, includes the evaluation of eye movements (i.e., allows the detection of LIS).

Another limitation of the CRS-R is its relatively long administration time (30-40 minutes) which may limit its use on a daily basis but also have an impact on patients' fatigue and motivation. In order to facilitate the evaluation of patients on a daily basis, a faster scale has been developed: the Simplified Evaluation of CONsciousness Disorders (SECONDs). This scale is based on the most frequently observed items of the CRS-R (Sanz et al. [2021]). It consists of six mandatory items: observation, command-following (i.e., score 6), oriented behaviors (i.e., score 5), visual pursuit (i.e., score 4) and fixation (i.e., score 3) and arousal (i.e., score 1 if eye opening). Communication intentional (i.e., score 7) or functional (i.e., score 8) and pain localization (i.e., score 2) are conditional items. Each item corresponds to a score, ranging from 0 to 8, that is linked to a specific diagnosis (i.e., 0 = coma, 1 = VS/UWS, 2-5 = MCS -, 6-7 = MCS+, 8= EMCS). It is also possible to calculate an additional index for each item which allows to follow more precisely the evolution of patients over time. The SECONDs have been validated in a study carried out on 57 DoC patients and has shown good intra and inter-rater reliability as well as good concurrent validity with the CRS-R (Aubinet et al. [2021]). Its administration time is about seven minutes (i.e., 2.5 faster than the CRS-R), which makes it easier to use on a daily basis. Moreover, this scale does not require a specific training (for complete guidelines, refer to Sanz et al. [2021]).

Neuroimaging tools

Behavioral assessment of consciousness in severely brain injured patients can be challenging when patients have deficits in motor, verbal, visual or auditory function. In order to avoid misdiagnosis as much as possible, it is ideal to complement these results with neuroimaging. There are different options for assessing the level of consciousness through the use of neuroimaging. First of all, it is possible to measure the patient's brain activity at rest (i.e., in the absence of any task or external stimulation). These techniques do not require the active participation of the patient, which makes it possible to bypass the potential presence of auditory, visual or motor deficits. Then, the use of passive paradigms allows

measuring the brain activity in response to external stimuli (i.e., auditory, tactile, nociceptive) without requiring the active participation of the patient. Finally, the use of active paradigms allows measuring the brain activity of the patient in response to a command. Some studies have also tried to use these active paradigms to establish communication with patients via the use of brain computer interface (BCI) technique. However, these techniques are not very sensitive and will be affected by fluctuations in vigilance as well as by the presence of language comprehension disorders.

One of the less expensive and most convenient techniques to use at the bedside is the electroencephalography (EEG). This non-invasive technique measures the electrical activity of the brain at rest, during sleep or in response to auditory, tactile or visual stimulation (event-related potential, ERP). Coupled with the BCI technique, it can also be a motor-independent communication tool for the patients with DoC or in LIS(Annen et al. [2018]). At rest, predominant EEG activity in theta or delta band have been observed in VS/UWS patients compared to MCS and healthy subjects (Piarulli et al. [2016], Estraneo et al. [2016]). Predominant EEG activity in theta and alpha band have been observed in MCS patient (Bai et al. [2021], Estraneo et al. [2016]). Patients in MCS* have lower delta power, higher theta and alpha power and higher connectivity than VS/UWS patients (Thibaut et al. [2021]). Periodic fluctuation in spectral entropy (about 70 min) similar to what is observed in healthy subjects have been observed in MCS patients. This fluctuation was not observed in VS/UWS patients and could be a marker of the vigilance fluctuations observed in some patients (Piarulli et al. [2016]). Recent studies have attempted to establish a resting state EEG score that can be used in clinical practice (Hirsch et al. [2013], Bagnato et al. [2015], Estraneo et al. [2016]). A 2019 study compared three different EEG classification methods and found that the EEG score based on the American Clinical Neurophysiology Society's terminology and combined with CRS-R scores have a good prognostic value (Scarpino et al. [2020]). EEG can also be used to study sleep in patients with DoC (for a review refer to Gottshall and Rossi Sebastiano [2020]). The presence of a sleep-wake cycles and sleep patterns are linked to a better outcome and allow to distinguish patients in VS/UWS from patients in MCS (Nekrasova et al. [2017]). Indeed, rapid eye movement and slow wave sleep patterns are less frequent in patients in VS/UWS compared to healthy subjects and patients in MCS (Mertel et al. [2020]). The search for these specific patterns is especially recommended by the EAN (Kondziella et al. [2020]). Active paradigms can also be used to try to differentiate between VS/UWS and MCS patients. The auditory oddball paradigm using subject's own name vs. other first name allows to detect the P300 component. This component is mainly observed in MCS patient rather than in VS/UWS and has a good prognostic value (Zhang et al. [2017], Wang et al. [2017]). Studies are still underway to optimize this paradigm. Indeed, a 2022 study using the own name paradigm showed that speech prosody could have an impact on the P300 latency (i.e., when the pitch prosody increases, the P300 latency decreases) suggesting that such parameters should be a standardized (Pruvost-Robieux

et al. [2022]). P300 can also be detected via an active vibro-tactile paradigm coupled with BCI technique (Heilinger et al. [2018], Murovec et al. [2020], Guger et al. [2018]). Counting the number of target stimuli provides an indication of whether the patient is able to follow a command independently of his motor abilities. One study observed an improvement in CRS-R scores after several sessions, paving the way for future research on the therapeutic impact of this technique (Murovec et al. [2020]). More recently, the use of EEG, fMRI and FDG-PET has made it possible to highlight a new category of patients who are behaviorally VS/UWS but have a typical brain activity. For instance, the use of EEG during a motor imagery task allows to detect cover t consciousness (CMD) (Edlow et al. [2017], Claassen et al. [2019]). A global functional connectivity and grey matter preservation was observed in patient in MCS* by using EEG and structural MRI (Thibaut et al. [2021]). The use of active paradigms such as mental imagery, oddball auditory evoked potentials and vibrotactile evoked potentials also allows the detection of LIS patients. Indeed, these patients show BCI responses close to what is observed in healthy subjects (Lesenfants et al. [2018], Chatelle et al. [2018b]).

Structural MRI provides information about the anatomy of the injured brain and subsequent lesion. A 2018 study assessing regional brain volume, showed that severely brain injured patients presented grey and white matter atrophy. In addition, VS/UWS patients were more likely to have grey and white matter loss than MCS patients, particularly in the thalamus (Rubeaux et al. [2015], Annen et al. [2018]). Patients in MCS* have higher gray matter volume than VS/UWS patients in the bilateral inferior frontal gyrus, fusiform gyrus, right temporal area, and right insula (Thibaut et al. [2021]). Regional brain volume assessments using structural MRI seems to be a good tool to disentangle VS/UWS and MCS patients and should be considered as a complementary assessment to improve the clinical diagnosis of DoC patients. The MRI-based Diffusion Tensor Imaging (DTI) technique allows to specifically visualize white matter fibers in the brain (Hulkower et al. [2013]). DTI has also been able to detect white matter lesions at the supra- and infratentorial level in LIS patients, which may partially explain why some LIS patients suffer from cognitive disabilities (Leonard et al. [2019]). Functional MRI is non-invasive and measures brain activity based on the blood oxygen level dependent (BOLD) changes at rest, during a passive stimulation or an active task. Using resting-state fMRI, studies have shown a decrease in default mode network (DMN) activity in DoC patients compared to healthy subjects (Vanhaudenhuyse et al. [2010]). DMN is activated when the subject is at rest. It involves the precuneus (involved in the internal network of consciousness), the medial prefrontal cortex and the lateral parietal cortex. Another theory involving the anterior forebrain mesocircuit suggest that prolonged DoC is due to an excess of thalamic inhibition resulting in a lack of excitation in the cortex (Schiff [2010]). It seems that the DMN and the mesocircuit are interconnected via thalamo-cortical and cortico-striatal pathways (Lant et al. [2016]). Indeed, a decrease in effective connectivity within the DMN and the anterior forebrain mesocircuit has been recently observed

in prolonged DoC (Coulborn et al. [2021]). This study shows that, in healthy subjects, the mesocircuit inhibits the DMN but this inhibition is impaired in patients with DoC. Other networks have also been studied such as the auditory, salience, frontoparietal, sensorimotor and visual networks. According to a 2015 study, it seems that the auditory network is the most sensitive to distinguish VS/UWS patients from MCS patients (accuracy rate of 80%) (Demertzi et al. [2015], Di Perri et al. [2016]). Functional MRI can also be coupled with passive stimulation (e.g., visual, auditory or nociceptive) (Calabrò et al. [2017], Kazazian et al. [2020]). Eventually, it is possible to perform active motor-independent fMRI paradigms by using mental imagery to detect command following (Kazazian et al. [2020], Wang et al. [2019]).

The Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) is currently the most precise neuroimaging tool for DoC patient's diagnosis. Indeed, a 2014 study investigated the sensitivity of FDG-PET and fMRI techniques with active paradigm in the diagnosis of DoC patients. Regarding FDG-PET, authors observed a sensitivity of 93% in the identification of MCS patients, and a congruence of 85% between the FDG-PET diagnosis and the CRS-R score. Moreover, this technique appears to have a good prognostic value as 74% of patients with VS/UWS and MCS diagnosed by FDG-PET remained respectively unconscious and conscious at follow-up (Stender et al. [2014]). FDG-PET allows the study of the cerebral metabolism rate of glucose (CMRglc) using radioactive fluorinated glucose. Studies have shown a global decrease in cerebral metabolism of approximately 40-50% in VS/UWS patients especially in the fronto-parietal network and the thalami but no clear cut-off was defined to disentangle VS/UWS from MCS (Levy et al. [1987], Tommasino et al. [1995], Laureys et al. [1999]). A study also found an average CMRglc level of 42% in VS/UWS patients and 55% in MCS patients, with a significant difference in regions involved in consciousness (i.e., frontoparietal cortex and precuneus). These results established that 42% of the global brain metabolism represents the minimal energetic requirement for sustained awareness in severely brain injured patients (Stender et al. [2015, 2016]). However, it seems that the recovery of consciousness is not only linked to an increase in cerebral metabolism in these regions, but also to the restoration of thalamo-cortical connectivity (Laureys et al. [2000]). In MCS patients, fronto-parietal regions and thalamo-cortical functional connectivity are preserved unlike what is observed in VS/UWS patients (Giacino et al. [2002]). The impairment of thalamo-cortical connectivity appears to correlate with a less favorable outcome (García-Panach et al. [2011]). At the regional level, a 2012 study showed an hypometabolism in both external (i.e., environment- related awareness) and internal (i.e., self-awareness) networks of consciousness in VS/UWS patients, while MCS and EMCS patients only had decreased metabolism in the internal network (Thibaut et al. [2012]). A 2009 study has shown that some MCS patients present an hypometabolism in brain regions involved in language processing, thus resulting in the onset of language disorders such as aphasia that can lead to misdiagnosis when using behavioral scales (Majerus et al. [2009]). FDG-PET revealed differences in brain metabolism between MCS- and

MCS+ patients. Indeed, MCS+ patients showed preserved brain metabolism in language processing areas and sensorimotor regions as compared to patients in MCS- (Bruno et al. [2012]). Among patients in MCS*, 67% of them showed metabolic preservation in the fronto-parietal regions (Thibaut et al. [2021], see **Figure 1.2**). Regarding LIS patients, global cortical metabolism is relatively preserved and close to what is observed in healthy subjects (Levy et al. [1987], Laureys et al. [2004, 2005]). Even if FDG-PET seems to have a better diagnostic precision than mental imagery fMRI, it should not be used as the only diagnostic tool, but rather as a complement to other means of assessment. The simultaneous use of EEG-PET-fMRI techniques allows to improve diagnosis and prognosis of severe brain injured patients (Golkowski et al. [2017], Hermann et al. [2021], Bodart et al. [2017]). In 2019, the Neurocritical Care Society launched the Curing Coma Campaign, which aims to identify gaps (i.e., in terms of diagnosis, prognosis, biomarkers, treatments...) that still exist in the field of DoC in order to provide directions for future research (Claassen et al. [2021]).

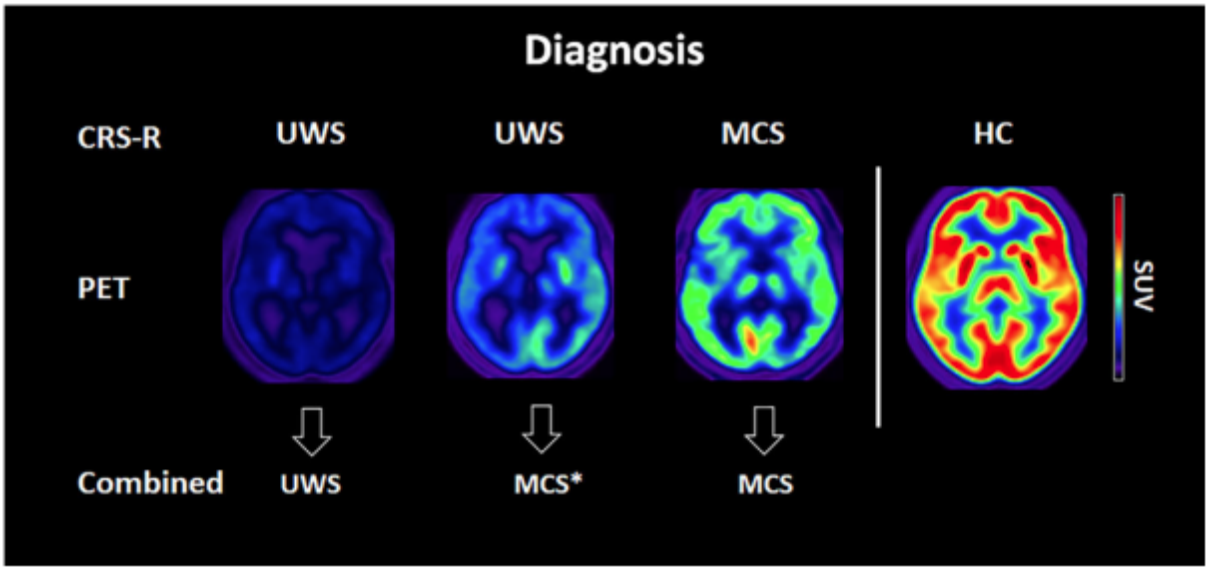


Figure 1.2: Global brain metabolism in VS/UWS, MCS*, and MCS and HC patients (from Thibaut et al. [2021]). In red = high brain metabolism, in blue = low metabolism, CRS-R = coma recovery scale-revised, VS/UWS = unresponsive wakefulness syndrome, MCS = minimal conscious state, MCS* = VS/UWS patient with atypical cortical metabolism preservation, HC = healthy control, SUV = standard uptake value.

1.2 Pain management and assessment in patients with DoC and LIS

1.2.1 Pain or nociception?

Definition

Nociception is a *"neuronal process allowing the encoding and processing of a noxious stimulus"* (Loeser and Treede [2008]). The consequences of this encoding may be autonomic (e.g., increased heart rate) or behavioral (e.g., flexion withdrawal). However, this does not necessarily imply a conscious perception of the stimulus.

According to the International Association for the Study of Pain definition, pain is an *"unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage"* (Raja et al. [2020]). Pain processing includes physiological, sensorial, cognitive and emotional aspects. Pain assessment is based on the subjective experience of the patient and requires that he or she is conscious. With DoC patients who cannot communicate about their subjective experience, it is difficult to infer the presence of a painful experience. However, verbal communication is not the only way to express pain, the absence of communication from the patient does not exclude the possibility that he or she is experiencing pain.

Neuronal correlates of pain and nociception

In case of tissue damage, the nociceptive signal is picked up by the nerve endings of the nociceptors (i.e., Aδ and C fibers). The A fibers are myelinated, so signal transduction is faster than for the amyelinated C fibers. Aδ fibers are involved in pain sensation and respond to mechanical or (non-noxious or noxious) thermal stimulation. The C fibers are polymodal nociceptors (i.e., responses to mechanical, thermal and chemical stimuli) and are involved in pain perception. The nociceptive signal is transduced towards the dorsal horn of the spinal cord. It is then transmitted to the thalamus through the spinothalamic pathway (also involved in thermal, and non-discriminative touch information transmission), which in turn sends the information towards cortical areas. All cortical and subcortical areas activated during the nociceptive stimulus processing constitute the pain neuromatrix (see **Figure 1.3**, Ingvar [1999], Zasler et al. [2022]). The pain neuromatrix is composed of the lateral and medial systems. The lateral system is responsible for the sensory processing of the nociceptive stimulus (i.e., localization, duration, intensity etc.). It involves the lateral thalamic nucleus, the primary somatosensory cortex (S1), the secondary somatosensory cortex (S2), the insula and the posterior parietal cortex (Hofbauer et al. [2001], Bushnell

et al. [1999]). The medial system is responsible for the affective processing of pain. It is composed of the medial thalamus nucleus, the prefrontal cortex, the anterior cingulate cortex (ACC), the posterior cingulate cortex (PCC) and the posterior medial cortex (Peyron et al. [2000]). Neuroimaging studies carried out in healthy subjects, have shown the importance of the S2, the insula and the ACC in pain processing and more particularly in acute pain. In 1997, Rainville et al set up a paradigm allowing to modulate the affective dimension of pain using hypnosis. They observed a positive correlation between the perception of this painful sensation and brain activity in the ACC (Rainville et al. [1997]). These results were confirmed in another study where the author found that if the ACC and the insula were activated five seconds before a nociceptive stimulation, then the perception of pain was increased (Boly et al. [2007]).

The activation of this the pain neuromatrix and the functional connectivity between the thalamus and these different regions is necessary and sufficient to allow conscious perception of pain (Dehaene and Changeux [2011], Baars et al. [2003]). However, neuroimaging studies have shown that the pain neuromatrix can also be activated during non painful stimulations, suggesting that these regions are more widely involved in multimodal processing (Mouraux et al. [2011], Mouraux and Iannetti [2018]).

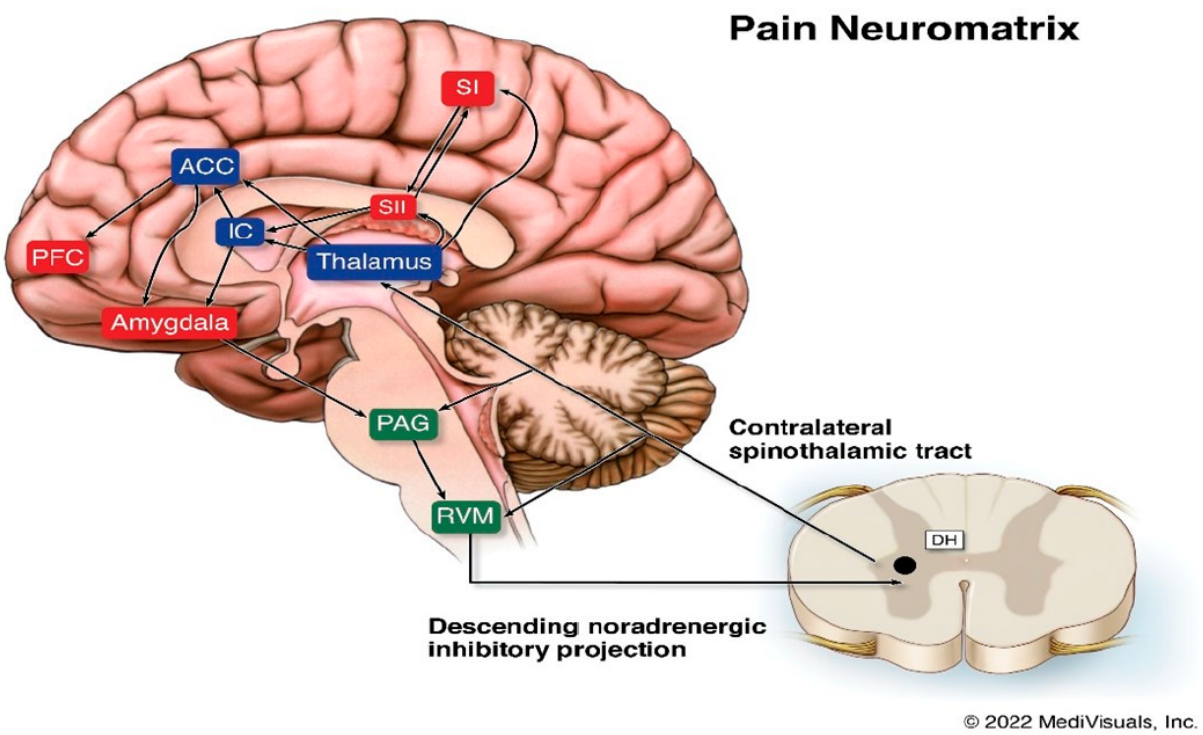


Figure 1.3: The pain neuromatrix (from Zasler et al. [2022]). ACC = anterior cingulate cortex, DH = dorsal horn, IC = insular cortex; PAG = periaqueductal gray, PFC = prefrontal cortex, RVM = rostral ventral medulla, SI = primary somatosensory cortex, SII = secondary somatosensory cortex.

1.2.2 Pain and nociception in severely brain injured patients

Sources of pain

There are many causes of pain in severely brain damaged patients. Pain can be acute (e.g., after a fractures, catheterization, wounds or surgery) or chronic such as spasticity, contractions, pressure sores, peripheral nerve damage or pain network disturbance (e.g., allodynia, neuropathic pain) (for a review refer to Zasler et al. [2022]). Pain is considered chronic if it lasts more than three months and may lead to functional modifications and mood disorders (e.g. depression, anxiety) (Turk et al. [2011], Grichnik and Ferrante [1991]). For instance, neuroimaging studies have found a positive correlation between the presence of anxiety and gray and white matter impairment in patients with chronic pain (i.e., neuropathic pain or fibromyalgia) (Malfliet et al. [2017], Gustin et al. [2011]). Few studies have looked specifically at pain in LIS. Despite their quadriplegia, touch sensitivity in all four limbs, head and face is preserved in these patients (León-Carrión et al. [2002b]). They are likely to develop spasticity, which may lead to long-term chronic pain (Pistoia et al. [2015]). Studies highlight the presence of chronic pain in 45% of patients in LIS (Rousseau et al. [2015], Bruno et al. [2011a]). Moreover, the presence of pain has a direct influence on the patients' quality of life (Bergés et al. [2007]). These different types of pain can disturb the level of arousal as well as the motivation of patients with DoC or in LIS which can have an impact on the clinical diagnosis.

Neuroimaging

Patients with DoC suffer from fronto-parietal network and functional connectivity damages, which lead to a disturbance in pain and nociception processing. However, neuroimaging studies carried out in this population have shown that some regions are preserved (Boly et al. [2008], Laureys et al. [2002]). Indeed, when nociceptive electrical stimulation is delivered to MCS patients, the activation pattern of the pain neuromatrix is close to the one observed in healthy subjects, especially in the secondary somatosensory cortex, the anterior cingulate cortex and the insula (Boly et al. [2008]). Functional connectivity within the pain neuromatrix is also preserved in MCS patients (Kupers et al. [2005], Boly et al. [2008]). Although more lateralized and with a smaller spatial range, these results suggests that MCS patient are able to consciously process pain (see **Figure 1.4**). In clinical setting, antalgic treatment should be used systematically in MCS patient, even if they cannot communicate about their pain. In contrast, in VS/UWS patients, nociceptive electrical stimulation will result in an isolated activation of the primary somatosensory cortex with an absence of functional connectivity with other regions involved in pain (Laureys et al. [2002]). However, a 2003 study used PET-H2150 (i.e., allows to follow cerebral blood flow) in seven VS/UWS patients. After a nociceptive electrical stimulation, they observed an increase in cerebral

blood flow in the primary and secondary somatosensory cortices and in the ipsilateral posterior insula (Kassubek et al. [2003]). Another study using fMRI showed that during nociceptive electrical stimulation, 50% of VS/UWS patients had activation of the sensory network and 30% had activation of the affective network (Markl et al. [2013]). These results suggest that residues of the pain processing network remain active in some patients considered "unconscious" from a behavioral point of view.

It is also relevant to mention that differences in terms of structures and physiological properties may exist between a severe brain injured patient and a healthy subject. Therefore, it is essential to perform a multimodal assessment not only based on neuroimaging but also on pain-related behaviors and physiological changes (i.e., increase of heart rate and respiratory rhythm, skin conductance etc.) (Cowen et al. [2015], Riganello et al. [2019], Devalle et al. [2018]).

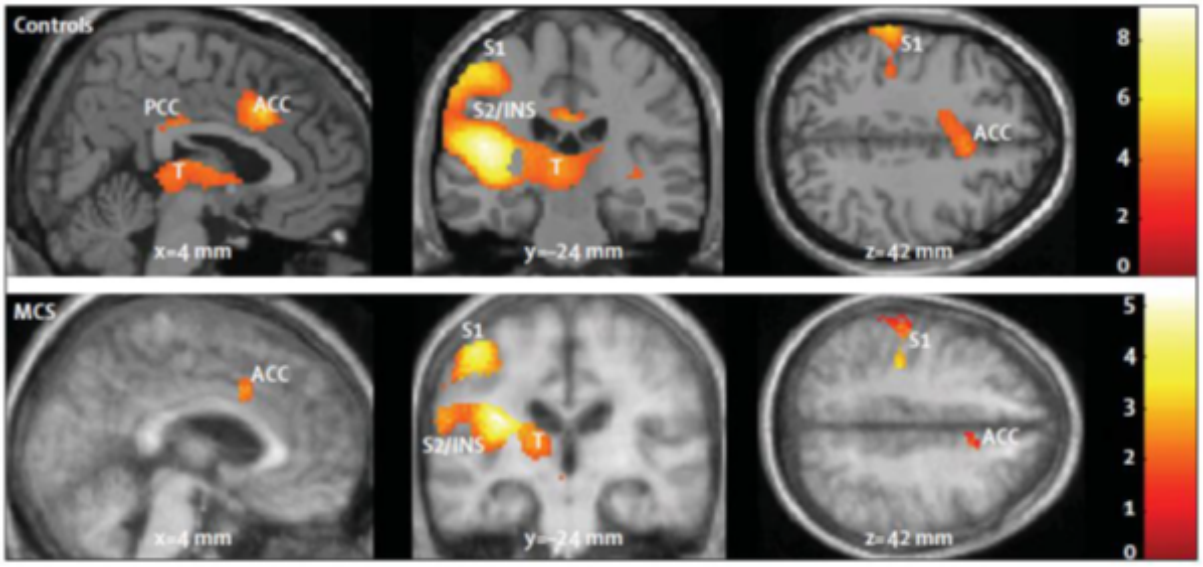


Figure 1.4: Brain regions activated after painful stimulation in healthy subjects (top) and MCS patients (bottom) (from Boly et al. [2008]). The activation pattern of patients is close to that observed in healthy subjects (uncorrected p value <0·001) . T = thalamus, PCC = posterior cingulate cortex, ACC = anterior cingulate cortex, S2/INS = secondary somatosensory cortex or insula, S1 = primary somatosensory cortex.

1.2.3 Assessment and management of pain and nociception in severely brain injured patients

Due to the absence of communication in DoC patients, pain assessment and management is a major clinical and ethical issue. A 2009 study asked healthcare professionals to give their opinions on the perception of pain in VS/UWS and MCS patients. The results varied according to their religious beliefs, professions and backgrounds. Among the physicians interviewed, 96% of them considered that MCS

patients could feel pain compared to 56% for VS/UWS patients (Demertzi et al. [2009]). Considering these results, it is logical to assume that the pain management strategies may vary according to the diagnosis and the health professional beliefs. However, as previously explained, residual activity in the pain network has been observed in some VS/UWS patients (Kassubek et al. [2003]). Moreover, MCS* patients, although behaviorally unresponsive also show brain activity close to that of MCS patients (Thibaut et al. [2021]). It is therefore important to set up pain assessment tools independent of the clinical diagnosis to avoid mismanagement of patients.

There are many behavioral scales to assess pain in non-communicative patients, such as: The Neonatal Infant Pain Scales (NIPS; assesses pain in infants), the Faces, Legs, Activity, Cry, Consolability pain scale (FLACC, assesses pain in children 2 months to 7 years of age), the Pain Assessment In Dementia Scale (PAINAD, assesses pain in patients with dementia), and the Checklist of Non-verbal Pain Indicator (CNPI, assesses pain in cognitively impaired older adults) (Warden et al. [2003], Lawrence et al. [1993], Voepel-Lewis et al. [2010], Feldt [2000]). However, none of these scales are specific to severely brain injured patients. The Nociception Coma Scale (NCS) was developed to allow pain and nociception assessment in DoC patients (Schnakers et al. [2010]). It consists of four subscales assessing motor, verbal, visual responses and facial expression. This scale is sensitive to the level of consciousness, with patients in MCS having higher NCS scores than VS/UWS patients. A 2014 study compared the NCS scores obtained after noxious stimulation (i.e., pressure on the nail bed) with those obtained after non-noxious (tactile) stimulation in VS/UWS and MCS patients. The total scores and subscores of the NCS were higher after a noxious stimulation compared to a non-noxious stimulation, except for the visual subscale which showed no difference between the two conditions (Chatelle et al. [2012]). This visual subscale was therefore excluded from the Nociception Coma Scale Revised (NCS-R, see **Table 1.1**). A positive correlation was observed between glucose consumption in the ACC and NCS-R scores suggesting that NCS-R scores are related to cortical processing of pain (Chatelle et al. [2014a]). The NCS-R is therefore a relevant behavioral tool for pain assessment and management in non-communicative brain-damaged patients. A recent study found that MCS patients have higher NCS-R total scores than VS/UWS patients suggesting that a strong correlation exists between the level of consciousness and the level of responsiveness to noxious stimulation (Chatelle et al. [2018a], Cortese et al. [2021]). Together, these studies confirm the experimental and clinical utility of the NCS-R in the assessment of pain in patient with DoC (Chatelle et al. [2016]). Nevertheless, there is a need for clear guidelines regarding its use.

In patients in LIS, pain is assessed using a communication code or a visual analogue scale (VAS) ranging from 0 to 10 (0 = no pain, 10 = most severe pain).

Table 1.1: The Nociception Coma Scale-Revised.

Motor response
3 - Localization to painful stimulus
2 - Flexion withdrawal
1 - Abnormal posturing
0 - None
Verbal response
3 - Verbalization (intelligible)
2 - Vocalization
1 - Groaning
0 - None
Facial expression
3 - Cry
2 - Grimaces
1 - Oral reflexive movement/Startle response
0 - None

In clinical practice, pharmacological treatments are favored to treat patients' pain. According to the World Health Organization (WHO) there are three main levels of analgesics treatments: level 1 refers to non-opioid analgesics, level 2 corresponds to weak opioids and level 3 is attributes to strong opioids (Ventafridda et al. [1985]). However, the systematic administration of pharmacological treatment is not always beneficial to patients with DoC or in LIS. Indeed, these types of treatments are often work with side effects, such as drowsiness, increased fatigue and cognitive slowing. In patient with DoC, an increase in the level of fatigue could lead to a decrease in patient's ability to recover and interact with clinicians. The patient's level of consciousness may then be underestimated. Since LIS patient's main means of interaction with the outside world are blinking, eye movements or residual movements of a finger, their everyday life requires strong cognitive engagement. The attentional resources needed to use communication tools are therefore major. On the other hand, not administering pain medication to a patient in need is also an important ethical issue. This shows how important it is to develop reliable pain assessment tools independent of clinical diagnosis and clear guidelines to improve pain management in this sensitive population.

This doctoral thesis aimed to improve guidelines regarding pain assessment and management in severely brain injured patients unable to communicate verbally such as patient with DoC and LIS. To this end, this work will be divided into two main axes:

1. *"How to improve pain assessment in patients with DoC and LIS?"*. In **Chapter 2**, we will provide some answers to this question by conducting two studies. First of all, we conducted a retrospective study on patient with DoC aiming to establish a conservative NCS-R cut-off score based on FDG-PET data. Then, we performed a survey focused on LIS patients in order to investigate and

better characterize pain (i.e., location, duration, frequency, management, impact on quality of life etc.) in this population.

2. *"How to reduce potential sources of pain?"*. In **Chapter 3**, we will first focus on a potential source of pain often observed in these patients unable to move: spasticity. This retrospective cross- sectional study investigated the relationship between the presence of pain and the presence of spasticity in DoC patients. The aim was to better characterize the spastic profiles most likely to induce pain in order to propose an adequate management. In a second part we presented the pilot results of a randomized placebo-controlled crossover trial carried out in DoC patients and evaluating the effects of an analgesic treatment on the NCS-R scores during physical therapy. The aim was to determine if the NCS-R can be used clinically to detect changes in behavior induced by pain medication and thus adapt the management of patients on a daily basis.

Chapter 2

How to improve pain assessment in patients with DoC and LIS?

Based on the following publications:

Bonin, E.A.C., Lejeune, N., Thibaut, A., Cassol, H., Antonopoulos, G., Wannez, S., Martial, C., Schnakers, C., Laureys, S., Chatelle, C. (2020). Nociception Coma Scale Revised allows to identify patients with preserved neural basis for pain experience. J Pain, S1526-5900(19)30063-X. https://doi.org/10.1016/j.jpain.2019.11.004

Bonin, E.A.C., Delsemme, Z., Blandin, V., Alnagger, N.L., Thibaut, A., Faymonville, M-E., Laureys, S., Vanhaudenhuyse, A., Gosseries, O. (2022). French survey on pain perception and management in patients with locked-in syndrome. Diagnostics, https://doi.org/10.3390/diagnostics12030769

2.1 Study 1: Nociception Coma Scale-Revised allows to identify patients with preserved neural basis for pain experience

2.1.1 Summary

The Nociception Coma Scale-Revised (NCS-R) was developed to help assess pain in patients with disorders of consciousness (DoC). Several studies have shown its sensitivity in assessing response to acute noxious stimuli. However, they failed to determine a reliable cut-off score that could be used to infer pain processing in these patients.

This retrospective cross-sectional study aimed to determine a NCS-R cut-off score supporting preserved neural basis for pain experience, based on brain metabolism preservation as measured by fluorodeoxyglucose positron emission tomography (FDG-PET). We included patients in unresponsive wakefulness syndrome (UWS) confirmed by the FDG-PET and examined the NCS-R total scores. As the highest score was 4, we defined the cut-off to be 5 and compared the brain metabolism of these patients to matched patients with DoC and a NCS-R cut-off score ≥ 5 (i.e., *potential pain*), as well as healthy subjects.

We found a higher global cerebral metabolism in healthy subjects compared to both patient groups and also in patients with *potential pain* compared with FDG-PET confirmed UWS. We observed a preserved metabolism in the left insula in patients with *potential pain* compared with FDG-PET confirmed UWS.

In terms of perspectives, our data suggest that using the cut-off score of 5 could be helpful to improve pain management in patients with DoC. Future studies should focus on patients showing scores below this cut-off to better characterize their profile and improve care.

Keywords: Nociception; Pain; Cut-off score; Nociception coma scale-revised; Disorders of consciousness.

2.1.2 Introduction

Assessing and treating pain in severely brain-injured patients unable to communicate such as patients with disorders of consciousness (DoC) is a real challenge. Indeed, DoC encompass the unresponsive wakefulness syndrome (UWS; eye opening periods and only reflexive movements Posner et al. [2007], The Multi-Society Task Force on PVS [1994]) and the minimally conscious state (MCS; purposeful responses without functional communication), two states associated with major ethical and clinical concerns with respect to pain treatment Chatelle et al. [2014b]. Neuroimaging studies investigating cortical responses to noxious stimuli in UWS and MCS patients, report preservation of thalamo-cortical connectivity and activation of areas involved in the cognitivo-affective dimension of pain (e.g., insula, anterior cingulate cortex [ACC]) in MCS patients that seem to be impaired in UWS patients Boly et al. [2007], Kassubek et al. [2003], Laureys et al. [2002]. However, other neuroimaging data reported remaining activity within the emotional pain network in about 30% of UWS patients Markl et al. [2013], suggesting that residual sensory and emotional pain processing can remain active even if the patient is unresponsive at bedside. Among the different techniques used to better document a patient's covert abilities, 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) has been shown to be a strong complement to bedside examinations for patients with DoC Stender et al. [2014, 2015, 2016], Thibaut et al. [2012] . A diagnostic precision study of FDG-PET reported that 32% of the behaviorally UWS patients showed signs of potential covert cognitive abilities, and 69% of them recovered signs of consciousness after 12 months Stender et al. [2014]. Moreover, the global metabolic rate of glucose has shown a good sensitivity (i.e., 95%) to distinguish UWS from MCS Stender et al. [2016].

The Nociception Coma Scale-Revised (NCS-R) was developed to assess potential pain in patients with DoC Chatelle et al. [2016, 2012], Schnakers et al. [2010], with several studies showing its sensitivity to nociception and to the level of consciousness Chatelle et al. [2012], Schnakers et al. [2010]. A study using FDG-PET reported a significant correlation between NCS-R total scores and resting brain metabolism in the ACC Chatelle et al. [2014a], supporting the correspondence between the score and the potential for cortical pain processing. If previous results support the experimental and clinical usefulness of this tool, there is currently no reliably defined cut-off score to determine the potential experience of pain and the need for treatment Chatelle et al. [2018a, 2012].

The aim of this study is to determine a NCS-R cut-off score using an approach based on neuroimaging data. Using FDG-PET, we investigated the NCS-R score ranges obtained in "FDG-PET confirmed UWS patients". The diagnosis was based on behavioral assessment and visual examination of brain metabolism (i.e., a severe bilateral hypometabolism of the associative frontoparietal cortex without preserved areas led to a diagnostic of UWS Stender et al. [2014]). We hypothesize that patients with a global hypometabolism of the whole cortical area (including the network known to be involved in pain process-

ing) cannot sustain conscious treatment of potentially painful stimuli. Therefore the highest behavioral responses observed in these FDG-PET confirmed UWS patients could be used as a conservative NCS-R cut-off score Chatelle et al. [2012], Stender et al. [2014, 2016]. We then considered the global and local differences in brain metabolism between FDG-PET confirmed UWS, patients with *potential pain* (i.e., MCS and UWS patients with a preserved brain metabolism on FDG-PET and a NCS-R score equal to or greater than the defined threshold) and healthy subjects. FDG-PET confirmed UWS and patients with *potential pain* were matched for age, gender and etiology, and healthy subjects were matched with both patients groups by age and gender. We hypothesized 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.

2.1.3 Materials and methods

Participants

This retrospective study included patients admitted to the intensive care and the neurology ward of the University Hospital of Liège. These patients were assessed as part of a week of diagnostic and prognostic assessment. Inclusion criteria were: (1) age $\geq$ 16 years, (2) no administration of neuromuscular function blockers and no sedation 24h before assessment, (3) a diagnosis of unresponsive wakefulness syndrome (UWS) or minimally conscious state (MCS), based on the behavioral assessment performed using the Coma Recovery Scale-Revised (CRS-R Giacino et al. [2004]), (4) pain assessment performed with the NCS-R, and (5) brain metabolism data assessed by FDG-PET. Exclusion criteria were: (1) documented history of prior brain injury; (2) premorbid history of developmental, psychiatric or neurologic illness resulting in documented functional disability up to time of the injury; and (3) upper limb contusions, fractures or flaccid paralysis. The study was approved by the Ethics Committee of the Hospital-Faculty Ethics committee of the University of Liege and written informed consent was obtained by the healthy subjects and the patients' legal representatives.

For FDG-PET analyzes, inclusion criteria for the healthy subjects were: (1) no history of neurologic or psychiatric disorders, (2) no head injuries, (3) absence of pregnancy, (4) absence of diabetes.

Different groups were used during this study, criteria of each group were:

- *FDG-PET confirmed UWS group:* (1) patients behaviorally diagnosed as UWS (2) with a global decrease of brain metabolism on FDG-PET based on visual examination of the FDG-PET Standardized Uptake Values (SUV; see below Stender et al. [2014]).

- *Patients with potential pain group:* (1) patients behaviorally diagnosed as UWS or MCS with (2) a preservation of brain metabolism on FDG-PET based on visual examination of the SUV and (3) a pain assessment performed by the NCS-R with a score equal to or greater than the threshold defined before.

Materials

The NCS-R is a behavioral scale used to assess acute pain in DoC patients. This scale includes three subscales assessing motor, verbal and facial responses. Each of these subscales is scored from 0 (no response) to 3 (highest response level) with a total score between 0 and 9. In this study, we included data obtained with the NCS-R at rest and following experimental noxious stimulation (i.e., deep pressure on the left and right nailbed of the middle finger for 5 seconds Schnakers et al. [2010]). Finger pressure as a noxious stimulation was used as it is widely implemented in different neurobehavioral scales to assess response to nociception Giacino et al. [2004], Teasdale and Jennett [1974]. To capture the patient's highest possible nociceptive response, NCS-R scores were also taken into account during mobilization (i.e., passive mobilization of the upper and lower limbs during a physical therapy session - stretching). If mobilizations are not comparable to an experimental noxious stimulus, several studies reported that mobilizations (i.e., care or physical therapy) could be potentially painful for patients with brain injury **?**Gélinas et al. [2019]. The NCS-R was administrated before the FDG-PET.

The clinical diagnosis of patients was determined using the CRS-R, consisting of 23 hierarchically arranged items that comprise six subscales addressing arousal, auditory, visual, motor, oromotor/verbal and communication functions. The lowest item on each subscale represents reflexive activity while the highest item represents cognitively-mediated behaviors Giacino et al. [2004]. At least 5 CRS-R were performed during the same week (i.e., between day 1 and day 5) by different examiners to reduce the potential misdiagnosis Wannez et al. [2017] and the CRS-R with the best diagnosis (i.e., the CRS-R with the highest level of consciousness) was used for final diagnosis.

For each patient, PET was performed during 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]. Statistical analyzes were performed using Statistical Parametric Mapping (SPM12; `https://www.fil.ion.ucl.ac.uk/spm/`). The FDG-PET and the NCS-R assessment were performed the same day.

Determining the cut-off score

We used the FDG-PET Standardized Uptake Values (SUV) to assess the cerebral metabolic rate of glucose consumption:

$$SUV = \frac{\text{(Decay corrected Voxel Intensity)}}{\frac{\text{(Injected Dose)}}{\text{(Body Weight)}}} \text{ at single subject level.}$$

The SUV images were used to select FDG-PET confirmed UWS patients through visual observation by three different expert examiners (i.e., neuropsychologist and physical therapist working with patients with DoC and PET imaging for at least 5 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 (see **Figure 2.1**). Full agreement was observed for typical UWS.

Then, we looked at the NCS-R highest score in this subgroup of FDG-PET confirmed UWS to determine the cut-off score.

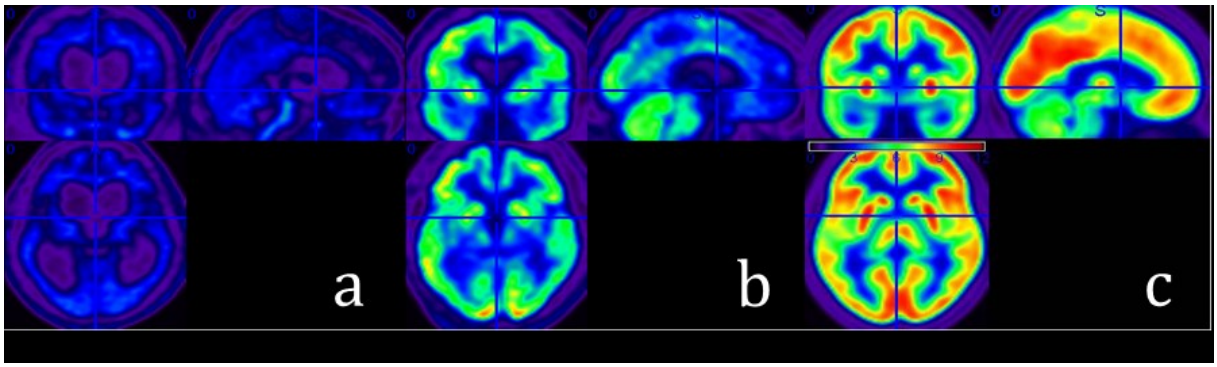


Figure 2.1: Brain metabolism at PET-FDG in a) unresponsive patient (UWS) with a global hypometabolism, b) patient in minimally conscious state (MCS) and c) healthy subject. The scale represents the cerebral metabolic rate of glucose (CMRglc; μmoL/g per minute) from 0 (blue) to 12 (red).

Brain metabolism and cut-off score

To investigate whether the cut-off score can be used to support preserved neural basis for pain experience, we used global and regional brain metabolism. A group of patients with DoC and potential pain was selected based on their age, gender and etiology (diagnosis of UWS or MCS) to match with the FDG-PET confirmed UWS patients. A group of healthy subjects (also matched for age and gender) was also included. We calculated the global mean value to compare global brain metabolism preservation between the three groups. The statistical analyzes were carried out using the R statistical software language (RStudio, Version 1.1.463). As the data distribution was not normal and the sample size was

small ($n < 30$), we used non-parametric tests. The comparison of the grey matter glucose consumption between the different groups was performed using a Kruskal-Wallis test.

To look for regional differences, we used a design matrix including the group of FDG-PET confirmed UWS patients, the group of patients with *potential pain*, and the group of healthy subject scans. We identified brain regions with preserved metabolism in the group of patients with *potential pain* vs FDG-PET confirmed UWS patients and in patients with *potential pain* vs healthy subjects. We also identified brain regions with decreased metabolism in the group of patients with *potential pain* vs healthy subjects and in FDG-PET confirmed UWS patients vs healthy subjects. Global normalization was performed by proportional scaling. Thresholding of results was done 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 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 DoC patients Boly et al. [2008], Chatelle et al. [2014a]).

A posteriori, we looked at regional brain metabolism preservation in patients MCS* (i.e., behaviorally diagnosed in UWS but with an atypical cortical metabolism preservation 15 who showed potential pain (i.e., a score $\geq$ cut-off)) at the single subject level, using the same approach as described above (compared with 33 healthy subjects, 18 men, mean age 43, SD 15 years).

Sensitivity and specificity

We calculated the sensitivity (i.e., proportion of patients who have received noxious stimulation and have a NCS-R score equal or above the threshold defined in the study) and specificity (i.e., proportion of patients who have not received noxious stimulation and have a NCS-R score below the threshold) of the threshold defined for all MCS patients assessed with the NCS-R since 2011 in order to determine the number of patients that may be underestimated by the identified threshold. Sensitivity and specificity was calculated for the defined threshold. For clinical interest, we also calculated sensitivity and specificity using the scores below that threshold.

2.1.4 Results

Of the 209 patients included in the database, 50 patients were diagnosed in UWS with the CRS-R (min 5 assessments). Out of these 50 patients, 26 showed a global cortical hypometabolism based on visual analysis (i.e., "FDG-PET confirmed UWS", see **Figure 2.1 and 2.2**) and 13 of these FDG-PET confirmed UWS patients were assessed with the NCS-R.

In a second step, these 13 FDG-PET confirmed UWS were matched with 13 patients with DoC presenting *potential pain* (i.e., NCS-R score ≥ 5; see **Figure 2.1** and **Supplementary Table A**) and 13 healthy subjects (18 men, mean age 43, SD 15 years). Of the 13 patients with *potential pain*, 10 were MCS and 3 were unresponsive at bedside (i.e., MCS* = UWS with atypical cortical metabolism preservation Gosseries et al. [2014]).

Determining the cut-off score

When looking at the range of NCS-R total scores for these 13 FDG PET confirmed UWS patients (8 men, age range: 27-73 years, aetiology: traumatic (n=1), post-anoxic (n=7), subarachnoid hemorrhage (n=1), stroke (n=2) and mixed etiology (n=2), see **Supplementary Table 1** and **Figure 2.2** for the flowchart) we found scores between 0 and 4 during potentially painful conditions (i.e., noxious stimulation and/or mobilization). Therefore, we set the cut-off score at ≥ 5.

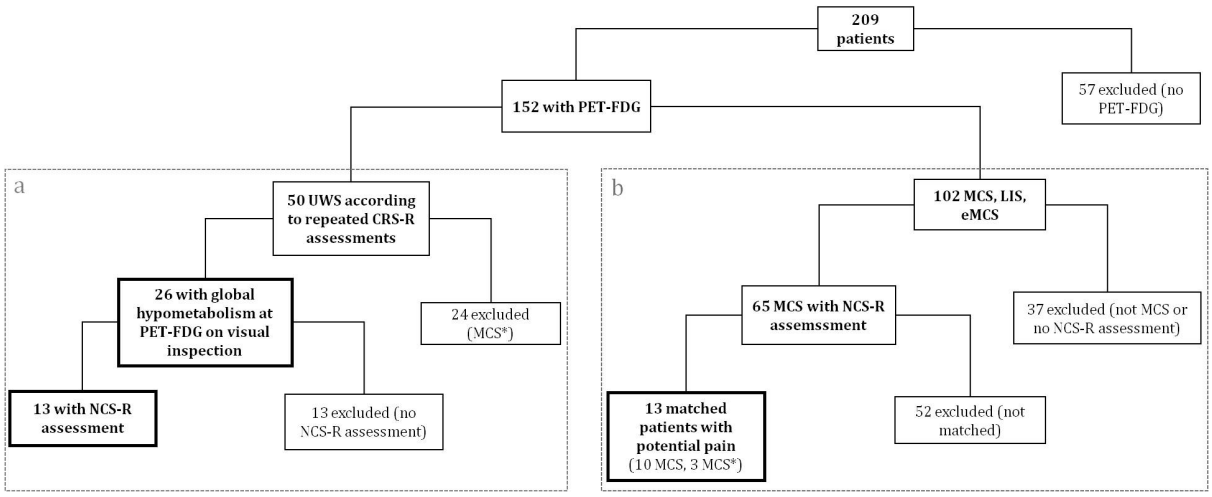


Figure 2.2: Flow chart representing the selection of patients in a) UWS with the CRS-R assessment and with a global hypometabolism at FDG-PET, b) MCS with *potential pain* defined based on threshold (LIS = Locked-in Syndrom, EMCS = Emergency Minimally Conscious State, UWS = Unresponsive Wakefulness Syndrom/Vegetative State, MCS* = UWS with atypical cortical metabolism preservation)

Brain metabolism and cut-off score

The data were not normally distributed according to Shapiro-Wilk tests ($W < 1$, $p = 0.012$). A non-parametrical statistical analysis was performed using a Kruskal-Wallis test to compare global metabolism in FDG-PET confirmed UWS patients vs those with *potential pain* vs healthy subjects. We found a higher global metabolism in healthy subjects compared to the group of FDG-PET confirmed UWS and the group of patients with *potential pain* ($\chi^2 = 33.80$; $df = 2$; $p < 0.0001$), and in patients with *potential pain* compared to FDG-PET confirmed UWS ($\chi^2 = 33.80$; $df = 2$; $p < 0.0001$; see **Figure 2.3**).

Locally, we observed a preservation in brain metabolism only in the left insula in patients with *potential pain* compared to FDG-PET confirmed UWS (Z = 3.31; corrected p = 0.016; MNI [Montreal Neurological Institute] coordinates x = -38, y = -20, z = 44; see **Figure 2.3**). This preservation was also observed in the group of patients with *potential pain* when compared to healthy subjects (Z = 5.95; corrected p = 0.004; MNI coordinates x = -32, y = -32, z = 40). We did not observe a preservation in brain metabolism in the right insula.

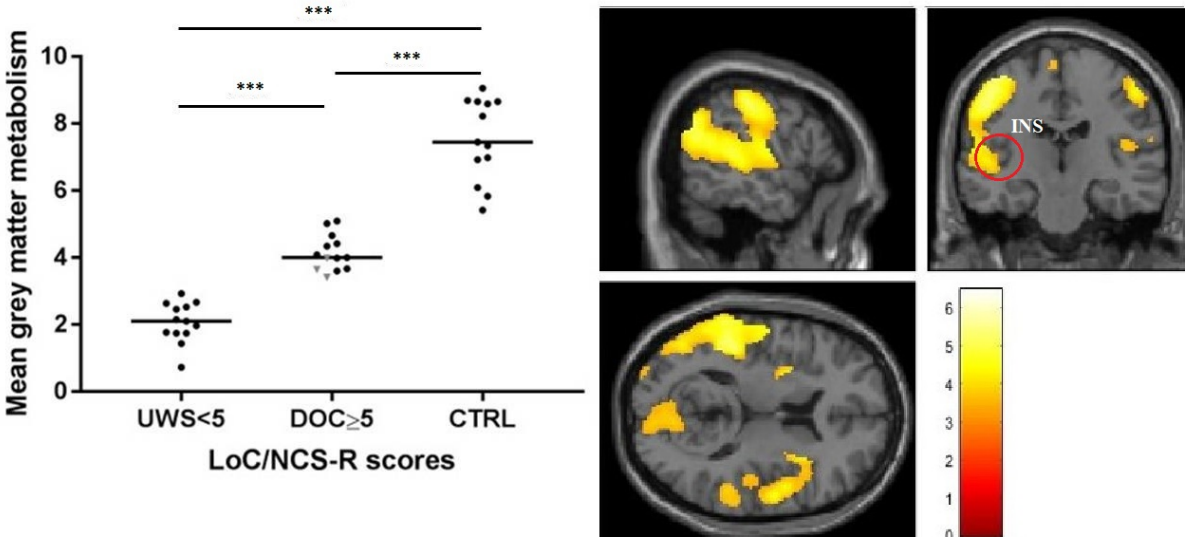
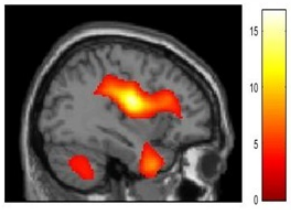
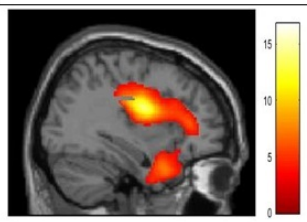
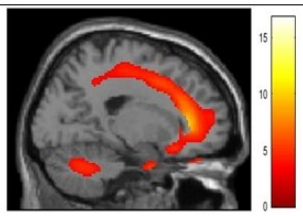
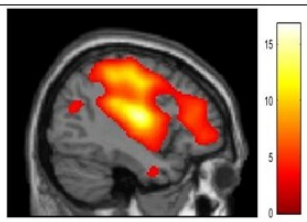
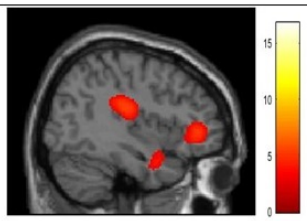
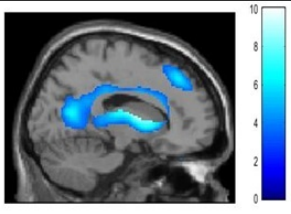


Figure 2.3: Left: Global brain metabolism preservation in FDG-PET confirmed unresponsive patients (UWS) with NCS-R score < 5, patients with disorders of consciousness (DoC) with NCS-R score ≥ 5 and healthy subjects (CTRL; grey triangles represent patients behaviorally diagnosed as UWS, Kruskal-Wallis, *** = $p < 0.0001$). Right: Regional brain metabolism preservation in patients with a NCS-R score ≥ 5 compared to FDG-PET confirmed UWS. Preservation of the brain metabolism was observed in the left insula (INS, x = -55mm, y = -20mm, z = 12mm).

A hypometabolism in the ACC was observed in FDG-PET confirmed UWS patients (Z = 5.06; corrected p = 0.011; MNI coordinates x = 6, y = 18, z = 36) compared to healthy subjects and between patients with *potential pain* and healthy subjects (Z = 4.01; corrected p = 0.012; MNI coordinates x = 4, y = 14, z = 32). When focusing on the three MCS* showing a score ≥ 5, we observed a preservation in brain metabolism in the insula bilaterally in all three patients, and in the ACC in two patients (see **Figure 2.4**).

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	0.003	
Anterior cingulate cortex	20	10	42	4.63	0.008	
MCS*2-preserved						
Left insula	-40	-24	28	6.88	.001	
Right insula	40	-24	28	4.52	0.009	
MCS*2-impaired						
Anterior cingulate cortex	16	2	34	4.14	0.003	

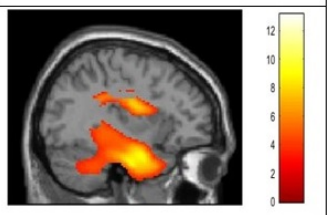
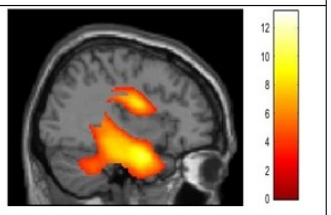
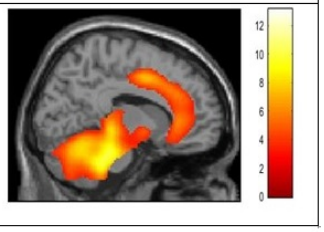
MCS*3-preserved						
Left insula	-34	-30	28	4.43	0.0001	
Right insula	36	-20	28	5.05	0.007	
Anterior cingulate cortex	12	4	44	5.53	0.001	

Figure 2.4: Coordinates of peak voxels (in standardized stereotaxic MNI space) showing preserved and impaired metabolism in patients behaviorally UWS with a NCS-R score $\geq$ 5 (MCS*) compared to healthy subjects (using a 10-mm radius spherical small volume correction in SPM—at voxel and cluster level, centred 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.

Sensitivity and specificity

Finally, when accounting for all patients with MCS 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 (NCS-R cut-off score $\geq$ 5).

A specificity of 73.8% (TN = 48/65, FP = 17/65) and a sensitivity of 73.8% (TP = 48/65, FN = 17/65) was also obtained for a NCS-R cut-off score of 2. A specificity of 90.8% (TN = 59/65, FP = 6/65) and a sensitivity of 55.4% (TP = 36/65, FN = 29/65) for a NCS-R cut-off score of 3. Finally, a specificity of 98.4% (TN = 64/65, FP = 1/65) and a sensitivity of 35.4% (TP = 23/65, FN = 42/65) was obtained for a NCS-R cut-off score of 4 (see **Supplementary Figure A.2**).

2.1.5 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.

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

As a second step, we compared the global brain metabolism between the FDG-PET confirmed UWS, patients with potential pain and healthy subjects. Patients with potential pain showed 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].

As a third step, we examined regional differences in brain metabolism in two areas known to be involved in pain processing, namely the insula (1) and the ACC (2).

(1) In the insula, we found a preservation only in the left insula in patients with *potential pain* compared to patients with FDG-PET confirmed UWS and healthy subjects 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 higher involvement of the right hemisphere in pain sensation Ostrowsky [2002], Vogt et al. [1996], several of them have also shown a bilateral activation of the insula during a noxious stimulation Brooks et al. [2002], Symonds et al. [2006].

(2) In the ACCs, however, a hypometabolism was also observed in patients with *potential pain* and patients in UWS when compared to healthy subjects. In neuroimaging studies on pain processing, the ACC is a region commonly linked 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], Stender et al. [2015]. Neuroimaging studies have shown that an increase of brain activity in the ACC is correlated with an increased pain sensation Rainville et al. [1997], Ingvar [1999]. 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. [2007, 2008].

While these results may be due to the MCS patients included in the 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 the presence of covert cognitive abilities in these patients Kassubek et al. [2003].

Previous studies aiming at determining NCS-R cut-off scores do not reliably distinguish behavioral responses that could be elicited by non-noxious vs noxious stimulations Chatelle et al. [2018a, 2012]. In fact, a recent study determined a cut-off score of 2, a total score achievable by purely reflexive/spinally-mediated behavior and that would inevitably lead to a large amount of false positive patients Chatelle et al. [2018a]. Nevertheless, we are aware that 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.

These findings must be interpreted in spite of several limitations: (1) the heterogeneity of the population (i.e., more anoxic patients in UWS than in MCS and time since injury); (2) this is a retrospective study, so it was difficult over confounding factors such as motor abilities (within the two patient groups (n = 26), 18 of them shown moderate to high spasticity), intensity of the noxious stimulation or heterogeneity in threshold for pain in each patient. Future studies should aim to control these factors as well as the stimulus intensity using a Newtonmeter, for example (as done in Chatelle et al. [2012], Schnakers et al. [2010]). (3) 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 a severe brain damage, DoC patients may have serious brain damage such as widening of the ventricles or hydrocephalus. However, when neuroimaging is performed on severely damaged brains, it is sometimes difficult to target our regions of interest. Some areas like the insula or the ACC may be damaged or "shifted" from a healthy brain template making group analysis complex. In our analysis, we used a customized template built with patients and healthy subjects scans to limit the effect of these deformations. (4) We only had the opportunity to analyze resting state brain metabolism data, 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], Laureys et al. [2002]). However, our results show that, at rest, there is a preservation of metabolism in some regions involved in pain processing, and therefore suggest pain processing may be possible in this patient group. Our results are a first step towards the development of guidelines on the use of the NCS-R for pain management in DoC. Additional studies using PET or fMRI during painful stimulation will be necessary to validate these results and to study effective connectivity in these patients during painful stimulus. (5) Although the scores of 2, 3 and 4 showed better sensitivity results (4 and 5 showed the same specificity and close sensitivity values, We here define the score of 5 as a conservative threshold as we have shown that the FDG-PET confirmed UWS are able to show scores of 4 at the NCS-R. However, clinicians should use this threshold with caution as we also showed that a better sensitivity was observed with lower scores (4, 3, 2, as also shown in another study Chatelle et al. [2018a]). We also acknowledge that more studies

are needed, especially as sensibility and specificity are very difficult to assess in this population (i.e., no self reporting for pain and pain experience, misdiagnosis of the level of consciousness,...). (6) FDG-PET during resting state and behavioral assessment can be influenced by the level of arousal of the patient and, even if we ensure the patient was as awake as possible during these evaluations, it can fluctuate in some patients, influencing the result Guenther et al. [2011], Monti et al. [2010]. One could also argue that one of the conditions used may not be nociceptive (i.e., mobilization). However, our aim was to first 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. [2015a]), we think 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 our approach. 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 a 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 have impairment in pain processing, it is also likely that for others the NCS-R failed to detect potential painful stimuli due to a patients' motor issue.

In conclusion, this retrospective study provided (1) 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, which suggest a better preservation of global and local (left insula and ACC) cerebral metabolism and (2) the suggestion that at least the preservation of the left insula and the left ACC are prerequisites to the conscious process of pain, supporting our hypothesis whereby behavioral responsiveness to pain necessitates a minimal preservation in regions involved in pain processing in patients with DoC. These data provide a first step towards providing better guidelines to clinicians for the management of pain in DoC. The very high specificity and low sensitivity of the score highlight the currently proposed score is very conservative, and that patients with a score ≥ 5 clearly should be targeted as potentially in pain. It also shows that patients with a lower score may still be in pain, in need of an appropriate treatment and should not be neglected.

Finally, this study also highlights the complex relationship between consciousness and pain processing. The use of the NCS-R in a clinical setting becomes clearer as we can now support that patients with a NCS-R score below 2 are not able to perceive a stimulus as being painful. On the other hand, patients scoring ≥ 5, during mobilization, cares or other situations, are likely able to perceive a stimulus as painful. Future studies should focus on better characterizing pain processing in patients with a NCS-R score between 2 and 5, and develop non-behavioral/paraclinical biomarkers of pain processing in DoC.

2.2 Study 2: French survey about pain perception and management in patients with LIS

2.2.1 Summary

Patients with locked-in syndrome (LIS) may suffer from pain, which can significantly affect their daily life and well-being. In this study, we aim to investigate the presence and the management of pain in LIS patients.

Fifty-one participants completed a survey collecting socio-demographic in-formation and detailed reports regarding pain perception and management (type and frequency of pain, daily impact of pain, treatments).

Almost half of the LIS patients reported experiencing pain (49%) that affected their quality of life, sleep and cognition. The majority of these patients re-ported that they did not communicate their pain to clinical staff. Out of the 25 patients reporting pain, 18 (72%) received treatment (60% pharmacological, 12% non-pharmacological) and described the treatment efficacy as 'moderate'. In addition, 14 (56%) patients were willing to try other non-pharmacological treatments, such as hypnosis or meditation.

This study provides a comprehensive characterization of pain perception in LIS patients and highlights the lack of guidelines for pain detection and its management. This is especially pertinent given that pain affects diagnoses, by either inducing fatigue or by using pharmacological treatments that modulate the levels of wakefulness and concentration of such patients.

Keywords: Survey; Locked-in Syndrome; Pain; Quality of life; Guidelines.

2.2.2 Introduction

The locked-in syndrome (LIS) is a neurological disorder that occurs after a brainstem lesion, most commonly due to a stroke Johncy and Shabaraya [2020], Laureys et al. [2005]. This results in paralysis of all four limbs, head, and face (quadriplegia or quadriparesis) and impaired speech (aphonia or hypophonia). LIS patients are conscious, have preserved cognitive functions and usually communicating through eye movements and blinking Maiser et al. [2016]. Also, despite their quadriplegia, LIS patients retain tactile sensitivity León-Carrión et al. [2002b]. The treating physician may erroneously diagnose the LIS patient as being in a coma, or as occupying one of several disorders of consciousness (DoC). These include the minimally conscious state (i.e., with discernible but fluctuating evidence of consciousness without effective communication; MCS Giacino et al. [2002], Demertzi et al. [2013]) or the unresponsive wakefulness syndrome (i.e., eye opening period but no sign of conscious-ness; UWS The European Task

Force on Disorders of Consciousness et al. [2010]) if not assessed adequately Gallo and Fontanarosa [1989], Schnakers et al. [2004].

Once a LIS patient becomes medically stable and receives appropriate medical care, life expectancy increases by several decades Laureys et al. [2005]. Therefore, the elapsed time between brain injury and LIS diagnosis is precious and finding the appropriate treatment both efficiently and effectively is of the upmost importance. A LIS diagnosis usually takes between 2.5 months and 4-6 years, and in the majority of the cases (55%) this diagnosis is made by the patient's relative and not the treating physicians (23%) León-Carrión et al. [2002a]. Pain management using pharmacological treatments in post-comatose patients can have deleterious side effects, such as increasing fatigue or decreasing awareness. This can lead to misdiagnosis and have important consequences on the continuation of care. The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" Gallo and Fontanarosa [1989], Schnakers et al. [2004], Raja et al. [2020]. LIS patients can suffer from acute pain (i.e., which usually occurs suddenly, most often due to inflammation or severe medical condition Michaelides and Zis [2019]) and/or chronic pain (i.e., pain lasting longer than 3 months or beyond the ex-pected period of healing of tissue pathology) Turk et al. [2011]. They can also suffer from neuropathic pain, which is defined as a "pain arising as a direct consequence of a lesion or disease affecting the somatosensory system" Bouhassira et al. [2005]. In addition, these patients are likely to develop spasticity, the severity of which correlates with pain intensity Pistoia et al. [2015]. Pain perception has been previously investigated in DoC patients. Neuroimaging studies have shown that painful stimulations applied to MCS patients can lead to the activation of brain regions involved in the sensorial and affective processing of pain (i.e., insula, anterior cingulate cortex and secondary somatosensory cortex), similar to observations in healthy subjects Boly et al. [2008], Laureys et al. [2002]. Meanwhile, in UWS patients, only the primary somatosensory cortex (i.e., involved in the sensory processing of pain) was activated following a painful stimulation, suggesting that pain processing in UWS could be compromised Giacino et al. [2018], Calabrò et al. [2017].

There are minimal scientific studies investigating the processing of pain in LIS. A survey conducted in 2008 showed that despite the fact that 46% of the LIS patients reported moderate or extreme pain, 72% of patients reported a good quality of life Bruno et al. [2011a]. These results should be interpreted with caution because only half of the patients who were contacted returned the completed questionnaire. It could be that the most painful patients or those with the lowest quality of life did not wish to answer the questionnaire and were therefore under-represented in the study. This bias can also lead to an overestimation of quality of life rates in LIS patients Bruno et al. [2011a]. Another study showed that pain was associated with a decrease of quality of life in LIS patients Bergés et al. [2007]. Furthermore,

it has been shown that patients' mental health and the presence of physical pain correlate with the frequency of suicidal thoughts, suggesting that pain has a significant impact on the quality of life of these patients as in other diseases like cancer, fibromyalgia syndrome or stoke Laureys et al. [2005], Bergés et al. [2007], Skevington [1998], Glare et al. [2014], Westerlind et al. [2020], Staud and Rodriguez [2006]. For example, stroke victims often complain of shoulder pain or other pain (such as central post-stroke pain or headache) that impacts their quality of life Hansen et al. [2012], Lindgren et al. [2007].

At present, no study in LIS patients provides a detailed description of pain and related issues (frequency, location, treatment protocols, etc.). In this survey, we investigated the presence and management of pain in LIS patients. The first aim was to evaluate the results obtained in previous studies regarding the presence of pain and the impact on quality of life. Indeed, as LIS patients often suffer from spasticity and long-term immobility, we hypothesise that the presence of chronic pain in these patients is frequent. We also hypothesise that the presence of pain will have a negative impact on their quality of life via affecting their cognition/mental abilities, emotional regulation and sleep quality. The second aim of this survey was to further characterise the pain experienced by this population (type and frequency of pain, means of communicating the pain, type of treatment used, degree of satisfaction with treatments, etc.). We expect that the majority of the patients will have used pharmacological treatments, with a minority who will have used non-pharmacological treatments.

2.2.3 Materials and methods

This survey was sent by email to LIS members of the Association du Locked-In Syndrome (ALIS, Paris, France). The survey was available in French, with a paper version and an online version. The study conformed to the principles of the Declaration of Helsinki and French Good Clinical Practices. According to the French law (Article L1121-1, Law n°2011-2012 29 December 2011 – art. 5) and the Belgian law (law of 7 May 2004), online surveys are not covered by the law on human experimentation. Therefore, ethical approval was not needed. Completion of the questionnaire was non-remunerated, voluntary, and anonymous. Completion was considered as consent for participation in the survey.

The questionnaire follows the CHERRIES checklist guidelines (see **Supplementary Material B**). The survey was developed on the basis of previous surveys carried out among LIS patients studying their well-being and quality of life Bruno et al. [2011a], Rousseau et al. [2015] and tested internally by the investigators before sending it to the participants. The online survey was done using a Google form and a paper version was also made available (PDF). The questionnaire was composed of 28 questions divided into two main sections: a first part collecting respondents' socio-demographic information and clinical status (i.e., age, sex, time since injury, aetiology, use of an electric wheelchair, presence of tracheotomy and gastrostomy, use of verbal or code communication; in compliance with the GDPR), and a second

part consisting of multiple select and closed questions on pain perception and management (e.g., type and frequency of pain, daily pain impact, tested treatments) (**Supplementary Table B**). Participants were also asked how they had completed the questionnaire (i.e., alone, with the help of a family member or health care professional).

For the first question, participants were asked to specify whether they had suffered from pain within the two weeks prior the survey completion. Only participants who had experienced pain within the last two weeks could continue with the questionnaire. Therefore, for patients who had not experienced pain, only socio-demographic data and clinical status were collected. Question 2 focused on the localisation of pain. In question 3 to 5, a visual analogue scales (VAS) ranging from 0 to 10 was used to assess pain intensity (0 = no pain, 10 = most intense pain). Questions 6 to 9 were based on the DN4 questionnaire Bouhassira et al. [2005]. The DN4 is a validated and simplified questionnaire administered by clinicians to detect neuropathic pain. Each question consists of a number of items (10 in total) and the participant is asked to indicate the presence or absence of each items by ticking "yes" or "no". An item that is present will have a score of 1 while an item that is absent will have a score of 0. The sum of all 10 items corresponds to the total score, and the threshold value for the diagnosis of neuropathic pain is 4/10. If patients show a score greater than or equal to 4/10, then they are considered to be suffering from neuropathic pain. In question 10, participants were asked to indicate how long they had been experiencing pain (if more than 3 months it could be considered as chronic pain). Questions 11 and 13 queried their experiences of pain prior to LIS. Participants who had experienced pain before LIS (Question 11) were asked to evaluate the pain intensity using the VAS (Question 12) and describe the evolution of this pain after LIS (Question 13). For question 14, patients were asked to indicate whether their pain was continuous (i.e., present all the time) or discrete (i.e., present at certain times of the day). For question 15, participants were asked to indicate how they expressed their pain. Questions 16 and 17 focused on elements that alter the experience of current pain: mood/emotion (i.e., negative or positive valence), temperature (negative or positive), position whilst sitting, type of care environment (e.g., nursing, physical therapy), touching the painful area, fatigue , engaging in physical exercises and available equipment (e.g., types of cushioning, using a wheelchair). Questions 18 to 22 were about the influence of pain on cognitive abilities, sleep quality and emotional regulation. For questions 19 and 22, patients used the VAS ranging from 0 to 10 to assess the influence of pain on their quality of sleep and emotional regulation (0 = no influence, 10 = strong influence). For questions 23 to 27, participants were asked to indicate whether they were taking pharmacological and/or non-pharmacological treatments for their pain, and if so to list them. The different pharmacological treatments were then classified into nine categories based on the WHO classification. Level 1 painkillers denoted non-opioids, level 2 painkillers referred to weak opioids and level 3 painkillers were strong opioids (more details in Table 2 for the others

categories) Ventafridda et al. [1985]. For questions 25 and 27, patients used the VAS ranging from 0 to 10 to assess the efficiency of these treatments (0 = not efficient, 10 = strongly efficient). Finally, for question 28, they were asked if they were willing to test new pharmacological or non-pharmacological treatments.

Data were exported and analyzed from the Google form in .csv format. Data collected from the paper version were added to these files. Statistical analyzes were performed using R studio (version 4.0.2) software. For the descriptive analyzes, we used subject counts and percentages to describe the categorical responses and means ± standard deviation (SD) when assessing pain intensity/influence or treatment efficiency. Regarding questions with multiple select answers, we based the calculation on the percentage of the number of participants who replied to each question. As the variables were categorical and/or ordinal variables, non-parametric statistics (i.e., Pearson's chi-squared test, Fischer test and Wilcoxon rank sum test) were performed.

2.2.4 Results

Demographic information and clinical status

From the 300 patients contacted by email, 59 completed responses were collected, 8 were excluded from the analysis as they were missing data. A total of 51 participants (41 from the online version and 10 from the paper version) were included in the analysis, representing a 17% acquisition rate. Seven participants (14%) completed the survey by themselves, 10 (20%) needed the help of a family member, eight (16%) needed the help of a health care professional (i.e., two psychologists, three occupational therapists, one nurse, one social worker and one ALIS member) and 26 (51%) did not answer to this question (i.e., which corresponds to patients who did not experienced pain).**Figure 2.5** provides an illustrative summary of the results.

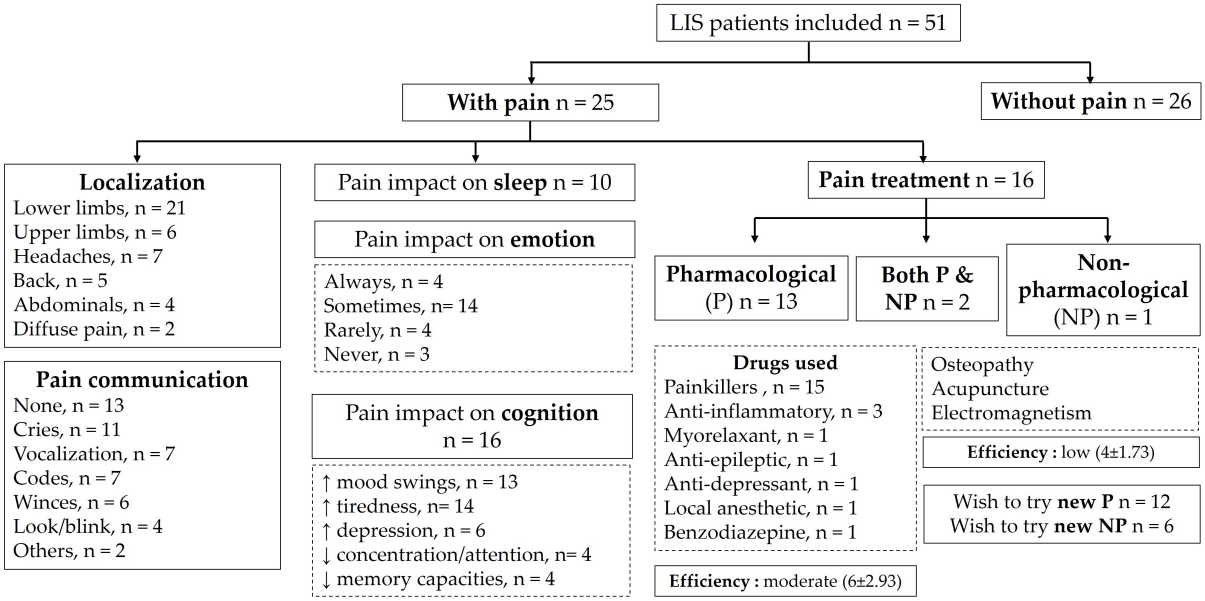


Figure 2.5: Summary of main results. (LIS = locked-in syndrome, P = Pharmacological treatment, NP = Non Pharmacological treatment).

Among the respondents that declared their sex, there the same number of women as men (n=18). Fifteen participants did not specify their sex. The mean age was 51±12 yo (range from 22 to 81 yo) and the mean time since injury was 11±8years (range from 1 to 36 years). The reported aetiology of the LIS was a stroke (40/51; 78%), a trauma (4/51; 8%), an infection (2/51, 4%) or other causes such as chemical intoxication, cervical mishandling, and meningioma (4/51; 8%). Eleven patients (22%) had been diagnosed with LIS for 20 years or more (up to 36 years), 14 (27%) had occupied a LIS for 10 years or more, and 26 (50%) of them were diagnosed within the last 10 years. Twenty-six participants (51%) had a tracheotomy and 34 (66%) had a gastrostomy. The majority of the participants owned a wheelchair (36/51; 71%) but only 19 actively used it (53%). Thirty-eight participants were unable to communicate verbally (38/51; 74%) and 35 used an alphabetic code to communicate (69%). The socio-demographic information and clinical status are summarised in **Table 2.1** (for more detail see **Supplementary Table B**).

Table 2.1: Summary of the socio-demographic information and clinical status for the whole sample and for the group of patient with and without pain.

Variable	Total Sample N = 51	Patients with pain N = 25	Patients without pain N = 26	P-value*
Sex, n (%)				0.31
Female	18 (50%)	10 (62%)	8 (40%)	
Male	18 (50%)	6 (38%)	12 (60%)	
Unknown	15	9	6	
Etiology, n (%)				0.57
Stroke	41 (80%)	21 (84%)	20 (77%)	
TBI	4 (7,8%)	2 (8,0%)	2 (7,7%)	
Infection	2 (3,9%)	0 (0%)	2 (7,7%)	
Other	4 (7,8%)	2 (8,0%)	2 (7,7%)	
Time since injury, Median (IQR)	9 (6 – 18)	6 (3 – 18)	10 (6 – 16)	0,26
Tracheotomy, n (%)	26 (51%)	15 (29%)	1 (22%)	0,21
Gastrostomy, n (%)	34 (67%)	19 (37%)	15 (29%)	0,17
Verbal communication, n (%)	13 (25%)	5 (9,8%)	8 (16%)	0,38
Use of an alphabetic code, n (%)	35 (69%)	19 (37%)	16 (31%)	0,27
Own a wheelchair, n (%)	36 (71%)	15 (29%)	21 (41%)	0,1
Survey completion, n (%)				-
Alone	7 (14%)	7 (14%)	0 (0%)	
With family member	10 (20%)	10 (20%)	0 (0%)	
With healthcare	8 (16%)	8 (16%)	0 (0%)	
No response	26 (51%)	0 (0%)	26 (51%)	

* Pearson's Chi-squared test; Fisher's exact test; Wilcoxon rank sum test

Past and current pain

There was no missing data for this part of the questionnaire. Half of the participants answered that they had experienced pain at some point during the last two weeks (25/51, 49%; median pain intensity: 6/10). The 26 participants who did not feel any pain did not complete the rest of the questionnaire. Pearson's chi-squared test and fisher tests were performed to see if there were any differences between the painful and the non-painful group according to sex, aetiology, presence of tracheotomy/gastrostomy (**Supplementary Table B**). For the time since injury, a Wilcoxon rank sum test was used to investigate the differences between the two groups. No significant differences between these different categories was found between the two groups (**Table 2.1**). Results regarding the localisation and communication method are summarised in **Figure 2.6** (for more details see **Supplementary Table B**.

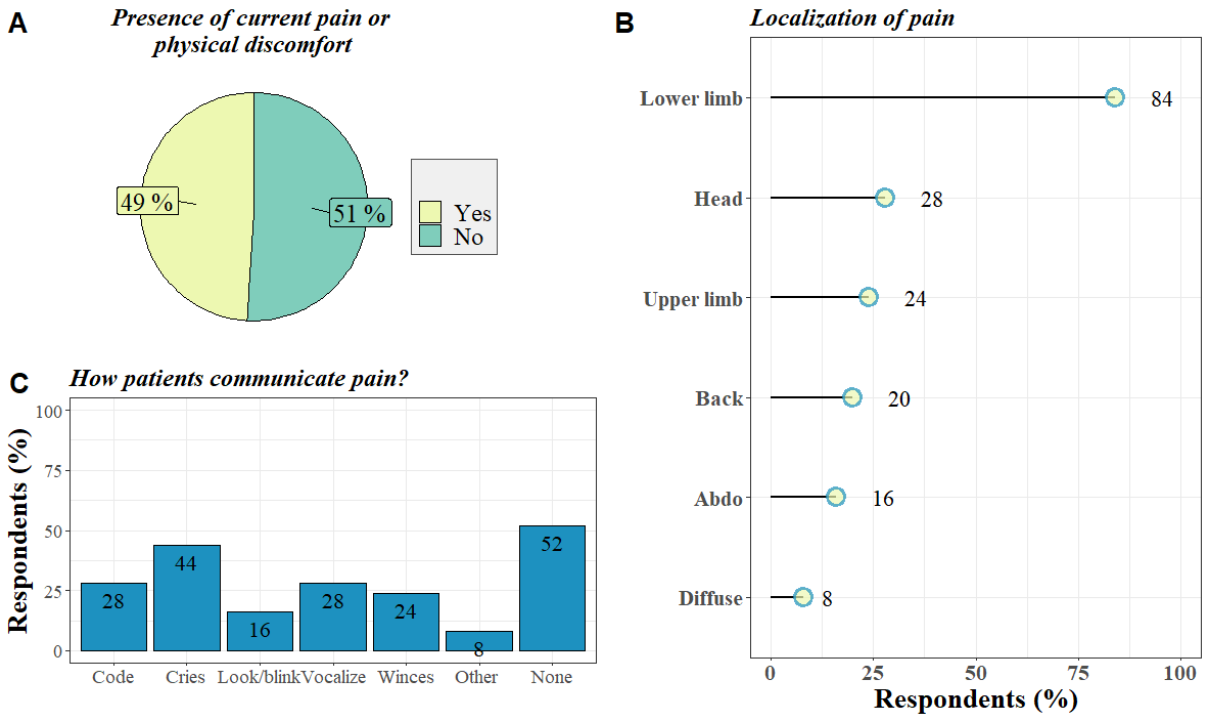


Figure 2.6: Summary of the results on past and current pain. A) Pie chart of the distribution (in percentages) of LIS patients who had experienced pain/physical discomfort versus LIS patients who had not experienced pain/physical discomfort in the last two weeks before the study. B) Lollipop graph representing the distribution (in percentages) of the different areas of the body reported as painful for these participants (multiple select answer). C) Bar plot showing the distribution (in percentages) of the different means of communication used by the participants to express their pain. (Other = Communication methods such as verbalisation via a speech valve and the presence of acute spasticity), (Code = Use of a communication code).

Questions 6 to 9 were based on the DN4 questionnaire. Regarding the features of the pains, 10 participants reported electrical shock-like sensations (40%), six experienced burning (24%), four experienced painful cold sensations (16%), and 10 reported none of these proposed features (40%). When participants were asked if they had experienced any other symptoms in the painful area, 12 participants (48%) had a vice-like pressurised feeling, six (24%) experienced sensation akin to pin-pricks, six (24%) felt a numbing sensation, five (20%) felt a tingling sensation, three (12%) had an itching sensation, and nine (36%) had none of the proposed symptoms. Twenty participants (80%) answered that they felt a decrease in touch sensitivity while sitting, three (12%) felt a decrease in touch sensitivity whilst being touched, and two (8%) did not feel any decrease in touch sensitivity. Seventeen participants (68%) reported that their pain was not caused, or altered by friction. From these questions, we identified nine participants out of 25 (36%) with neuropathic pain (i.e., total score > 4).

Participants were then asked to indicate when they felt these painful sensations.Twelve participants out of 25 felt these pain for more than one year (48%), five be-tween 6 months and one year (20%), six between 3 and 6 months (24%) and two between 1 and 3 months (8%). As pain is considered chronic when it lasts for more than three months, almost all the participants suffered from chronic pain (23/25;

92%). The majority of the participants claimed not to have experienced any pain before the LIS (21/25; 84%). Whereas, some participants reported already experiencing pain prior to their brain injury (2/25; 8%) in addition to some participants who were unsure of whether or not they experienced pain prior to their brain injury (2/25; 8%). Finally, two participants noted a decrease in this pain following the LIS and one did not feel any change.

When describing the current pain experienced, 21 participants out of 25 reported hav-ing discrete episodes of pain (84%) and four reported continuous pain (16%). Four participants out of 21 felt discrete episodes of pain less than once a day (19%), two once a day (10%), three more than once a day (14%) and 12 did not know (57%). Six participants out of 21 felt discrete episodes of pain during the evening (29%), five during the morning (24%), five during the afternoon (24%) and five could not give an estimation of the time of painful experiences (24%).

Factors that influence pain

Participants were asked to select from different elements of daily life that could increase or decrease their pain levels. (**Figure 2.7**). It should be noted that eight (32%) participants reported that certain elements such as type of care (3/25; 12%), temperature (2/25; 8%), touching (2/25; 8%), tiredness (2/25; 8%), supine (2/25; 8%) and sitting (1/25; 4%) positions could both decrease and increase pain (**Figure 2.7**).

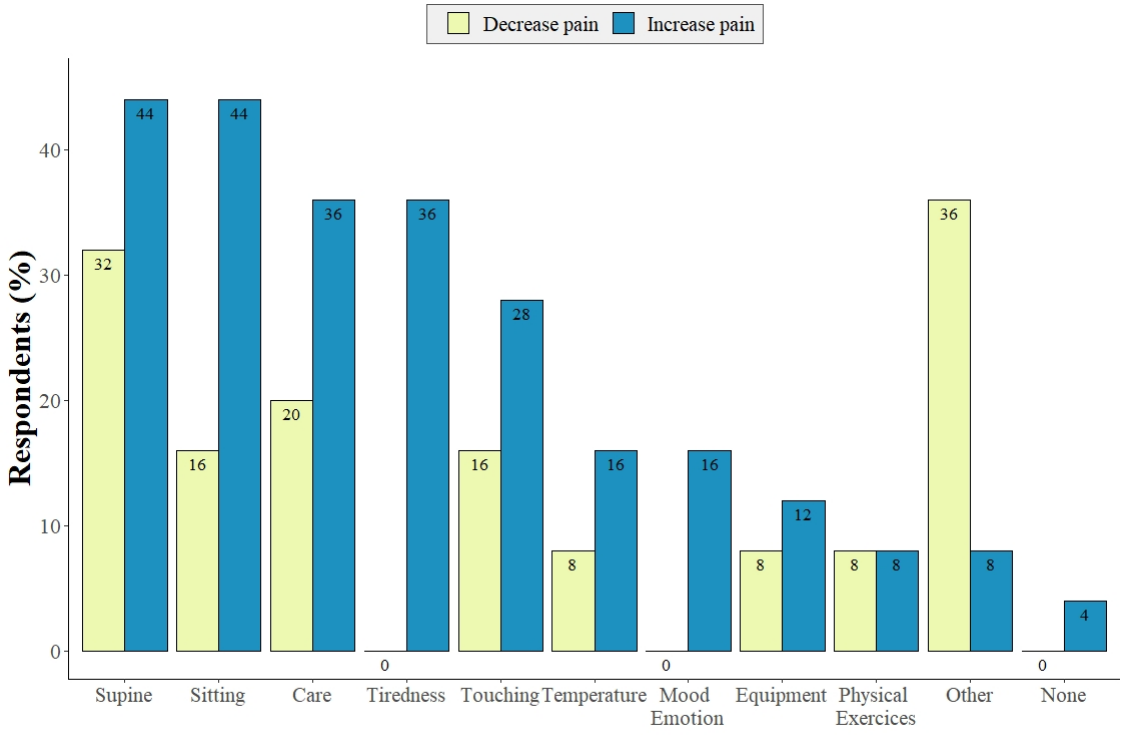


Figure 2.7: Distribution of the different elements that can increase (in blue) or decrease (in yellow) pain in LIS patients (multiple choices answer). Others = feeding, daily handling increase pain but being busy, Botox injection, use of medication decreases pain).

Regarding the effects of pain on cognition/mental abilities, emotional regulation and sleep, results are summarised in **Figure 2.8**. Fisher tests were performed to see if there were any relationship between the use of pain treatment and the presence of sleep disturbance or cognitive disabilities (**Supplementary Table S4**). No link could be established between the presence/absence of pain treatment and the presence/absence of sleep or cognitive disturbances.

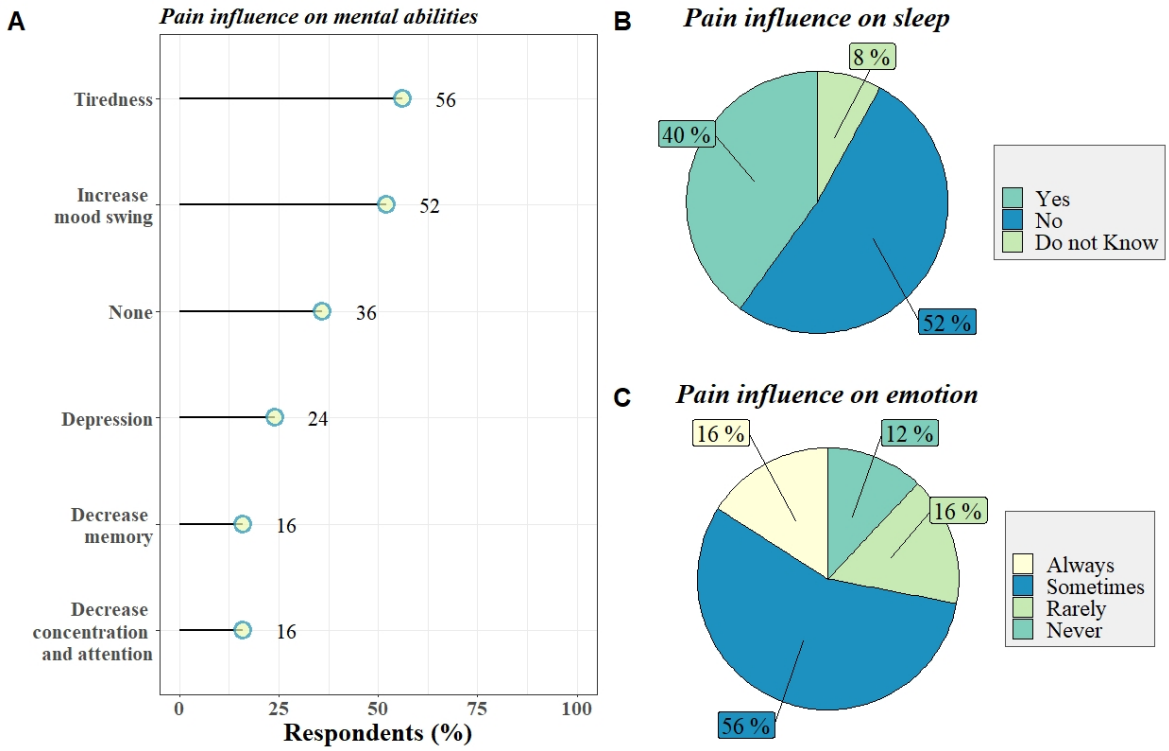
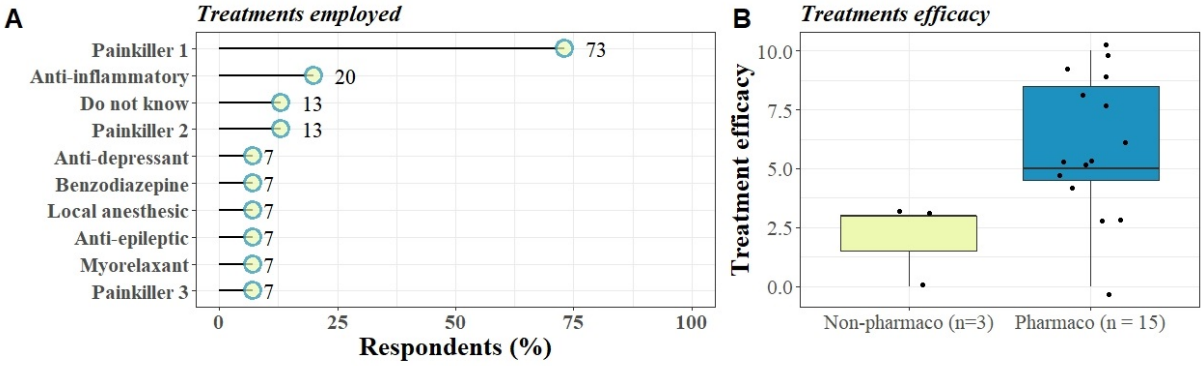


Figure 2.8: Influence of pain on A) mental abilities (multiple choice answers), B) sleep, and C) emotions in LIS patients.

Treatments

Details of the use and efficacy of pharmacological and non-pharmacological treatments are summarised in **Figure 2.9**. Fisher tests were performed to see if there were any relationship between pain intensity (VAS score greater or equal to 5 compare to VAS score lower than 5) and the use of pain treatments (**Supplementary Table S4**). No significant link could be established between pain intensity and the presence/absence of pain treatments.



C *Interest in new treatments*

Variable	Yes	No	No response
Pharmacological n (%)	12 (48%)	7 (28%)	6 (24%)
Non-pharmacological, n (%)	6 (24%)	14 (56%)	5 (20%)

Figure 2.9: Pain treatment in LIS patients. A) Lollipop plot representing the type of pharmacological treatments used by patients to treat pain (based on the WHO classification Ventafridda et al. [1985]). More than half of the patients used at least two types of medication (5/15; 53%) B) Boxplot representing the treatments efficacy according to the participants opinion (0 = not effective, 10 = very efficient) and the type of treatments used (pharmacological vs non-pharmacological). Additional information: Only three out of 25 (12%) participants reported taking non-pharmacological treatments (osteopathy, acupuncture and electromagnetic therapy), among these three, two patients were also using pharmacological treatments to manage pain. C) Table shows the patients' opinion about willing to try new pharmacological and/or non-pharmacological treatments to prevent pain.

2.2.5 Discussion

The results of the survey show that half of the participants had experienced pain during the two weeks before the completion of the questionnaire. The other half of the participants did not report suffering from pain during that time, suggesting that an effective pain management plan was in place. Among the participants who reported pain, 92% suffered from chronic pain. A large proportion of the participants reported that their sleep and cognition/mental abilities were affected by their pain. These two findings confirm our first hypotheses and previous results obtained in former studies Bruno et al. [2011a], Bergés et al. [2007], Skevington [1998]. The second purpose of this observational study was to describe in more detail the characteristics, assessment and management of pain in LIS patients by directly interviewing the persons concerned. Importantly, more than half of the participants did not communicate their pain with clinical staff, which raises questions about the proper detection and management of pain. Regarding the treatments employed, painkiller level 1 (non-opioids) was the most commonly used (73%). Only a minority of participants had tried non-pharmacological treatments such as osteopathy, acupuncture and electromagnetic therapy, yet more than half were willing to try such options.

Past and current pain

Half of the participants interviewed in this study reported pain (49%). The vast majority of the participants reported pain in the lower limbs (84%), followed by headache (28%) and pain in the upper limbs (24%). It can be assumed that the pain in the limbs could be related to the fact that LIS patients remain completely immobile and are unable to move by themselves (71% of the participants were in wheelchairs).

Regarding the type of pain experienced by the participants, the majority occurred after the brain injury therefore being more likely to be a direct result of the patient's condition. Moreover, 92% had chronic pain, 84% reported discrete episodes of pain, including 19% who experienced pain more than once a day. Neuropathic pain is category of chronic pain, but not all chronic pain conditions are neuropathic. In our study, 36% of the respondents had neuropathic pain Bouhassira et al. [2005]. The characteristics of neuropathic pain that stand out the most were sensations of electric shocks (40%), a vice-like pressurized feeling (48%) and decrease of touch sensitivity (80%). Out of the nine participants identified as having neuropathic pain, seven were in LIS following a stroke. Several studies have ventured to develop treatments for post-stroke neuropathic pain, including by employing motor cortex stimulation. However, the results have yet to be replicated in LIS patients Hamani et al. [2021].

Our study also highlights that 52% of participants experiencing pain did not communicate it to others. Of those who communicated their pain, 68% did so using an alphabetical code. Forty-four percent of the participants stated that they expressed their pain through crying. Notably, other means of communication (e.g. wincing) are not always easy to detect or may be confounded with reflexive behavior. Only 28% of the participants used a communication code to communicate their pain. This could be explained by the fact that, in the case of acute pain in particular, using an alphabetical code requires a significant concentration, which can be tiring and discouraging for the patient. The development of new means of communication such as the use of brain computer interface requiring less cognitive effort could be interesting for these patients who do not communicate their pain Annen et al. [2020].

Factors that influence pain

Interestingly, we found a number of factors that can either increase or decrease perceived pain depending on the individual. For example, in some patients, supine and sitting position can increase or decrease pain depending on the patient. This exemplifies the high variability between subjects, pain is subjective and therefore the treatment and management of pain approached on a case-by-case basis. Importantly, 36% of patients reported that tiredness worsened their pain and 40% claimed that pain disrupted their sleep. In this sense, tiredness, sleep quality and pain perception may constitute a positive

feedback cycle of pain-influencing factors. There are several studies that support this proposed bidirectional relationship, for example one study indicated that 50-70% of patients with chronic pain suffer from sleep disorders Rousseau et al. [2015]. Furthermore, patients who suffer from poor sleep quality during the proceeding night have an increase in pain during the following day. Conversely, a significantly painful day has been showed to be followed by subsequent sleep disturbances the following night O'Brien et al. [2011]. It is therefore important to find a balance between the management of these pains and the impact of these such treatments on their levels of arousal and quality of sleep.

Regarding the deleterious effects of pain on mental health, cognitive abilities and emotional regulation, our results are in line with previous surveys. One survey shows that 55% of LIS patients reported experiencing significant anxiety and/or other comorbid mood disorders, including, 13% of LIS patients reporting being depressed Bergés et al. [2007] and 27% reporting experiencing suicidal thoughts Rousseau et al. [2015].

Additionally, previous research has reported that perceived pain and life satisfaction are inversely correlated León-Carrión et al. [2002a], Skevington [1998]. Exploring this, some authors have highlighted a number of variable associated with life satisfaction of LIS patients. These include a lack of mobility concerning recreational activities and those within the community and particularly the non-recovery of speech production Bruno et al. [2011a]. Preserved communication is likely to and significantly improve the quality of life of LIS patients. Indeed, a previous survey associated LIS patients reporting a good quality of life with the ability to produce speech and lower subsequent rates of anxiety Demertzi et al. [2013].

Treatments

Our results show that most LIS patients use pharmacological treatments, in particular level 1 painkillers (73%) to reduce perceived pain. In general, the subjects seem to be moderately satisfied with pharmacological treatments but it is important to point out that, like pain sensitivity, there is a large inter-individual variability. In addition, the treatments may cause several deleterious side effects, such as increased fatigue and cognitive diminution. These can deteriorate their quality of life and impact their diagnoses. Indeed, LIS patients are confronted daily with substantial cognitive and attentional demands since their only means of interaction with the outside world is via blinking, eye movements or residual finger movements. Consequently, the attentional resources required to master the communication technology are significant.

Avoiding the undesirable effects of heavy analgesic medication, may facilitate more efficient communication by allowing the patients to take advantage of all communication tools at their disposal. Therefore,

the improvements in comfort, potentially combined with a reduction in medication, will significantly improve the quality of life of these patients.

In our survey, few participants (12%) tried osteopathy, acupuncture and electromagnetic therapy as complementary/non-pharmacological treatments. Researchers have also demonstrated the effectiveness of physical therapy (combined with other treatments) in the management of LIS patients Akınoğlu [2018]. Surprisingly none of the participants who reported pain had ever tried complementary approaches such as hypnosis, relaxation or meditation. These complementary techniques have yielded positive effects in other patient populations suffering from acute or chronic pain Vanhaudenhuyse et al. [2020]. Neuroimaging studies carried out in meditation experts have shown that a decrease in pain sensitivity was associated with decreases in brain activity in brain regions involved in emotional processing and executive functions, in conjunction with increases of brain activity in regions involved in pain processing. This decrease in the cognitive and emotional control of pain could facilitate the an alteration in the processing of pain as a neutral stimulus rather than an unpleasant one Grant et al. [2011], Gard et al. [2012]. Regarding the use of hypnosis to modulate pain, studies have shown a decrease of brain activation in areas involved in sensory and affective processing of pain during hypnosis Vanhaudenhuyse et al. [2009], Casiglia et al. [2012]. This technique has also been shown to be effective in reducing pain in patients with chronic pain Bicego et al. [2021], Vanhaudenhuyse et al. [2018]. Therefore, it would be relevant to further investigate the potential benefit of hypnosis and meditation in the management of LIS patients. Proposing a global non-pharmacological approach could help reducing pain while preserving patient's level of arousal.

Another important factor in the management of LIS is the speed of finding appropriate treatment. Previous studies confirm that there is a reduction in mortality and improvements in functional recovery in the case of early and intensive rehabilitation (management within approximately 1 month after the morbid event) Bruno et al. [2011a], Casanova et al. [2003].

Limitations and future directions

Our study has several caveats that limit the generalisability of our results. Firstly, the sample size is small (n=51 in total but only 25 who completed the entire questionnaire). It would be interesting to follow-up with a larger representative sample. Unfortunately, as LIS is a rare pathology gathering a large sample is remains a challenging consideration for future studies. Nevertheless, the vast majority of studies in the literature on LIS patients are case studies and published group studies are approximately the same size as our sample size Bruno et al. [2011a], Branco et al. [2021], Rousseau et al. [2013], Khalili-Ardali et al. [2021], Svernling et al., Corallo et al. [2017], Leonard et al. [2019], Lugo et al. [2015]. The response rate however was low (17%) but this should be put into perspective with the context in which the study was carried out and the type of population targeted. Indeed, we do not know how many participants did

actually read the email; some LIS patients may not have had the tools and help to fill the questionnaire; ALIS sends several questionnaires per year, and this can become discouraging for the LIS patients because it requires time and effort. However, to our knowledge, this is the first survey that directly addresses LIS patients. Secondly, only patients who had pain in the two weeks prior the completion of the survey were included. Longitudinal data are required to see if there is a correlation between the presence of these painful sensations and the diagnosis of these patients (i.e., a patient whose attention is diminished because of pain could be misdiagnosed as UWS). Thirdly, some questions lack precision, especially regarding the type of care, the influence of temperature or the dosage of pain treatments. In particular, it would have been interesting to know the dose of pain treatments, since it can impact the diagnosis of the patient, their sleep quality and cognitive abilities. Indeed, the use of a too high dose of painkillers can lead to an increase in tiredness and thus a decrease in the level of arousal and communication abilities. Interventional studies testing the effects of specific painkillers should be conducted. Additionally, future studies should use alternative response format (e.g., Likert scales) to collect more detailed data than binary format (e.g., yes/no).

Conclusion

This survey is one of the first studies that provides an overview of pain and its management in LIS by directly interviewing patients. This study highlights that (1) half of LIS patients experience pain, in particular chronic pain. (2) LIS patients do not communicate often about their pain perception, which does not facilitate good management. This means that caregivers must be vigilant about detecting signs of pain. The systematic implementation by clinical teams of a specific communication code for pain could help detect these signs more quickly. (3) Pain in LIS patients influences quality of life and more specifically sleep and emotion. This will have an impact on patients' diagnosis by affecting their level of concentration and motivation. (4) Pharmacological treatments are still the mainstay of pain management for these patients. Such pharmacological treatments can lead to various side effects, including drowsiness, increased fatigue, and cognitive slowing. An exciting other option to reduce pain and avert these side effects would be to use non-pharmacological treatments, but this requires further investigation in future studies.

Chapter 3

How to reduce potential sources of pain?

Based on the following publications:

Bonin, E.A.C., Fossati, M.L.B., Chatelle, C., Martens, G., Martial, C., Briand, M-M., Bejor, M., Laureys, S., Thibaut, A. (2021) Pain and spastic features in chronic DoC patient: A cross-sectional retrospective study. Annals of Physical and Rehabilitation Medicine journal. https://doi.org/10.1016/j.rehab.2021.101566

Bonin, E.A.C., Fossati, M.L.B., Filippini, M-M., Bornheim, S., Lejeune, N., O'Brien , A.T., Bodart, O., Laureys, S., Thibaut, A., Chatelle, C. (2021) Evaluation of the effect of analgesic treatment on signs of nociception-related behaviors during physiotherapy in patients with disorders of consciousness: a pilot crossover randomized controlled trial. PAIN, https://doi.org/10.1097/j.pain.0000000000002367

3.1 Study 3: Pain and spastic features in chronic DoC patients: A cross-sectional retrospective study

3.1.1 Introduction

Based on clinical and neuroimaging data, it is now widely admitted that post-comatose patients who remain in minimally conscious state (MCS; consistent but fluctuant signs of consciousness) or patients diagnosed in vegetative state/unresponsive wakefulness syndrome (VS/UWS; awake but no behavioral sign of consciousness) but with atypical brain activity may retain the ability to perceive pain Gosseries et al. [2014]. Therefore, appropriate monitoring and analgesic treatment should be provided. A positive correlation has been reported between the level of spastic muscle overactivity (SMO), as measured by the Modified Ashworth Scale (MAS), and signs of pain, as assessed by the Nociception Coma Scale-Revised (NCS-R), in this population Thibaut et al. [2015a]. The immobility and the lack of voluntary movement in this population could elicit contracture that could lead to a more difficult bedside evaluation of the SMO and induce pain, which can reduce the patients' motor abilities, especially during physical therapy. Consequently, the management of SMO is crucial in order to avoid pain and to enable the physical therapy in this population. It could also allow the patient to show signs of consciousness and thereby prevent diagnostic errors Martens et al. [2019].

In this cross-sectional retrospective study, we aim to evaluate the correlation between pain and SMO for each individual joint, thereby allowing a better characterization of spastic profiles that are more prone to induce pain. Furthermore, we explore the correlation between SMO in the upper and lower limbs and age, time since injury and diagnosis, pain scores during mobilization, tendon and joint retractions and equinovarus foot, to identify factors influencing SMO severity.

3.1.2 Materials and methods

The study followed STROBE guidelines and was approved by the Ethics Committee of the Hospital-Faculty Ethics committee of the University of Liege and written informed consent was obtained by the healthy subjects and the patients' legal representatives. Patients included in the study were diagnosed as being in VS/UWS or in MCS (both considered a disorder of consciousness – DoC –) based on a minimum of five assessments using the Coma Recovery Scale-Revised (CRS-R; Giacino et al. [2004]). SMO and pain were measured with the MAS (during mobilization) and the NCS-R (at rest, during a nociceptive stimulation and during mobilization), respectively. The MAS score was assessed for each segment of each limb (i.e., fingers, wrists and elbows for the upper limbs, hips, knees and ankles for the lower limbs). Then, the mean score for each limb was used for the analyzes. Spearman correlation was used to

study the correlation between pain and SMO during mobilization. Multiple univariate and multivariate linear regression analyzes were performed to identify the relationship between SMO and demographic and clinical factors (i.e., NCS-R, tendon/joint retraction, equinovarus foot, age, time since injury, etiology, CRS-R diagnosis and gender).

3.1.3 Results

We included 73 chronic DoC patients (27 women; mean age: 40 (13) years; 50 MCS, 23 VS/UWS; 42 traumatic etiology; time since injury: 39 (39) months; see **Table 3.1** for details). Seventy out of 73 patients (96%) showed signs of SMO on a least one muscular group (**Figure 3.1**). We found a positive correlation between NCS-R scores during mobilization and MAS scores for the wrist ($p = 0.0491$) and fingers' flexors ($p=0.0240$) and hip adductors ($p = 0.0196$), while no significant correlation was found for elbow flexors, knee flexors and ankle plantarflexors. There was a positive correlation between SMO in the upper and lower limbs and tendon/joint retractions ($p<0.001$ and $p=0.009$, respectively) and between SMO of ankle plantarflexors and equinovarus foot ($p < 0.001$), while there was no correlation between SMO and etiology or diagnosis. Etiology and CRS-R diagnosis did not influence NCS-R scores either. We found a positive correlation between time since injury and maximum SMO in the upper limbs ($p = 0.0005$) or lower limbs ($p = 0.0010$), tendon/joint retractions ($p = 0.03$) and equinovarus foot ($p = 0.001$). There was no significant correlation between time since injury and NCS-R scores. A younger age was associated with higher SMO in the lower ($p < 0.001$) but not the upper limbs ($p = 0.0620$), while there were no differences between genders. In the first multivariate model, there was a significant effect of longer time since injury on maximum MAS scores in the upper limbs (adj $R^2 = 0.0721$); when adding age, we observed an improvement of the model (adj $R^2 = 0.1482$), likewise when adding NCS-R scores (adj $R^2 = 0.2155$) and tendon/joint retractions (adj $R^2 = 0.4566$). In the second multivariate model on lower limbs, there was a significant effect of time since injury on maximum MAS scores in the lower limbs (adj $R^2 = 0.0623$); when adding age, we observed an improvement of the model (adj $R^2 = 0.1696$). Details regarding the statistical analyzes can be found in **Supplementary material D**.

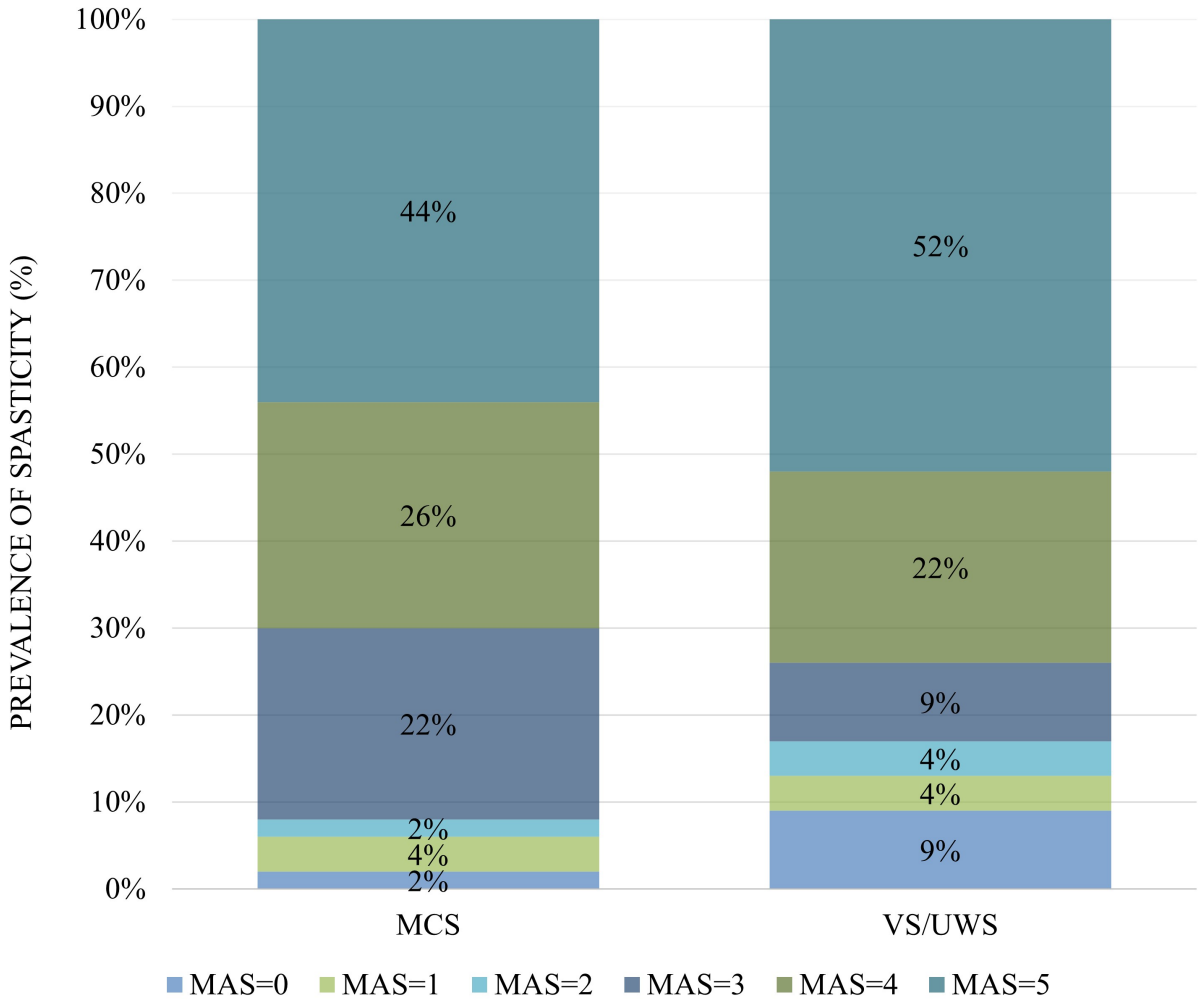


Figure 3.1: Prevalence of spastic muscle overactivity (maximum MAS score for each patient). MAS= Modified Ashworth Scale, MCS= Minimally Conscious State, UWS/VS = Unresponsive Wakefulness Syndrome/Vegetative State.

Table 3.1: Demographic and clinical data of the patients. IQR = Interquartile Range; MAS = Modified Ashworth Scale; MCS = Minimally Conscious State; UWS/VS = Unresponsive Wakefulness Syndrome/Vegetative State; SD = Standard Deviation.

Data	VS/UWS (n=23)	MCS (n=50)	All
Mean age (SD)	37 (13)	41 (17)	40 (15)
Gender (males%)	70 (n=16/23)	60 (n=30/50)	63 (n=46/73)
Etiology (traumatic brain injury%)	48 (n=11/23)	62 (n=31/50)	58 (n=42/73)
Mean Time Since Injury (SD) (months)	39 (41)	39 (39)	39 (39)
Median Spasticity Elbow Flexors (IQR1;3) (MAS total score)	4 (2;4)	3 (2;4)	3 (2;4)
Median Spasticity Wrist Flexors (IQR1;3)	4 (2;4)	3 (2;4)	3 (2;4)
Median Spasticity Fingers' Flexors (IQR1;3)	3 (2;4)	3 (2;4)	3 (2;4)
Median Spasticity Hip Adductors (IQR1;3)	2 (1;4)	2 (1;3)	2 (1;3)
Median Spasticity Knee Flexors (IQR1;3)	2 (1;4)	3 (2;4)	3 (1;4)
Median Spasticity Dorsiflexors (IQR1;3)	3 (0;5)	3 (1;5)	3 (1;5)
Median NCS-R (IQR1;3)	3 (2;3)	3 (2;4)	3 (2;4)

3.1.4 Discussion

The aim of this study was to identify spastic features more likely to induce pain and to analyze the relationship between SMO and pain as well as between SMO and demographic and clinical factors. We confirmed that the prevalence of SMO in DoC patients is extremely high and that there is a correlation between SMO and pain as previously reported Thibaut et al. [2015a]. Here we highlight again the importance of tackling SMO by evaluating it in the early stage and regularly afterwards while providing treatment as soon as possible when detected.

Our regression models highlighted several factors linked to SMO. We found that the muscle's groups more prone to induce pain were wrist and fingers' flexors and hip adductors. In regard to this finding, for the wrist and fingers' flexors, it could be recommended to use soft splints daily, since they are well tolerated and have shown promising effects on reducing hand SMO and do not require supervision Thibaut et al. [2015b]. It is difficult to find a similar strategy for the SMO of the hip adductors, as available treatments are based on passive mobilizations and positioning Elliott and Walker [2005]. As previously observed, we identified a correlation between SMO in the lower limbs and equinovarus foot Martens et al. [2019]. As in

other populations (e.g., stroke patients), the MAS scores for lower limb tend to increase over time in DoC patients, probably because of ongoing nervous system reorganization as well as increasing muscle stiffness Baude et al. [2019]. This could also be an argument to introduce splints and optimal positioning as soon as possible in DoC patients daily care. Focal injections of botulinum toxin, or the use of baclofen could also be used to treat SMO. Moreover, these treatments could lead to a decrease in signs of pain and an increase in the level of consciousness Marque et al. [2019]. However, in contrast to studies in post-stroke patients, the evidence for the efficacy of these antispastic treatments is still limited in post-traumatic patients Laxe [2019]. In addition, age was a determinant factor, with younger patients being more prone to be spastic. The reason could be that muscle force generation from tendon reflexes is slower and weaker with increasing age; this also is the case for tonic reflexes associated with SMO, spastic responses may be weaker in older patients Opheim et al. [2015]. On the other hand, we did not find any correlation between SMO and etiology (traumatic brain injury or not) or CRS-R diagnosis (MCS versus VS/UWS), which is in line with the results obtained in previous studies Thibaut et al. [2015a]. This is probably due to the extended brain lesions of DoC patients, preventing us to identify specific patterns in the onset of SMO. Our study showed that the most frequent posture observed across etiology involved the upper limbs (i.e. wrists and fingers), which is in line with the results of a recent study that investigated postural pattern in post- traumatic and post-stroke patients Doussoulin et al. [2020]. In other conditions, such as stroke, the prevalence of SMO is relatively high, arising in about 30% of patients Thibaut et al. [2013]. Contracture is even more frequent in patients with DoC than in patients with milder brain injury, probably due to more extensive brain lesions, prolonged immobility, weakness, disuse and absence of movements of muscles in contracted positions Martens et al. [2019]. The association of immobilization and SMO could cause adaptive anatomical muscles changes and reflexes modifications (e.g., muscle atrophy, loss of sarcomeres and accumulation of connective tissue and fat) constituting a self-reinforcing negative effect Baude et al. [2019]. In addition, patients with post-stroke SMO may suffer from higher pain levels than post-stroke patients who have not developed SMO, stressing the link between pain and SMO Thibaut et al. [2013] and the importance of taking these two factors into consideration in rehabilitation programs and in long-term care.

This study has some limitations: (1) patients were assessed once for SMO and pain, but we know it may fluctuate over time; (2) as the MAS was used (measures the resistance to high velocity passive movement), the velocity of passive joint movement, the angle of contraction outbreak or potential tendon retraction were not assessed; (3) the population was heterogeneous (e.g., different etiologies, time since injury). Future longitudinal studies should assess SMO and pain several times in a more homogeneous population and acquire additional data in patients with DoC in order to classify patients according to their specific etiology, brain lesion, rehabilitation and time since insult.

In this study, we found a high prevalence of SMO and pain during mobilization in DoC patients. This highlights the importance of preventing SMO and of a proper and careful assessment of pain, to obtain the best rehabilitative outcome in the most comfortable conditions for the patient.

3.2 Study 4: Evaluation of the effect of analgesic treatment on signs of nociception-related behaviors during physical therapy in patients with DoC: a pilot crossover randomized controlled trial

3.2.1 Summary

Neuro-orthopedics disorders are common in patients with DoC and can lead to potential pain. However, the patients' inability to communicate makes pain detection and management very challenging for clinicians. In this crossover randomized double-blind placebo-controlled study, we investigated the effects of an analgesic treatment on the presence of nociception-related behaviors.

At baseline, the NCS-R was performed in three conditions: a non-noxious stimulation, a noxious stimulation and during a physical therapy session. Patients with a NCS-R total score during physical therapy equal or above the score observed following the noxious stimulation could participate to the clinical trial, as well as patients with a score above 5. They received an analgesic treatment and a placebo on two consecutive days in a randomized order followed by an assessment with the NCS-R.

Out of 18 patients, 15 displayed signs of potential pain during physical therapy. Patients showed higher NCS-R scores during physical therapy compared to the other conditions, suggesting that mobilizations were potentially painful. Out of these 15 patients, 10 met the criteria to participate to the placebo-controlled trial. We did not find any effect of analgesic treatment on the NCS-R scores.

This study highlights that physical therapy may be potentially painful for DoC patients, while analgesic treatments did not reduced NCS-R scores. Therefore, careful monitoring with appropriate assessment and treatment before and during mobilization should become a priority in clinical settings. Future studies should focus on the development of assessment tools sensitive to analgesic dosage to manage pain in DOC.

Keywords: Consciousness disorders, Pain management, Nociception Coma Scale-Revised, Physical therapy, Minimally conscious state, Unresponsive wakefulness syndrome/vegetative state, Randomized controlled trial.

3.2.2 Introduction

Nociception is the neural process of encoding noxious stimuli. It includes physiological and behavioral aspects and does not require an access to consciousness **?**. *Pain* is defined as "an unpleasant sensory and emotional experience associated with potential or actual tissue damage" Task Force on Taxonomy of the International Association for the Study of Pain (IASP) [1994]. Pain is therefore subjective and requires conscious processes. Severely brain-injured patients can suffer from disorders of consciousness (DoC) such as unresponsive wakefulness syndrome (UWS; eye-opening periods and reflexive responses to stimuli Posner et al. [2007], Riganello et al. [2012], Schnakers et al. [2010], Schnakers and Zasler [2015], Seel et al. [2010], The Multi-Society Task Force on PVS [1994]), minimally conscious state (MCS; reproducible but fluctuating signs of consciousness without communication Giacino et al. [2002]) and emergence from the MCS (EMCS; functional communication/use of objects Seel et al. [2010]). These patients are unable or may have severe difficulties to reliably communicate and express their pain experience Giacino et al. [2002, 2004], Gosseries et al. [2014], Laureys et al. [2002]. Nevertheless, the IASP specified that "the inability to communicate verbally does not negate the possibility that an individual is experiencing pain" Task Force on Taxonomy of the International Association for the Study of Pain (IASP) [1994]. Sources of pain can be heterogeneous and vary depending on the patient's condition. For instance, in the acute setting, pain can be a consequence of fractures, soft tissue injuries, and inserted tubes. On the other hand, during the subacute and chronic phases, pain is more likely to be caused by spasticity, myostatic contracture, arthrosis or dystonia Schnakers and Zasler [2015]. Therefore, pain and nociception management in this population is a clinical challenge and an ethical responsibility. The Nociception Coma Scale revised (NCS-R) was developed to assess pain and nociception in DoC patients Schnakers et al. [2010]. This behavioral scale comprises three subscales (motor, verbal and facial expressions) respectively scoring from 0 (no response) to 3 (highest response level) with a maximal total score of 9. Several studies reported its good concurrent validity as well as sensitivity to nociception and level of consciousness Bagnato et al. [2018], Chatelle et al. [2018a, 2012]. The clinical value of the NCS-R in pain management for DoC patients was evaluated in a study conducted in the acute setting (i.e., intensive care unit). The results suggested that the NCS-R seems to be an appropriate tool to monitor analgesic administration and ensure that the provided analgesic treatment reduces signs of pain without influencing the level of consciousness Chatelle et al. [2016]. However, there is currently no reliable guideline that has been defined regarding its use in a clinical setting Chatelle et al. [2018a]. Moreover, these studies showed some limitations regarding the analgesic administration (i.e., lack of blinding and absence of placebo Chatelle et al. [2016, 2018a]).

In this study, we first aim to investigate whether mobilizations (i.e., physical therapy – PT) are associated with increased signs of nociception-related behaviors in DoC patients, as measured with the NCS-R. We expected that mobilizations would not induce as much of these signs as a noxious stimu-

lation. However, if patients display NCS-R scores during PT equal or higher than following a noxious stimulation, this may suggest that PT could potentially be painful. In a second phase, we wanted to assess the sensitivity of the NCS-R to analgesics treatment in the context of a potentially painful condition in a clinical setting by conducting a crossover randomized double-blind placebo-controlled study. We hypothesized that analgesic treatment administration would induce a decrease in the NCS-R scores during both PT and noxious stimulation.

3.2.3 Materials and methods

Standard protocol approvals, registrations, and patient consents

The study was registered (NCT04330547) and approved by the Ethics Committee of the Hospital-Faculty Ethics committee of the University of Liege and written informed consent was obtained by the patients' legal representatives in accordance with the Declaration of Helsinki.

Study design

This study is a two arms, two periods crossover randomized double-blind placebo-controlled trial.

Participants and eligibility criteria

In this crossover randomized double-blind placebo-controlled study, patients were assessed as part of a week of diagnostic and prognostic assessment. Inclusion criteria were: (1) age $\geq$ 16 years; (2) $>$ 28 days post-injury; (3) no administration of neuromuscular function blockers and no sedation 24h before assessment; (4) a diagnosis of UWS, MCS, or EMCS based on the behavioral assessment performed using the Coma Recovery Scale-Revised (CRS-R Giacino et al. [2004]). Exclusion criteria were: (1) documented history of prior brain injury; (2) premorbid history of developmental, psychiatric, or neurologic illness resulting in documented functional disability up to time of the injury; and (3) upper limb contusions, fractures, or flaccid paralysis.

Note that medication was documented to control for drug effect on the central nervous system (**Appendix C**).

Randomization and blinding

Each patient included in the clinical trial received once the placebo and once the analgesic treatment, in a randomized order. The medical doctor in charge of the randomization provided to the nurse the type and the dose of the analgesic treatment or the placebo to administrate (see section on Clinical trial for more details). Investigators and patients were blinded to the treatment allocation.

Procedures

The study was performed within three days and was divided into two phases (baseline assessments at T0 and the clinical trial at T1 and T2).

Baseline (T0)

The level of consciousness was assessed using the CRS-R. This scale is the gold standard for the behavioral assessment of the level of consciousness and is widely used in patients with prolonged DoC Giacino et al. [2004]. It is composed of six subscales that assess the following domains: visual, motor, auditory, and oro-motor/verbal functions, as well as communication, and arousal. The score ranges from 0 to 23, in which the lowest score indicates coma and the highest score indicates the emergence from the minimally conscious state. The diagnosis is made according to the presence/absence of particular behavioral responses Gosseries et al. [2014]. Then, the Modified Ashworth Scale (MAS; Bohannon et al. [1987]) was used to assess spasticity (i.e., reflex contraction in response to passive stretching) at the main joints of upper and lower limbs. This scale ranges from 0 (no increase in muscle tone) to 4 (very strong increase in tone, stiffness). Spastic hypertonia, by definition, depends on the speed of execution of the movement (the faster the limb is mobilized, the more hypertonia increases). The evaluation should be performed at least three times for each joint. After that, patients' pain responsiveness was assessed using the NCS-R during a tactile stimulation (i.e., five taps on the dorsal part of the hand), during a noxious stimulation (i.e., deep pressure on the nail bed of the left and right middle fingers for five seconds Schnakers et al. [2010]). Then, the presence of potential pain was assessed with the NCS-R during 15 minutes of passive mobilizations of the upper and lower limbs performed by always the same trained physical therapist (i.e., physical therapy; PT **?**).The physical therapist (i.e., SB; see co-authors) stopped the movement when the articulation could no longer be mobilized, and maintained that posture for a certain time (i.e., between 30 and 90 sec depending on the state of the stretched muscle and the patient's discomfort).

If the NCS-R score observed during PT was higher or equal to the one observed during a noxious stimulation, or if it was higher or equal to the cut-off score of 5 defined in a previous study Bonin et al. [2020b], the patient was eligible for the second phase of the study, the clinical trial.

Clinical trial (T1 and T2)

This randomized double-blind placebo-controlled clinical trial was performed on the subset of patients identified on T0. Analgesic and placebo treatments were administered 24 hours apart (T1 and T2) by a nurse, at least a half-hour before each assessment. The placebo administered was Folavit® capsules (folic acid, 0,4mg). The nature and dose of the analgesic administered were chosen according to the patient's

needs, based on the World Health Organization guidelines (i.e., non-opioid as level 1, weak as level 2, and strong opioid as level 3 Ventafridda et al. [1985]):

1. If the patients had no analgesic treatment, they received a non-opioid analgesic (level 1).
2. If the patient was already receiving pain medications, we added the lowest effective dose of the level above the level of the regular pain medications of the patient:
    - If they already had a level 1-drug, they received a level 2-drug.
    - If they already had a level 2-drug, they received a level 3-drug.
    - If the patient was already a level 3-drug, we increased the dosage by steps (reference: 5mg oxycodone as first choice for level 3-drugs, also see **Appendix C.2** lists of the suggested medications (from first to last choice by category)).

The medication was administered by oral intake or gastrostomy feeding tube, according to the habits of the patient regarding his/her usual medication intake.

The same assessments were performed as described in T0 for T1 and T2 (i.e., NCS-R during tactile, noxious, and PT, CRS-R, and spasticity assessment).

Statistical analyzes

As our data (i.e., NCS-R total scores as well as the CRS-R total scores) were ordinal and our sample was rather small ($n < 30$), we used non-parametric tests for analyzes.

Baseline: a Kruskal-Wallis test was performed to test baseline differences (T0) between the three stimulations (i.e., tactile, noxious, PT). Then, a Dunn–test was performed as a post-hoc analysis to investigate differences in NCS-R scores between each stimulation.

Clinical trial: we investigated differences in NCS-R scores and sub-scores between each stimulation (i.e., tactile, noxious, PT) and each condition (i.e., placebo and treatment) using Kruskal-Wallis and Dunn-tests (post hoc analysis). Finally, we used Kruskal-Wallis and Dunn tests to investigate the effect of treatment and placebo administration on the level of consciousness (CRS-R scores).

Analyzes were performed using R studio (version 4.0.2) and were considered significant at $p < 0.05$. For each of the Dunn-tests, p-values were adjusted using the Benjamini-Hochberg (BH) method.

3.2.4 Results

Baseline (T0)

From February 2018 to February 2019, 18 patients were included in the study (8 women; age 44 ± 15 years), of which two patients were UWS/VS, 14 were MCS and two were EMCS according to the CRS-R assessment (see **Figure 3.2** for the flow diagram). Etiology was traumatic (n = 8), ruptured aneurysm (n=6) and post-anoxic encephalopathy (n=5) (see **Table 3.2.4** for demographic and clinical data).

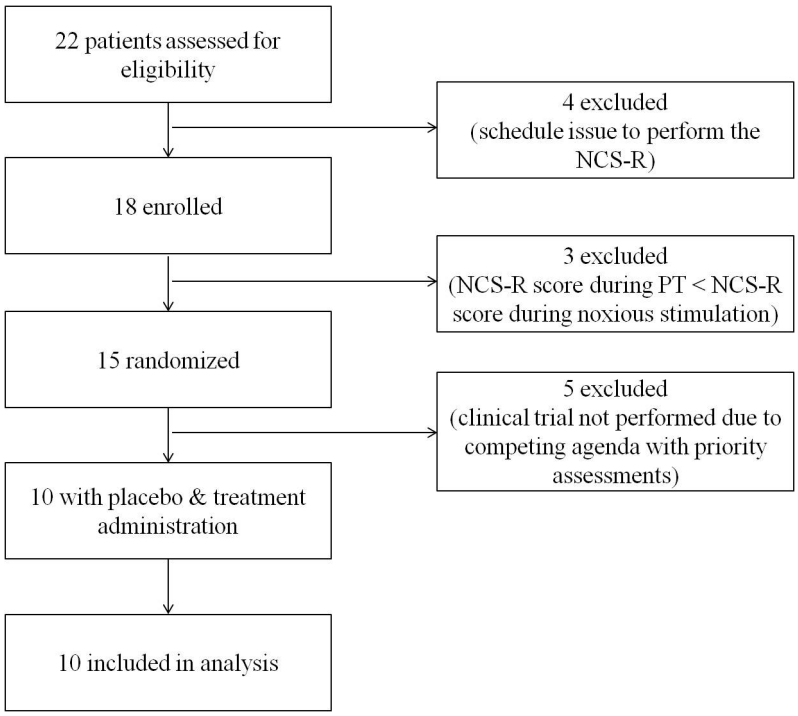


Figure 3.2: CONSORT Flow diagram. (NCS-R = Nociception Coma Scale-Revised, PT = physical therapy).

During baseline, 15/18 showed signs of potential pain (i.e., NCS-R $\geq$ 5) during PT (15/18, 83.3%). Given the initial population, most of the patients with potential pain were MCS (13/15, 86.7%), only one UWS (1/15, 6.6%), and one EMCS (1/15, 6.6%). Out of these 15 patients, 11 had a NCS-R total score during PT higher or equal to the one observed following a noxious stimulation (11/15, 73.3%) and four showed a NCS-R total score during PT higher or equal to the cut-off score of 5 (4/15, 26.6%) Bonin et al. [2020b]. All the patients that were considered as potentially painful were spastic and 13 of them (13/15, 86.7%) were severely spastic with a MAS score higher or equal to 3 (i.e., corresponding to a considerable increase in tone with difficulty to perform passive movement 13/15, 86.7%) for one or more of the tested joints (see **Appendix C** for more details on the MAS scores). Out of the 18 patients included in the study, 8 of them received an anti-spastic treatment (i.e., baclofen per os (n=7) or intrathecal (n=1); see **Table 3.2.4**). Finally, two-thirds of these patients were not under any analgesic treatment before their inclusion in the study (10/15, 66.6%).

Table 3.2: Demographics of patients included in the study. (LoC = Level of Consciousness, EMCS = Emergence from the Minimally Conscious State, F = Female, MCS = Minimally Conscious State, M = Male, UWS/VS = Unresponsive Wakefulness syndrome/Vegetative State, na = not applicable).

									Analgesic treatment			**Antipastic treatment**	
ID	Sex	Age	Etiology	LoC	Times since injury (d)	Tracheotomy	Inclusion phase 2	Reason of exclusion	Before the study	During the study	Dosage (per os)	Nature and administration mode	Dosage (per os)
1	M	23	Traumatic	UWS/VS	97	Yes	Yes	na	na	Paracetamol	1g	No	na
2	M	73	Postanoxia encephalopathy	MCS	645	No	No	NCS-R score during PT < NCS-R score during noxious stimulation	na	na	na	Lioresal (oral)	25 mg, 3 per day
3	M	37	Traumatic and hypoxia	MCS	2923	Yes	Yes	na	na	Paracetamol	1g	No	na
4	F	71	Ruptured aneurysm	MCS	412	Yes	No	Competing agenda with priority assessments	na	na	na	No	na
5	F	45	Ruptured aneurysm	MCS	1340	No	Yes	na	na	Paracetamol	1g	Lioresal (oral)	25 mg, 3 per day
6	F	50	Ruptured aneurysm	MCS	93	No	Yes	na	Fentanyl and paracetamol	Oxycodone	10 mg	Lioresal (oral)	25 mg 3 per day

									Analgesic treatment			Antipastic treatment	
ID	Sex	Age	Etiology	LoC	Times since injury (d)	Tracheotomy	Inclusion phase 2	Reason of exclusion	Before the study	During the study	Dosage (per os)	Nature and administration mode	Dosage (per os)
7	M	40	Traumatic	MCS	766	No	Yes	na	Paracetamol and tramadol	Tramadol	100 mg	Lioresal (oral)	25 mg, 3 per day
8	M	60	Postanoxia encephalopathy	MCS	341	Yes	Yes	na	na	Paracetamol	1g	No	na
9	M	30	Traumatic	EMCS	2412	No	Yes	na	Tramadol	Tramadol	50 mg	No	na
10	M	63	Traumatic	MCS	169	Yes	Yes	na	na	Paracetamol	1g	No	na
11	F	47	Ruptured aneurysm	MCS	124	Yes	Yes	na	Paracetamol	Tramadol	50 mg	No	na
12	F	33	Postanoxia encephalopathy	EMCS	7363	No	No	EMCS not assess with the NCS-R	Paracetamol	na	na	Lioresal (intrathecal)	na
13	M	34	Traumatic	MCS	165	Yes	No	Competing agenda with priority assessments	na	na	na	No	na

									Analgesic treatment			Antipastic treatment	
ID	Sex	Age	Etiology	LoC	Times since injury (d)	Tracheotomy	Inclusion phase 2	Reason of exclusion	Before the study	During the study	Dosage (per os)	Nature and administration mode	Dosage (per os)
14	F	40	Ruptured aneurysm	MCS	1290	No	No	Competing agenda with priority assessments	na	na	na	Lioresal (oral)	10 mg 3x2 per day
15	M	23	Traumatic	MCS	125	Yes	No	Competing agenda with priority assessments	na	na	na	No	na
16	M	52	Postanoxia encephalopathy	MCS	337	Yes	Yes	na	Morphine and midazolam	Oxycodone	5 mg	No	na
17	F	27	Traumatic	UWS/VS	765	No	No	NCS-R score during PT < NCS-R score during noxious stimulation	na	na	na	Lioresal (oral)	na
18	F	39	Ruptured aneurysm	MCS	239	No	No	Competing agenda with priority assessments	na	na	na	Lioresal (oral)	15 mg 1x3 per day

The influence of PT on NCS-R total scores was assessed with a Kruskal-Wallis test performed on the 18 patients included at baseline (T0). A difference in the NCS-R total scores was observed between the type of stimulation (i.e., tactile, noxious, PT; $\chi^2 = 32.11$, $p < 0.0001$). Additional analyzes using Dunn-tests corrected with BH method showed a difference (see **Figure 3.3**) in the NCS-R scores between PT and tactile stimulation (Z score = 5.55, p-value adjusted < 0.0001) as well as between tactile and noxious stimulation (Z score = 3.74, p-value adjusted < 0.001). However, these additional analyzes did not allow us to found a significant difference in the NCS-R scores between PT and noxious stimulation.

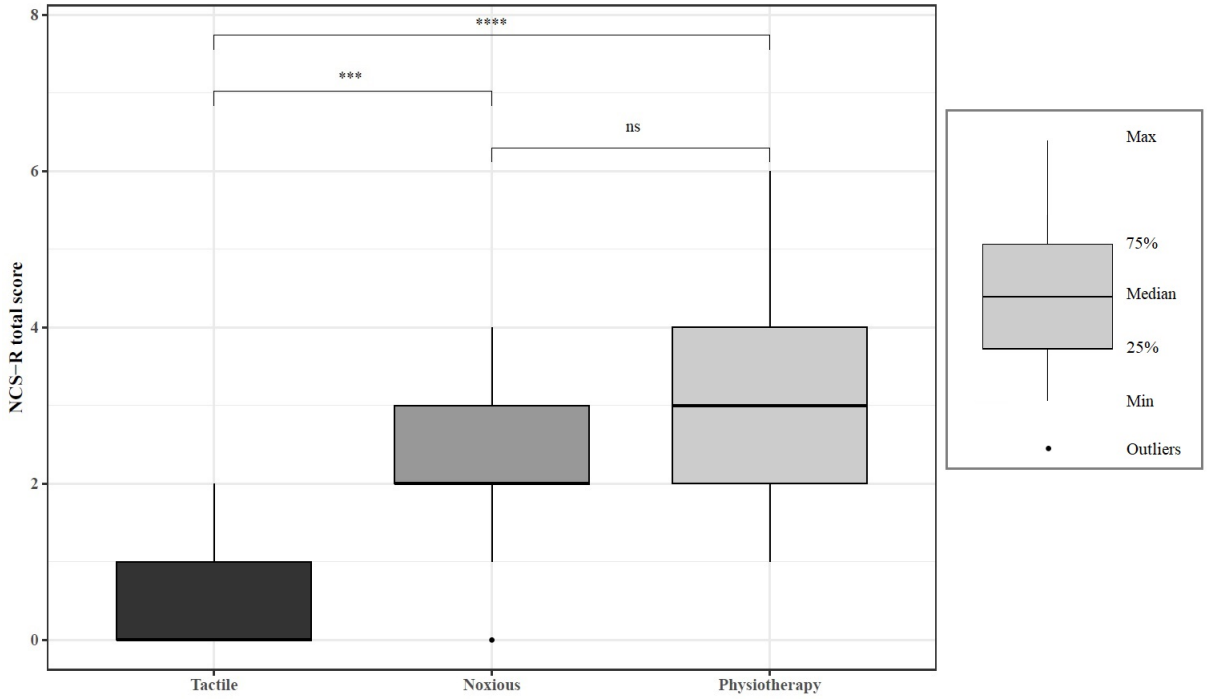


Figure 3.3: Demographic table of patients included in the study. (F = Female, M = Male, UWS = Unresponsive Wakefulness Syndrome, MCS = Minimally Conscious State, EMCS = Emergence from the Minimally Conscious State, ns = no significance, *** = p <0 .001, **** = p <0 .0001).

Clinical trial (T1 and T2)

Ten patients were included in the clinical trial (3 women, 5 TBI, 44.7 ± 12.6 years). Out of the 15 patients identified as potentially painful during baseline, five were not included in the clinical trial due to schedule issues (i.e., the clinical trial could not be scheduled due to competing agenda with priority assessments).

For both conditions (i.e., placebo and treatment), we found a difference in NCS-R total scores between the type of stimulation (placebo: $\chi^2 = 14.01$, $p < 0.001$; treatment: $\chi^2 = 10.31$, $p < 0.01$; **Figure 3.4**). During the placebo condition, additional analyzes using Dunn-tests corrected with BH method showed a difference in the NCS-R total scores between PT and tactile stimulation (Z score = 3.74, p-value adjusted < 0.001). During the treatment condition, we also found a difference between PT and tactile stimulation (Z score = 3.20, p-value adjusted < 0.01).

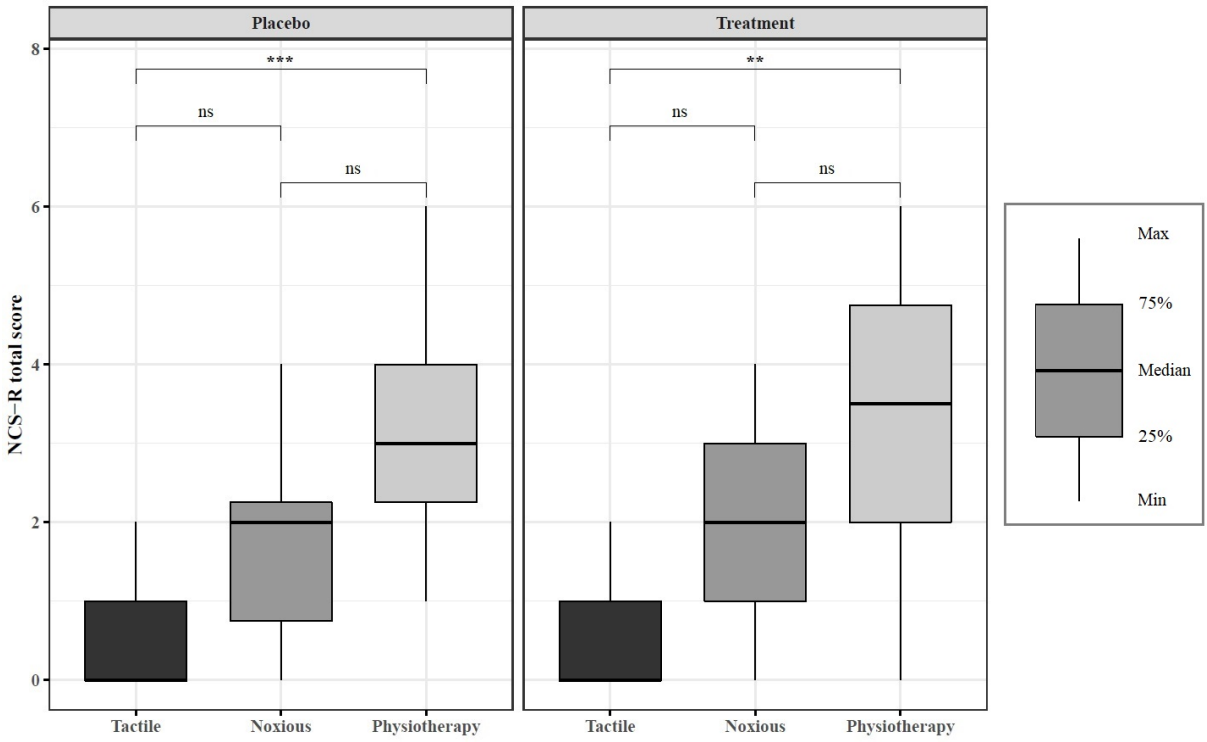


Figure 3.4: Changes in the NCS-R total score following tactile, noxious stimulation and physical therapy during the clinical trial (T1 and T2 n=10) after the placebo/treatment administration (NCS-R = Nociception Coma Scale-Revised, ns = no significance, ** = $p < 0.01$, *** = $p < 0.001$).

Moreover, regarding the NCS-R subscales (i.e., motor, verbal, facial expression), we found a difference on the facial expression NCS-R subscores for both condition (placebo: $\chi^2 = 10.87$, $p < 0.01$; treatment: $\chi^2 = 7.65$, $p < 0.05$). Dunn-tests corrected with BH method showed a difference on the NCS-R facial expression subscores between PT and tactile stimulation for both condition (placebo: Z score = 3.28, p-value adjusted < 0.01; treatment: Z score = 2.76, p-value adjusted < 0.05; see **Supplementary Figure C.1**). We did not find differences between conditions on the motor and verbal NCS-R subscores. Statistical analyzes used to investigate the effect of treatment administration on the NCS-R total scores during noxious stimulation and PT did not show any significant difference between each condition (i.e., placebo, treatment; see **Supplementary Figure C.2**).

At the single-subject level, a decrease of the NCS-R total score after the treatment administration was observed in two patients during PT. Four patients showed a higher NCS-R total score after treatment administration than after placebo administration (see **Supplementary Table C**). Kruskal-Wallis analysis did not show any effect of treatment administration on the level of consciousness (i.e., CRS-R total scores). At the single-subject level, no changes were observed in terms of diagnosis between placebo and treatment condition.

3.2.5 Discussion

The aim of this study was first to investigate the influence of PT on signs of potential pain in DoC patients, and then to determine the effects of an analgesic treatment on those signs using a double-blind randomized placebo-controlled study. We can highlight two main findings: (1) PT could be potentially painful for DoC patients. Patients displayed higher NCS-R total scores during PT than during tactile stimulation and we did not observe differences between NCS-R total scores during PT vs. noxious stimulation. Our findings support the hypothesis that PT could potentially be painful for DoC patients and could be at least as painful as noxious stimulation. The presence of spasticity could partially explain these results as all of the patients suffered from spasticity and the majority of them had severe spasticity (i.e., MAS score ≥ 3, 13/15, 86.7%). A previous study showed that 89% of chronic DoC patients develop spasticity Thibaut et al. [2015b]. Spasticity was also associated with increased signs of pain, particularly during nursing cares and mobilizations Thibaut et al. [2015b]. Intrathecal baclofen pump is frequently used as an anti-spastic treatment in patients with severe brain injury. Beside reducing the signs of pain related to spasticity, several openlabel studies have shown that intrathecal baclofen may also improve patient's behavioral responsiveness Margetis et al. [2014], Pistoia et al. [2015], Thibaut et al. [2019]. To our knowledge, no such study achieved to show such results with oral baclofen. In our study 7/8 patients included in the clinical trial received baclofen per os; therefore, the risk that pain or behavioral responsiveness could be influence by the administration of intrathecal baclofen is limited. Importantly, 15/18 patients included during baseline showed signs of potential pain during PT (i.e., NCS-R > 4) and only five of them (33%) were treated with analgesics before inclusion. These results are in line with a previous clinical study which showed that 59% of patients with an identified potentially painful condition (polytrauma, wounds) did not have analgesic treatments during care Chatelle et al. [2016]. We also found that the facial expression subscale was particularly important, being the only one showing higher scores during PT compared to tactile stimulation. Spasticity and motor limitations can explain the absence of difference on the motor subscale. For the verbal subscale, 60% of the patients included in the clinical trial had a tracheotomy, which severely limits verbal interactions and could influence this subscore Lejeune et al. [2020]. These results highlight the fact that behavioral tools such as the NCS-R should be used more routinely to monitor potential pain during PT or other potentially painful care. This may allow the implementation of appropriate pain treatment and potentially also facilitate the management of spasticity in DoC patients. Additionally, futures studies will need to focus on the definition of objective indicators of facial responses reflecting pain-related response in these patients, as it seems to be a key behavior in the assessment of pain Arif-Rahu and Grap [2010].

(2) Analgesic treatment did not decrease signs of nociception during PT. During the double-blind randomized placebo-controlled study, no effect of analgesic treatment on NCS-R total scores and subscores

were found. Surprisingly, at an individual level, we observed an increase in the NCS-R total score during PT after treatment administration for 4 patients and a decrease in only 2 patients. This study did not support the hypothesis that analgesic treatment could decrease signs of pain during PT (i.e., NCS-R scores), as it was suggested in a previous open-label study showing a decrease of the NCS-R total score after administration of an analgesic treatment in acute patients Chatelle et al. [2016]. However, the difference in terms of the methodology (e.g., open-label vs double-blind, treatment) and the population (e.g., acute vs chronic) should be investigated in the future. The absence of analgesic effect in our study could be explained either by the fact that the NCS-R is not sensitive enough to detect the effect of the analgesic treatment, or that the medication administered did not efficiently reduce pain. Indeed, the majority of the patients received, as analgesic treatment, a non-opioid agent (i.e., paracetamol). The use of such non-opioid medication may not have been strong enough to induce a noticeable effect on pain and therefore on the NCS-R. Hence, an optimal pain management should account for a titration period to adapt the medication to the patient's needs and condition. Future studies should also take into consideration the duration of the presence of signs of nociception during care, to better detect the change after the administration of the treatment. Optimal pain management involves that the nature and the dose of the treatment is appropriate to the patient's needs to avoid a reduction in vigilance and a slow down in the recovery of consciousness Pistoia et al. [2015]. Our results suggest that analgesic treatment did not have a negative influence on the level of consciousness of patients included in the study. However, our results could not support the case study suggesting that an appropriate analgesic treatment may promote behavioral responsiveness Laureys et al. [2002]. The absence of a titration allowing for optimal medication for each patient may explain this result.

Our findings must be interpreted in spite of several limitations. First, the sample size of this study is small (n=18 and 15 for the clinical trial). Secondly, this study falls within the field of translational research and needed to be adapted to the clinical context. Consequently, the nature of the analgesic treatments already in place in these patients was not controlled and heterogeneous. Moreover, the administration of the analgesic was provided through the gastrostomy feeding tube for 87% of the patient. However, it was done at distance from the enteral feeding, diminishing the risk of malabsorption that could be caused by the formation of complexes between the medication and molecules contained in the enteral feeding (e.g., proteins or ions). These factors could have influenced the results and could explain the absence of difference between placebo and treatment. Another limitation is the possible variability in amplitudes of the movement during PT at baseline and after treatment administration, as analgesic may have influenced the patients' range of motion. Finally, the presence of dysautonomia may be a limitation. However, according to the medical files of each patient included in the present study none of them showed any signs of this syndrome such as paroxysmal hypertension, tachycardia, hyperthermia. Therefore, it is

unlikely that signs of dysautonomia would appear during the duration of our protocol. Moreover, based on demographical information, the shorter time since injury in our sample was about 97 days, which decreases the risk of dysautonomia. To our knowledge, there is only one validated tool that allow to assess dysautonomia Baguley et al. [2014] and the NCS-R does not evaluate the same clinical signs, so the risk of misdiagnosis is rather low. In the future, treatments administration should be better controlled (i.e., control of premedication and use of titration method, intravenous administration) and these results should be repeated in a multicenter study with a larger number of patients. In addition, we only used the NCS-R to assess pain responsiveness. However, the use of physiological measures of pain such as skin conductance Critchley et al. [2000], Wijnen et al. [2006] or heart rate Riganello et al. [2012] could have provided additional information on pain related physiological metrics, which should not be neglected in clinical setting. Moreover, at baseline, pain was only assessed once, while in a clinical setting pain and nociception assessments would have to be repeated on a daily basis in order to be able to detect fluctuations in pain responsiveness. Finally, the CRS-R was performed the day of the assessment for each condition, but not simultaneously with the NCS-R and we know that DoC patients may fluctuate during the day which could have an influence on pain sensitivity/responsiveness Monti et al. [2010]. However, we did not find any significant change regarding the CRS-R total score at each day of assessment suggesting that the level of consciousness assessment of these patients was relatively stable.

In conclusion, the present study highlights that (1) PT is associated with potential pain in most of the DoC patients and only a few of them were receiving an analgesic treatment prior to the study. (2) The study did not allow us to show an effect of a first-intention analgesic treatment on the NCS-R scores, suggesting that either the NCS-R score lacks of sensitivity or that NCS-R responsiveness to analgesics should be evaluated with different level of analgesic drugs. Our results highlight a lack of clear guidelines for (i) the implementation of such behavioral scales (e.g., assessments frequency) and (ii) the administration of optimal treatment in this population. Indeed, appropriate assessment (e.g., systematic NCS-R assessment during care and mobilizations) and treatment (e.g., titration allowing for optimal treatment for each patient) of pain before and during mobilizations should become a priority in clinical setting.

Chapter 4

General discussion

In this last chapter, we will start by summarizing the results obtained in the four above-mentioned studies. Then, these results will be put into perspective with the existing literature. Finally, we will conclude this discussion and present the perspectives for future research.

4.1 Summary of the main results

This work was centered around two main questions: 1) how to improve pain assessment in patients with DoC and patients in LIS? (study 1: **NCS-R/PET** and study 2: **LIS survey**) and 2) how to reduce potential sources of pain? (study 3: **SMO/pain** and study 4: **PT/antalgic**).

The first study aimed to determine a NCS-R cut-off score based on neuroimaging data (FDG-PET). In spite of a certain number of limitations, it allowed to establish a conservative NCS-R cut-off score of 5. This cut-off score makes it possible to identify patients with preserved neural basis for pain processing (e.g., MCS*). The study highlights brain metabolism differences between "FDG-PET confirmed UWS" patients, patients with potential pain (i.e., UWS and MCS patients with NCS-R score ≥ 5) and healthy subjects at both global and regional levels. Indeed, in addition to a relatively preserved global cerebral metabolism, patients with potential pain show a metabolic preservation in the left insula, a region known to be involved in the processing of the affective dimension of pain. These results suggest that the cut-off score of 5 is specific to cortical process of pain and could be use in clinical as a red flag for pain processing independently of the clinical diagnosis.

The second study was a French survey targeting patients in LIS. The aim of the study was to interview directly patients about the presence or absence of pain and better characterize it, particularly with regard to the type, frequency, duration, impact on quality of life, means of communication and treatments used to manage this pain. Half of the patients surveyed (49%) reported experiencing pain, with the majority suffering from chronic pain (92%). The study showed that pain had a negative influence on the quality of life, particularly in terms of sleep and emotions. It was noted that more than half of the participants (52%) did not communicate their pain to caregivers. This alarming finding highlight the importance of good detection of behavioral and physiological signs of pain by clinical teams. Despite the many possible side effects, the survey showed that level 1 pharmacological treatments (i.e., non-opioids analgesics) were currently the most widely used to manage pain in patients in LIS. However, some patients seem to be interested in the implementation of new non-pharmacological treatments.

The third study aimed to evaluate the relationship between pain and SMO for each joint in order to identify the spastic profile most likely to cause pain in patients with DoC. The results showed a high prevalence of SMO during mobilizations and a positive correlation between the presence of SMO and the presence of pain. We also showed that mobilizations involving the upper limbs (i.e., fingers and wirsts) were the most likely to cause pain in patients with DoC. Then, the relationship between upper and lower limb SMO and clinical and demographic data was investigated in order to identify factors that might influence the severity of SMO. The results showed that, contrary to the diagnosis and the etiology, age is a determining factor of SMO, young people being more prone to become spastic.

The fourth study was a randomized, double-blind, placebo-controlled trial designed to evaluate the effects of analgesic treatment on nociception and pain signs during physiotherapy. Although preliminary, these results showed that physiotherapy induced pain in the majority of patients (83%) and that only a minority of them were already receiving pain medication before inclusion in the study (33%). We also noted that the facial expression subscale of NCS-R was the only subscale that showed higher scores during physical therapy compared to tactile stimulation. Another important point of this study was the lack of effect of analgesic treatments on NCS-R scores. This suggests a lack of sensitivity of the NCS-R in detecting behavioral changes related to analgesic administration or a lack of effectiveness of the treatments used.

4.2 What have we learned from the literature?

NCS-R cut-off scores and pain assessment

Differentiating pain and nociception in the context of DoC is not an easy task. A 2017 study sought to assess the pain threshold (Newton/cm^2) of patients with DoC compared to healthy subjects aiming to assess sensitivity to pain. Using an algometer, they applied a standardized nociceptive pressure to determine the minimum pressure to induce a behavior in the patient (i.e., pressure response) (Sattin et al. [2018]). The results showed that patients with severe brain injury (i.e., UWS, MCS and EMCS) had a lower pressure response than healthy subjects. The NCS scores of the patients in UWS were lower than the NCS scores of patients in MCS but the average value of pressure response were the same for both diagnoses. Moreover, a very large variability in behavioral response was observed following nociceptive pressure, the NCS scores ranging from 1 (i.e., associated with reflex behavior such as oral reflex movements) to 7 (i.e., associated with higher level behaviors such as pain localization). This could mean that pressure response is not necessarily associated with pain perception (i.e., related to high NCS score) but could be related to a touch sensitization inducing reflex behaviors (i.e., link to low NCS score) in some patients. The pressure response may also be biased by the presence of chronic pain or SMO which could prevent behavioral responses (e.g., SMO will impact the motor response of patients). These results highlight the increased sensitivity of patients with DoC to tactile or nociceptive stimuli. A specific attention should be paid to patients with a low pressure response value, especially in terms of preventive treatment of pain. The pressure response value does not determine whether the patient is processing the information as a pain signal or whether it is related to touch sensitization. The scores obtained on the revised version of the NCS (i.e., NCS-R) allow to differentiate between reflex behaviors (e.g., groaning, oral reflex movements etc.) and higher-level behaviors (e.g., pain localization, cry, intelligible verbalization etc.) but a cut-off score to disentangle pain from nociception processing is still needed. A

2012 study aiming to investigate the sensitivity of the NCS had found a cut-off score of 4 (Chatelle et al. [2012]). This cut-off score differentiated patients with DoC (i.e., any diagnosis) who received noxious stimulation (i.e., nail pressure) from those who received non-noxious stimulation (i.e., touch). However, different NCS cut-off scores were observed in UWS (i.e., NCS cut-off score of 3) and MCS (i.e., NCS cut-off score of 4). In this study, no UWS patient had shown a score greater than 4 following noxious stimulation. In accordance with these observations, in our NCS-R/PET study, we observed in UWS patients unable to process pain (i.e., with global cortical hypometabolism) a maximum NCS-R score of 4 (Bonin et al. [2020b]). However, our study included three patients diagnosed as UWS behaviorally but with NCS-R scores of 5 during mobilizations. Neuroimaging results showed in these patients a preservation of global metabolism as well as areas involved in pain processing (i.e., left insula) suggesting a diagnosis of MCS* (Gosseries et al. [2014], Thibaut et al. [2021]). Additionally, a 2018 study highlighted an NCS-R cut-off score of 2 involving reflex behaviors (i.e., flexion withdrawal, oral reflex movement, etc.) and thus allowing the detection of nociception in patient with DoC (Chatelle et al. [2018a]). However, the presence of these reflex behaviors does not necessarily imply a conscious perception of pain. Our cut-off score of 5 was then used in a randomized placebo-control study (PT/antalgic, Bonin et al. [2022]) to select patients who might experience pain for inclusion in the clinical trial. Due to the low sensitivity of this cut-off score, patients with NCS-R scores at mobilization $\geq$ than those during noxious stimulation were also included. Of the patients included in the clinical trial, only four had NCS-R scores ≥ 5, confirming its low sensitivity. However, although less sensitive than these two previous cut-off scores, our cut-off score of 5 is based on neuroimaging data and is specific to cortical pain processing. Moreover, it is independent of clinical diagnosis and could also serve as a marker of covert consciousness, which makes it useful in clinical setting. Taken together, the different cut-off scores should be interpreted as follow: the cut-off score of 2 is related to nociception (i.e., obtainable by reflex behaviors) whereas the cut-off score of 5 is associated to cortical processing of pain and could allow the detection of patient in MCS*.

Among the patients who might experience pain included in PT/antalgic study, only 33% were already on analgesic treatment before inclusion in the study. This means that signs of pain were probably not properly assessed or detected in these patients before their inclusion, which raises an important ethical problem. An online international survey about needs and beliefs of healthcare staff regarding pain assessment and management in patients with DoC is currently ongoing at the Coma Science Group. By allowing a better understanding of their expectations this study could improve the management of pain in this sensitive population and decrease psychological distress of health professionals. This survey is divided in 2 sections: a first part collecting respondents' socio-demographic information (age, gender, nationality, religion, year of experience with DoC patients) and a second part collecting data on respondents' knowledge and expectations on pain assessment and management of DoC patients (questions

regarding pain perception, behavioral signs of pain, pain assessment tools or pain treatment). Preliminary results of this survey included 78 healthcare professionals and highlighted a lack of knowledge regarding pain assessment tools in patients with DoC (Bonin et al. [2021]). Indeed, despite the fact that the majority of the healthcare professionals (90%) find pain assessment in patient with DoC important, only 36% report using standardized tools and 40% feel they do not have the adequate tools. Less than half of respondents reported using the NCS-R to assess pain in patients with DoC (46%) and 32% said they did not know about this tool. Research has shown that this tool is the most suitable for assessing pain in DoC patients, but its implementation in clinical setting does not seem to be a given. Work is needed within clinics and hospitals to properly train clinical teams to use this tool.

PT/antalgic study highlighted the importance of the facial expression subscale in the detection of nociception signs (Bonin et al. [2022]). Indeed, for this subscale we observed a significant difference between the NCS-R subscores during PT and the NCS-R subscores during tactile stimulation. Indeed, in patients with severe spasticity or limited oral response due to intubation/tracheostomy/anarthria, the use of motor or verbal responses may be limited and facial response is sometimes the only remaining response that can be used. If grimacing is considered as an indicator of pain, the Multi-Society Task Force on PVS does not consider it as a necessary sign of conscious perception as they can occur reflexively through subcortical pathways in the thalamus and limbic system (The Multi-Society Task Force on PVS [1994]). Patients showing no sign of consciousness except for grimaces to nociceptive stimuli can therefore be diagnosed as being in UWS. Nevertheless, very few studies have investigated such behaviours in conscious (MCS) versus non-conscious (UWS) patients. Previous study reported that grimaces were more frequently displayed in response to nociceptive stimuli than in response to non-nociceptive stimuli in both MCS and UWS patients (i.e., 48% vs 4% for UWS and 65% vs 3% for MCS, respectively Schnakers et al. [2012]). However, grimace to pain were not observed more frequently in MCS than in UWS patients. Moreover, even if it is commonly used for pain assessment in non-communicative patients (Chatelle et al. [2012], Gelinas et al. [2006], Chanques et al. [2009], Feldt [2000]), facial responses are clinically scored based on gross observation of facial expression to a nociceptive stimulus. Indeed, the NCS-R facial expression subscale, for example, assesses only the presence of cries, grimaces and oral reflex movements. It would be interesting to develop tools, such as the Facial Action Coding System (Bartlett et al. [2014]), to define objective indicators of facial responses reflecting pain-related response in this population (Arif-Rahu and Grap [2010]).

Regarding LIS, our LIS survey revealed that the majority of these patients interviewed suffered from chronic pain (92%), 36% of which was neuropathic pain Bonin et al. [2020a]. This non-nociceptive pain (i.e., does not involve peripheral nociceptors) can be due to a lesion in the central or peripheral nervous system and impact the patient's quality of life. Our survey highlighted that pain impacted patients' sleep

in 40% of cases and their moods in 72% of cases. Neuropathic pain is assessed using the DN4 questionnaire but requires that the patient be able to communicate and therefore cannot be used in DoC (Bouhassira et al. [2005]). For patients with DoC, the NCS-R specifically evaluates acute pain that appears following a standardized stimulation or during mobilization. However, to our knowledge, no study has investigated its validity in non-nociceptive pain such as allodynia or neuropathic pain. LIS survey also showed that half of the patients in LIS experiencing pain did not spontaneously communicate about it. This shows how important it is for the clinical team to assess the signs of acute and chronic pain on a daily basis through the use of communication codes or BCI techniques (Annen et al. [2020]).

Spastic muscle overactivity, physiotherapy and pain

Detecting and treating potential sources of pain is of utmost importance, especially for patients with DoC. NCS-R/PET study highlighted that a large proportion of patients experienced pain during mobilizations (i.e., during physiotherapy, PT). These results were also confirmed in PT/antalgic study with a rate of 83% of patients experiencing pain during PT. This echoes a study conducted in 2019, which showed that the use of personalized stimuli (i.e., determined on a case-by-case basis by clinical teams during patient mobilizations) could allow for better pain assessment compared to standardized stimuli (e.g., nail pressure) (Formisano et al. [2020]). Pain during PT could be explained by the fact that the majority of these patients showed signs of moderate to severe spasticity (87% in PT/antalgic study). Spasticity is part of the SMO and correspond to an velocity-dependent increase in muscle tone resulting from the hyper-excitability of the stretch reflex. SMO also encompasses other forms of hypertonia such as spastic dystonia (i.e., inability to relax the muscle during resting state), spastic co-contraction (i.e., inappropriate activation of antagonist muscles during active mobilization of agonists) and spastic myopathy (i.e., muscle modification following a brain injury, does not require the presence of spasticity, (Martens et al. [2019]). SMO occurs due to a lesion of the first motor neuron and the absence of voluntary movement and the immobilization of patients with DoC could increased it expression (Gracies [2005]). Previous studies showed that the proportion of patients with DoC suffering from SMO ranging from 59 to 95% (Martens et al. [2017], Zhang et al. [2021]). SMO will mask some of the patient's motor responses which may lead to an underestimation of the level of consciousness (Monti et al. [2010], Cruse et al. [2011]). Moreover, a 2014 study aimed to investigate the clinical impact of SMO in patient with DoC. They found a positive correlation between the MAS and the NCS-R scores suggesting that SMO could induce pain in this population (Thibaut et al. [2015a]). This is why it is crucial to better characterize SMO in patients with DoC and identify a in order to be able to propose targeted treatments at a early stage to reduce spasticity and thus pain. In SMO/pain study, we investigate the relationship between SMO and pain and confirmed the previous result by observing a positive correlation between the presence of SMO and the presence of pain. We also found that the wrist and finger flexors and the hip adductors

were the muscle groups most likely to induce pain. These results were confirmed by a recent study which showed that the muscles most affected by SMO were the shoulder internal rotators, wrist flexors, elbow flexors, finger flexors, ankle plantar flexors, hip adductors, knee flexors (Zhang et al. [2021]). Identifying the muscle groups most likely to develop SMO could allow better prevention and treatment of SMO, as well as the associated pain, during the mobilization of these muscles.

In order to treat SMO, physical, pharmacological and surgical treatments exist (for a review see Martens et al. [2017]). Among pharmacological interventions, the use of baclofen is the most common in clinical setting. Several studies and case reports showed beneficial effects of baclofen use on reducing signs of SMO as well as on improving recovery of consciousness (Shrestha et al. [2011], Francois et al. [2001]). Indeed, the reduction of SMO through the use of appropriate treatment could either improve recovery of consciousness or either enhance the ability of a patient to express signs of consciousness by improving motor function and/or reducing pain (Pistoia et al. [2015], Lanzillo et al. [2016]). It is also possible to perform local toxin injections such as phenol (i.e., target a peripheral nerve of the upper and/or lower extremities which will decrease the SMO of the innervated muscle group) and botulinum (i.e., blocks the nerve impulses that control muscle contraction in order to induce relaxation of the targeted muscle) toxin to reduce SMO of a specific group of muscles (Martens et al. [2017]). A case study conducted in 2020 showed an improvement in SMO in a UWS patient following the use of levetiracetam (i.e., anti-epileptic) combined with other treatments such as physiotherapy, toxine botulinium or baclofen (Pingue et al. [2020]). Regarding physical intervention, the use of soft splints and passive muscle stretching during PT is recommended to prevent SMO in the upper limbs (Thibaut et al. [2015b]). There are also other techniques such as: use of orthoses or serial casts, bed positioning, wheelchair seating, tilt-tables and standing frames. A 2017 study also showed the importance of PT in improving SMO by highlighting a negative correlation between PT frequency and MAS scores (Thibaut et al. [2018]). Regarding surgical intervention, the use of transcranial and spinal cord magnetic stimulation or transcutaneous electrical nerve stimulation or intrathecal baclofen pumps also allow a to reduce SMO in patients (Mahmood et al. [2019], Korzhova et al. [2018], Halbmayer et al. [2022], Nardone et al. [2020]). It is recommended to perform a combination of these three different types of interventions for a better efficiency (Zhang et al. [2021]).

By decreasing the signs of SMO these treatments could have an impact on the signs of pain. LIS survey showed that a minority of painful LIS patients (12%) used PT or osteopathy as a non-pharmacological complementary treatment to reduce pain. Regarding patient with DoC, preliminary results from our ongoing survey on healthcare professionals indicate that among the patient who were treat for pain with non-pharmacological treatment, 94% use PT (see **Figure 4.1**). Unfortunately, in our PT/antalgic study, eight patients already had an anti-spastic treatment before inclusion but still had high MAS and NCS-R

scores suggesting that these treatments are not effective enough to treat spasticity and do not seem to prevent pain during mobilization (Thibaut et al. [2015b]). Indeed, given the small number of existing randomized clinical trials, there is still a lack of evidence regarding the real effectiveness of the anti-spastics treatments currently in place (Laxe [2019]). Therefore, it is necessary to set up randomized clinical trials on a large number of patients in order to investigate the effectiveness of these treatments on SMO and pain. In addition, a recent study showed that SMO could evolve differently depending on the type of brain damage and patient's diagnosis. Indeed, in this study TBI patients seemed to develop SMO more gradually than non-TBI patients. SMO/pain study also highlighted that age could have an impact on the patient's level of SMO (older patients being at greater risk of developing a spastic profile). This shows that it is important to improve the tools to assess SMO and to adapt the treatments according to the patient (Winters et al. [2022]).

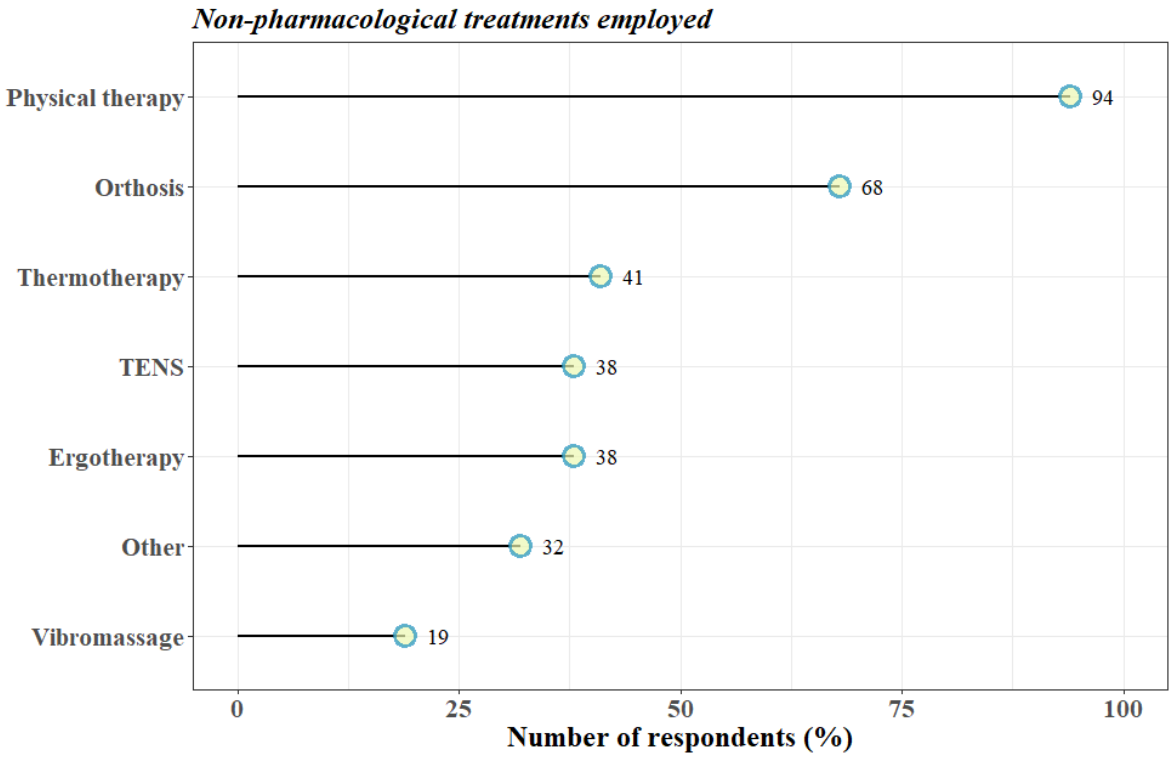


Figure 4.1: Pain management of patients with DoC part I Lollipop plot representing the type of non pharmacological treatments used by health care professionals to treat pain (TENS = transcutaneous electrical nerve stimulation).

Pain treatment

Appropriate treatment of pain can have an impact on the patient's quality of life but also on the clinical diagnosis. Indeed, a case study of a patient diagnosed with CSM showed that reducing signs of pain resulted in an improvement in the patient's condition as well as an increase in these CRS-R

scores (Lanzillo et al. [2016]). This indicates that the use of appropriate pain treatment could lead to improved outcomes and reduced misdiagnosis. NCS-R/PET and PT/antalgic studies demonstrated the clinical utility of the NCS-R and its cut-off score of 5 in detecting patients who might experience pain. Regarding pain treatment in patient in LIS, LIS survey highlight that the majority of the patients were under pharmacological treatments (73% non-opioids, 20% non-inflammatory and 13% weak opioids, see **Figure 4.2**). Regarding pain treatment in patient with DoC, preliminary results from our ongoing survey on healthcare professionals indicate that 87% of clinicians reported using pharmacological treatments for pain (74% non-opioids, 61% weak opioids and 46% strong opioids). In our PT/antalgic study, we wanted to evaluate the effects of analgesic treatment on signs of pain during PT in patient with DoC. Unlike a previous open label study (Chatelle et al. [2016]), our results did not allow us to observe a change in the NCS-R scores after administration of the treatment except for two patients.

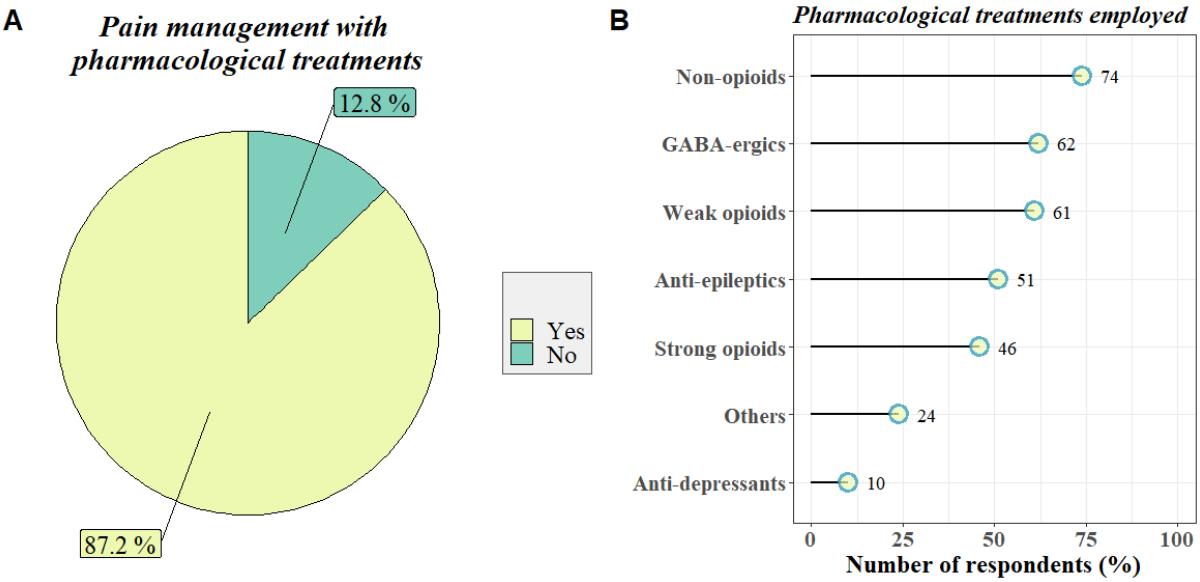


Figure 4.2: Pain management of patients with DoC part II. A) Pie chart shows the habits of health care professionals regarding the use of pharmacological treatments to prevent pain in patients with DoC. B) Lollipop plot representing the type of pharmacological treatments used by health care professionals to treat pain (based on Ventafridda et al. [1985]).

Several hypotheses could explain this lack of results. First of all, the NCS-R may not be sensitive enough to detect subtle changes induced by analgesic treatment. Therefore, it would be interesting to investigate the effects of analgesic treatment on other physiological markers of pain such as heart rate, skin conductance or pupillary diameter to see if they are more appropriate. The second hypothesis refers to the possibility of ineffectiveness of the treatments administered. The most commonly used treatment in PT/antalgic study was paracetamol. This is a non-opioid analgesic (level 1), which acts at the peripheral level by inhibiting the synthesis of prostaglandins responsible for fever and sensitization of peripheral nociceptors. It also stimulate the descending serotonin pathways, which are involved in the inhibition

of pain sensations (Jóźwiak-Bebenista and Nowak [2014]). Next comes tramadol, a weak opioid (level 2), which is a milder form of morphine that acts centrally by inhibiting noradrenergic and serotoninergic reuptake (Bravo et al. [2017]). It is often used in addition to paracetamol for both central and peripheral action. Finally, oxycodone was also used as a level 3 treatment. It is a strong opioid (i.e., morphine) that act centrally on the opioid receptors of the spinal nociceptors as well as peripherally. The binding of morphine to these receptors activate the descending inhibitory pathways of the central nervous system and inhibit the afferent nociceptive neurons of the peripheral nervous system, in order to decrease the nociceptive transmission (Murphy et al. [2022]). It is possible that the dosage of these treatments was not strong enough to induce a visible response to NCS-R or reduce pain. In the future, it would be preferable to perform a titration period in order to determine the optimal dose to administer (i.e., that induces a visible response to the NCS-R without decreasing the level of consciousness at the CRS-R). We may also wonder if the mode of action of these treatments is adapted to patients with severe brain damage such as DoC or LIS patients. Indeed, the brain lesions of these patients may prevent the action of these drugs on their therapeutic targets. It is therefore necessary to conduct studies to better understand the mechanism of action of these drugs in this specific patient population. Moreover, it could be that the processing of pain differs according to sex. Indeed, one study showed that women are more sensitive to pain than men. Women are also more likely to develop chronic pain and respond differently to antalgic treatments (Bartley and Fillingim [2013]). In our study, we did not observe any difference between men and women in the NCS-R scores during PT even after administration of the analgesic. The study of sex differences is still underdeveloped in patients with DoC but deserves to be studied more in the future in order to offer therapeutic projects adapted to each patient.

4.3 Conclusion and future perspectives

In this work, we aimed to improve guidelines regarding pain assessment and management in severely brain injured patients unable to communicate verbally such as patient with DoC and LIS. We are now going to conclude on the knowledge brought thanks to the studies realized in the course of this work and to evoke the future perspectives that this brings.

How to improve pain assessment in patients with DoC and LIS?

In order to improve the guidelines for pain assessment in DoC patients, we aimed to establish a NCS-R cut-off score based on neuroimaging data. One of the hypotheses of this study was that patients with global cortical hypometabolism were not able to consciously process pain. On the basis of this assumption, we set a NCS-R cut-off score of 5 specific to cortical pain processing. However, this study has some limitations and further studies are still needed to validate this score. Indeed, in this retrospective

study only the resting brain metabolism could be analyzed. In the future, it would be interesting to measure the brain activation of the patients directly during a standardized pain stimulation (i.e., use of an algometer or by electrical stimulation) using fMRI or FDG-PET techniques. The NCS-R scores would be collected before and during image acquisition in order to compare brain patterns between patients with NCS-R ≥ 5 scores and those with lower scores. Indeed, We know that the cut-off score of 2 is associated with nociception whereas the cut-off score of 5 is related to cortical pain processing but with a very low sensibility. We should now investigate what happens to brain metabolism (at rest and after noxious and non-noxious stimulation) in patient in UWS and MCS with NCS-R scores of 3 and 4 to ensure that we do not underestimate the number of patients with preserved brain activity at the neuromatrix level.

In addition, we also confirmed our second hypothesis that patient that might experienced pain (i.e., with scores greater than or equal to this cut-off score) had preservation of global metabolism as well as regions of the neuromatrix (in this case the left insula). In clinical setting, this cut-off score could allow the identification of patients with a metabolic preservation of the regions usually involved in pain processing. However, studies have shown that the neuromatrix may not be specific to pain processing but also involved in the processing of non-nociceptive stimuli (Iannetti and Mouraux [2010], Mouraux et al. [2011]). Therefore, the demonstration of preservation of this neuromatrix alone does not guarantee that these patients feel pain. It is important to couple this neuroimaging information with the behavioral results of the NCS-R as well as physiological markers to be able to stipulate a potential pain in DoC patients. Without a subjective verbal report from the patient we can only make assumptions about the actual pain experienced by the patients. However, for ethical and clinical reasons, it is still necessary to treat any patient with a suspected pain perception. In addition, for better pain management, it would be interesting to develop new tools allowing patients to communicate about their pain independently of their motor and verbal abilities (e.g., BCI techniques, use of specific pain eye codes for LIS). Moreover, in this work we were also able to highlight the importance of the facial expression NCS-R subscale in pain assessment especially in the case of patients suffering from severe spasticity or having a tracheotomy. Future studies should attempt to better characterize the facial response to a painful stimulus in patient with DoC. This could allow the identification of additional key features that could be implemented in the NCS-R.

Among patients in LIS included in our survey, we found that some of them felt that pain had a deleterious impact on their sleep. Some of the patients interviewed also stated that fatigue could increase pain. It is known that the fluctuation of vigilance during the day can have an impact on behavioural responses (i.e., a tired patient will be less inclined to respond to the requested examinations) (Piarulli et al. [2016]). This is why it is advisable, for the CRS-R for instance, to carry out repeated assessments at different times of the day in order not to avoid misdiagnosis (Wannez et al. [2017]). This fluctuation of

vigilance, in addition to having an impact on the clinical diagnosis, can therefore also influence the level of pain in certain patients. It would be interesting to further investigate the effect of this fluctuation of vigilance on the NCS-R scores in patients with DoC and in LIS in order to confirm this result and to determine whether repeated NCS-R assessments throughout the day could better identify patients with pain.

How to reduce potential sources of pain?

Preliminary results from our ongoing survey on healthcare professionals and the LIS survey show that in terms of pain treatment, pharmacological treatments are favored. However, this work, contrary to a previous open-label study (Chatelle et al. [2016]), did not make it possible to demonstrate the utility of the NCS-R in the detection of the behavioral changes following the taking of analgesic treatments. This difference in results can be explained by the fact that Chatelle et al. [2016] performed an open label study focus on acute patients with DoC whereas our study was a randomized double-bling placebo-control trial targeting patients with chronic DoC. The lack of change in NCS-R scores could therefore be related to the lack of effectiveness of the treatments in the chronic stage. Indeed, due to their condition, patients with chronic DoC will present different pain profiles than patients with acute DoC (e.g., development of SMO, neuropathic pain etc.) and thus be more refractory to antalgic treatments. It would therefore be interesting to better characterize these profiles and study the effect of analgesic treatments in patients with acute DoC compared with patients with chronic DoC. In addition, future studies could use the titration method (i.e., pharmacological method that consists of administering successive boluses of analgesic until the patient is relieved, while respecting the dosage) to better assess the effects of analgesics on NCS-R scores (Aubrun et al. [2004]). It would also be interesting to study more specifically the effect of anti-spastic treatments on NCS-R scores.

Moreover, depending on the type of pain, these treatments are not always suitable. For instance, in the case of patients suffering from neuropathic pain (such as patient in LIS), opioid analgesics are not the most suitable treatments (Foley [2003]). It is therefore important to identify the sources of pain in DoC and LIS patients in order to best adapt treatments. Regarding the management of patients in LIS, 24% of the participants interviewed in our survey stated that they were willing to try non-pharmacological treatments. Indeed, pharmacological treatments are often accompanied by side effects (i.e., drowsiness, increased fatigue, cognitive slowing, impact on emotions, etc.) that can impact the behavioral responses of patients during evaluations. Being able to propose a global non-pharmacological approach therefore seems essential for these patients. By avoiding the undesirable effects of analgesic treatment, these patients in LIS could take maximum advantage of the tools made available to them to communicate. Among the existing non-pharmacological treatments that have been proven in other

patient populations, hypnosis could be developed for patients in LIS. Indeed, numerous studies have shown that hypnosis allows the modulation of pain perception, both acute and chronic, especially in healthy subjects or those suffering from chronic pain (Rainville et al. [1997], Bicego et al. [2021], Vanhaudenhuyse et al. [2018]). The use of hypnosis for patients in LIS could allow to modulate the pain experienced by these patients while decreasing a pharmacological medication sometimes deleterious. The improvement of comfort, potentially combined with a decrease in medication, will significantly improve the quality of life of these patients.In addition, hypnosis does not require a motor response from the patient, which makes it particularly interesting for patients in LIS, unlike other non-pharmacological approaches such as aerobic exercises, which are known to relieve pain (Rice et al. [2019]).

In conclusion, the use of the NCS-R remains the most appropriate way to assess pain in severely brain damaged patients unable to communicate. Communication and training are needed to implement this tool in clinical teams. The threshold score of 5 can be used as a red flag to detect patients who might experience pain and covert consciousness. However, patients with scores below this threshold should not be neglected and the use of neuroimaging and other physiological markers can be a good complement to the assessment. It is also necessary to emphasize the importance of treating pain in all DoC patients regardless of their diagnosis. Indeed, we now know that most patients in UWS have residual brain activity, including in the prefrontal areas, which means that they can, at least partially, process pain and not only nociception. We recommend using the NCS-R as soon as the patient appears painful and during care/mobilization in order to be able to follow its evolution over time and revise the pharmacological treatment if needed. Regarding patients in LIS, even if they do not communicate their pain spontaneously, it is important to actively and regularly verify this through the use of simple communication codes. When signs of pain are detected, it is essential to identify the source of the pain (i.e., spasticity, neuropathic pain, injuries etc.). To treat these pains, pharmacological treatments are currently favored despite the side effects (i.e., decrease in attention) that they generate. The development of new non-pharmacological treatments, such as hypnosis, could avoid these side effects.

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Appendix A

Nociception Coma Scale-Revised allows to identify patients with preserved neural basis for pain experience

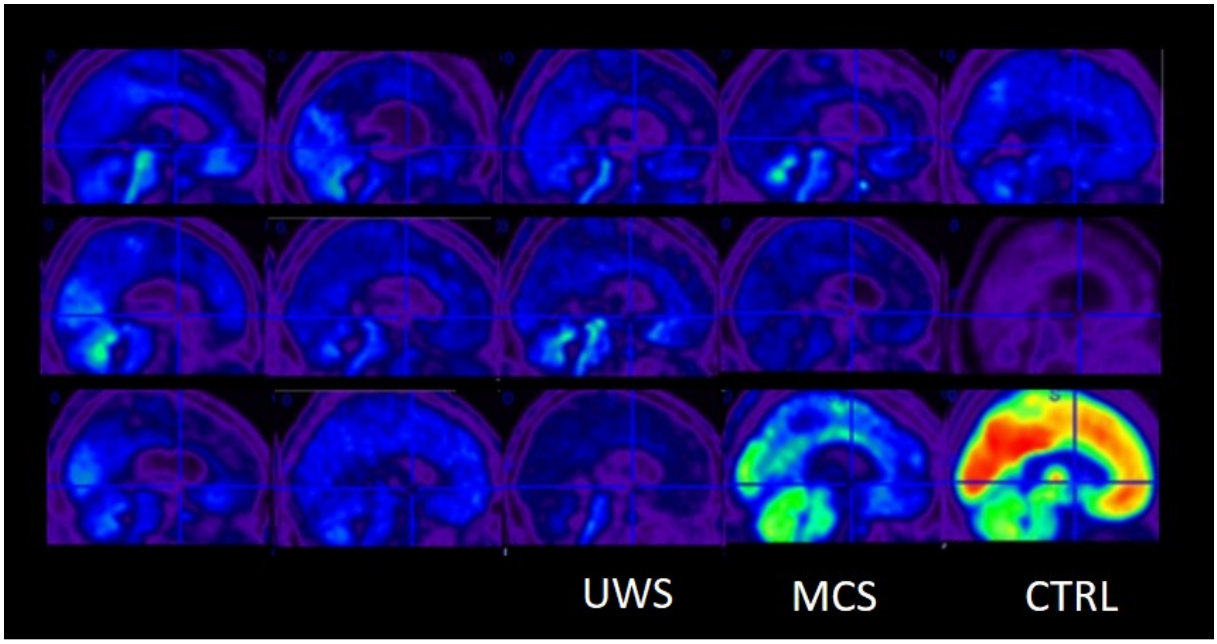

Figure A.1: PET-FDG showing the brain activation in the 13 well-documented UWS selected for the study. (MCS = Minimally Conscious State, CTRL = Healthy Control).

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 A.2: 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).

Table A.1: Demographic table of patients in UWS with the CRS-R assessment and with a global hypometabolism at PET-FDG, based on visual inspection and matched patients with NCS-R score 5. (F = Female, M = Male, TBI = Traumatic Brain Injury, SAH = Subarachnoid Hemorrhage, UWS = Unresponsive Wakefulness Syndrome, MCS = Minimally Conscious State, MCS* = behaviorally UWS with atypical brain metabolism on FDG-PET na = not applicable. In red = highest score observed during the NCS-R).

ID	Sex	Age	Etiology	LoC	Time since injury	Analgesic treatment before study	Tracheotomy	Spasticity	NCS-R during baseline	NCS-R during stimulation	NCS-R during mobilization
1	F	40	Anoxia	UWS	392	Dafalgan (4 x 1g)	yes	4	0	3	4
2	F	44	TBI	UWS	337	na	no	na	0	0	4
3	F	47	Anoxia	UWS	149	Dafalgan (1g)	yes	2	0	1	0
4	F	48	Stroke	UWS	557	na	yes	2	1	2	2
5	F	63	Anoxia	UWS	1213	Spasfon, Lyrica (75mg), Contramal (100mg)	yes	na	0	0	3
6	M	27	Anoxia	UWS	130	Dafalgan (1g)	yes	4	1	3	3
7	M	30	Anoxia	UWS	739	na	yes	2	1	3	1
8	M	31	Mixed	UWS	121	Rivotril (2 x 5 drops)	yes	2	0	2	3
9	M	51	SAH	UWS	430	Paracetamol (500mg)	no	3	2	2	2

ID	Sex	Age	Etiology	LoC	Time since injury	Analgesic treatment before study	Tracheotomy	Spasticity	NCS-R during baseline	NCS-R during stimulation	NCS-R during mobilization
10	M	55	Stroke	UWS	1460	Durogesic 25 (1 patch/ 3 days)	yes	4	2	2	2
11	M	57	Mixed	UWS	205	na	yes	na	1	4	3
12	M	65	Anoxia	UWS	11	Dafalgan (4 x 1g)	yes	0	0	1	0
13	M	73	Anoxia	UWS	99	na	yes	1	0	3	1
14	F	26	Mixed	MCS*	231	Phenobarbital (70mg), Rivotril (2,5mg/mL)	yes	1	1	4	5
15	F	30	TBI	MCS +	565	Diazepam, Transtec (35 g), Dafalgan	no	3	2	5	5
16	F	43	SAH	MCS +	100	na	yes	2	0	5	2
17	F	44	Anoxia	MCS*	97	Dafalgan (3 x 1g), Durogesic patch (50g/72h), Oxynorm (5mg)	yes	na	5	5	5
18	F	68	Anoxia	MCS*	728	Dafalgan (1g)	no	3	1	4	5

ID	Sex	Age	Etiology	LoC	Time since injury	Analgesic treatment before study	Tracheotomy	Spasticity	NCS-R during baseline	NCS-R during stimulation	NCS-R during mobilization
19	M	24	TBI	MCS +	2686	Rivotril (2 x 2,5mg)	yes	4	0	0	5
20	M	29	Hypoxémia	MCS +	405	Aspegic (100mg)	no	na	2	6	7
21	M	46	TBI	MCS +	648	Dafalgan (1g)	no	2	2	5	5
22	M	50	TBI	MCS +	253	na	yes	1	2	4	5
23	M	52	Stroke	MCS -	1459	na	yes	na	3	5	3
24	M	54	Stroke	MCS -	27	Dafalgan (3 x 1g), Rivotril (3 x 7 drops)	yes	1	0	5	4
25	M	61	TBI	MCS +	1991	Paracetamol (3 x 1g)	yes	0	1	6	6
26	M	60	TBI	MCS +	131	na	yes	3	0	5	2

Appendix B

French survey about pain perception and management in patients with LIS

Checklist for Reporting Results of Internet E-Surveys (CHERRIES) (ns = non specify, na = non applicable)

Checklist Item	*Explanation*	*Page Number*
Describe survey design	Describe target population, sample frame. Is the sample a convenience sample? (In "open" surveys this is most likely.)	3
IRB approval	Mention whether the study has been approved by an IRB.	3
Informed consent	Describe the informed consent process. Where were the participants told the length of time of the survey, which data were stored and where and for how long, who the investigator was, and the purpose of the study?	ns
Data protection	If any personal information was collected or stored, describe what mechanisms were used to protect unauthorized access.	na
Development and testing	State how the survey was developed, including whether the usability and technical functionality of the electronic questionnaire had been tested before fielding the questionnaire.	3-4
Open survey versus closed survey	An "open survey" is a survey open for each visitor of a site, while a closed survey is only open to a sample which the investigator knows (password-protected survey).	na
Contact mode	Indicate whether or not the initial contact with the potential participants was made on the Internet. (Investigators may also send out questionnaires by mail and allow for Web-based data entry.)	3
Advertising the survey	How/where was the survey announced or advertised? Some examples are offline media (newspapers), or online (mailing lists – If yes, which ones?) or banner ads (Where were these banner ads posted and what did they look like?). It is important to know the wording of the announcement as it will heavily influence who chooses to participate. Ideally the survey announcement should be published as an appendix.	3
Web/E-mail	State the type of e-survey (eg, one posted on a Web site, or one sent out through e-mail). If it is an e-mail survey, were the responses entered manually into a database, or was there an automatic method for capturing responses?	3-4
Context	Describe the Web site (for mailing list/newsgroup) in which the survey was posted. What is the Web site about, who is visiting it, what are visitors normally looking for? Discuss to what degree the content of the Web site could pre-select the sample or influence the results. For example, a survey about vaccination on a anti-immunization Web site will have different results from a Web survey conducted on a government Web site	na
Mandatory/voluntary	Was it a mandatory survey to be filled in by every visitor who wanted to enter the Web site, or was it a voluntary survey?	3
Incentives	Were any incentives offered (eg, monetary, prizes, or non-monetary incentives such as an offer to provide the survey results)?	3
Time/Date	In what timeframe were the data collected?	4
Randomization of items or questionnaires	To prevent biases items can be randomized or alternated.	na
Adaptive questioning	Use adaptive questioning (certain items, or only conditionally displayed based on responses to other items) to reduce number and complexity of the questions.	na

Number of Items	What was the number of questionnaire items per page? The number of items is an important factor for the completion rate.	3
Number of screens (pages)	Over how many pages was the questionnaire distributed? The number of items is an important factor for the completion rate.	3
Completeness check	It is technically possible to do consistency or completeness checks before the questionnaire is submitted. Was this done, and if "yes", how (usually JAVAScript)? An alternative is to check for completeness after the questionnaire has been submitted (and highlight mandatory items). If this has been done, it should be reported. All items should provide a non-response option such as "not applicable" or "rather not say", and selection of one response option should be enforced.	na
Review step	State whether respondents were able to review and change their answers (eg, through a Back button or a Review step which displays a summary of the responses and asks the respondents if they are correct).	na
Unique site visitor	If you provide view rates or participation rates, you need to define how you determined a unique visitor. There are different techniques available, based on IP addresses or cookies or both.	ns
View rate (Ratio of unique survey visitors/unique site visitors)	Requires counting unique visitors to the first page of the survey, divided by the number of unique site visitors (not page views!). It is not unusual to have view rates of less than 0.1 % if the survey is voluntary.	ns
Participation rate (Ratio of unique visitors who agreed to participate/unique first survey page visitors)	Count the unique number of people who filled in the first survey page (or agreed to participate, for example by checking a checkbox), divided by visitors who visit the first page of the survey (or the informed consents page, if present). This can also be called "recruitment" rate.	ns
Completion rate (Ratio of users who finished the survey/users who agreed to participate)	The number of people submitting the last questionnaire page, divided by the number of people who agreed to participate (or submitted the first survey page). This is only relevant if there is a separate "informed consent" page or if the survey goes over several pages. This is a measure for attrition. Note that "completion" can involve leaving questionnaire items blank. This is not a measure for how completely questionnaires were filled in. (If you need a measure for this, use the word "completeness rate".)	4
Cookies used	Indicate whether cookies were used to assign a unique user identifier to each client computer. If so, mention the page on which the cookie was set and read, and how long the cookie was valid. Were duplicate entries avoided by preventing users access to the survey twice; or were duplicate database entries having the same user ID eliminated before analysis? In the latter case, which entries were kept for analysis (eg, the first entry or the most recent)?	na
IP check	Indicate whether the IP address of the client computer was used to identify potential duplicate entries from the same user. If so, mention the period of time for which no two entries from the same IP address were allowed (eg, 24 hours). Were duplicate entries avoided by preventing users with the same IP address access to the survey twice; or were duplicate database entries having the same IP address within a given period of	na

	time eliminated before analysis? If the latter, which entries were kept for analysis (eg, the first entry or the most recent)?	
Log file analysis	Indicate whether other techniques to analyze the log file for identification of multiple entries were used. If so, please describe.	na
Registration	In "closed" (non-open) surveys, users need to login first and it is easier to prevent duplicate entries from the same user. Describe how this was done. For example, was the survey never displayed a second time once the user had filled it in, or was the username stored together with the survey results and later eliminated? If the latter, which entries were kept for analysis (eg, the first entry or the most recent)?	na
Handling of incomplete questionnaires	Were only completed questionnaires analyzed? Were questionnaires which terminated early (where, for example, users did not go through all questionnaire pages) also analyzed?	4
Questionnaires submitted with an atypical timestamp	Some investigators may measure the time people needed to fill in a questionnaire and exclude questionnaires that were submitted too soon. Specify the timeframe that was used as a cut-off point, and describe how this point was determined.	ns
Statistical correction	Indicate whether any methods such as weighting of items or propensity scores have been used to adjust for the non-representative sample; if so, please describe the methods.	ns

Table B.1: Questions included in the survey (VAS = Visual Analogue Scale, SD = Standard Deviation).

Items and responses	Total number of respondents	Mean VAS score ± SD	Frequency (%)
1. Have you felt pain or physical discomfort these last two weeks?	51		
Yes	25		49
No	26		51
2. If you feel pain, at which place(s) of your body is it? (several choices possible)	25		
Lower limb	21		84
Upper limb	6		24
Abdominals	4		16
Back	5		20
Head	7		28
Diffuse	2		8
3. Indicate on a scale ranging from 0 to 10 the intensity of the MOST INTENSE PAIN felt during the last two weeks (0 = no pain, 10 = most intense pain)	25	6.16 ± 2.44	
4. Indicate on a scale ranging from 0 to 10 the intensity of the LEAST INTENSE PAIN felt during the last two weeks (0 = no pain, 10 = most intense pain)	25	1.88 ± 2.55	
5. Indicate on a scale ranging from 0 to 10 the intensity of the MEAN PAIN felt during the last two weeks (0 = no pain, 10 = most intense pain)	25	4.20 ± 1.98	

Items and responses	Total number of respondents	Mean VAS score ± SD	Frequency (%)
6. Does the pain have one or more of the following characteristics? (several choices possible)	25		
0 = no features	10		40
1 = burn	6		24
2 = painful cold sensation	4		16
3 = electrical shocks	10		40
7. Do you experience other symptom(s) in the painful area? (several choices possible)	25		
None	9		36
Tingling	5		20
Pinprick	6		24
Numbness	6		24
Itches	3		12
Vice-like pressurized feeling	12		48
8. Do you feel a decrease in touch sensitivity in areas where pain is present?	25		
Decreased sensitivity during sitting	20		80
Decreased sensitivity during simple touch	3		12
No decrease in sensitivity	2		8
9. Is pain caused or increased by friction?	25		
Yes	8		32

Items and responses	Total number of respondents	Mean VAS score ± SD	Frequency (%)
No	17		68
10. Since when did you feel these pains:	25		
1-15 days	0		0
15 days - 1month	0		0
1 -3 months	2		8
3 -6 months	6		24
6 months -1 year	5		20
> 1 year	12		48
11. Before the locked-in syndrome, did you already have these pains?	25		
Yes	2		8
No	21		84
I do not know	2		8
12. If you already had pain before the locked-in syndrome, indicate on a scale ranging from 0 to 10 the intensity of this pain (0 = no pain, 10 = most intense pain)	25	2.5 ± 2.08	
13. If you had this pain before, how did your pain progress following the locked-in syndrome?	4		
Aggravation of pain	0		0
Decrease of pain	2		50
Same pain	1		25
I do not know	0		0

Items and responses	Total number of respondents	Mean VAS score ± SD	Frequency (%)
14. The pain you are currently experiencing is:	25		
Discrete	21		84
Continuous	4		16
If discrete, do you feel it:	21		
More than once a day	4		19
Once a day	2		9
Less than once a day	3		14
I do not know	12		57
If discrete, do you feel it more:	21		
The morning	5		23
The afternoon	5		23
The evening	6		28
I do not know	5		23
15. When you are in pain, how do you express pain? (several choices possible)	25		
Winces	6		24
Cries	11		44
Look/blinking	4		16
Vocalizations	7		28
Communication code	7		28
No expression of pain	13		52

Items and responses	Total number of respondents	Mean VAS score ± SD	Frequency (%)
Other	2		8
16. When you feel pain, do you feel that certain elements increase the pain? (several choices possible)	25		
Mood/emotions	1		4
Temperature	4		16
Supine	3		12
Sitting	7		28
Care (nurses, physiotherapy, ...)	8		32
Touching	10		40
Tiredness	10		40
Physical exercises	8		32
Equipment (specific cushion, . . .)	2		8
None	2		8
Other	3		12
17. When you feel pain, do you feel that certain elements decrease the pain? (several choices possible)	25		
Mood/emotions	0		0
Temperature	1		4
Supine	4		16
Sitting	6		24
Care (nurses, physiotherapy, ...)	2		8

Items and responses	Total number of respondents	Mean VAS score ± SD	Frequency (%)
Touching	10		40
Tiredness	5		20
Physical exercises	6		24
Equipment (specific cushion, ...)	3		12
None	3		12
Other	9		36
18. When you have pain, do you feel that pain affects your mental abilities? If yes, specify (several choices possible)	25		
Decreased concentration/attention	4		16
Increased mood swings	13		52
Decreased memory capacity	4		16
Depression	6		24
Tiredness	14		56
None	9		36
19. Does the pain disrupt your sleep?	25		
Yes	10		40
No	13		52
I do not know	2		8
20. If yes, indicate on a scale ranging from 0 to 10 how much the pain disturbs your sleep (0 = no influence, 1 = strong influence)	25	6.42 ± 3.23	

Items and responses	Total number of respondents	Mean VAS score ± SD	Frequency (%)
21. When you are in pain, do you feel that pain affects your emotions?	25		
Always	3		12
Sometimes	4		16
Rarely	14		56
Never	4		16
22. If yes, indicate on a scale ranging from 0 to 10 how much the pain affects your emotions (0 = no influence, 1 = strong influence)	25	4.68 ± 2.92	
23. Do you take any pharmacological treatment to ease your pain?	25		
Yes	15		60
No	10		40
24. If yes, how often do you take this treatment?	15		
Several times a day	5		33
Once a day	8		53
Occasionally, when the pain is too strong	2		13
25. If yes, indicate on a scale ranging from 0 to 10 how effective this (these) treatment(s) is (are) (0 = no influence, 1 = strong influence) 25 6 ± 2.93 26. To relieve your pain, have you ever tried non-pharmacological treatments (example: meditation, hypnosis, etc.)?	25		
Yes	3		12

Items and responses	Total number of respondents	Mean VAS score ± SD	Frequency (%)
No	21		84
I do not know	1		4
27. If yes, indicate on a scale ranging from 0 to 10 how effective this (these) treatment(s) is (are) (0 = no influence, 1 = strong influence)	25	2 ± 1.73	
28. Do you want to test a new pharmacological or non-pharmacological treatment? (several choices possible)	25		
Yes, I would be ready to test a new pharmacological treatment	12		48
Yes, I would be ready to test a new non-pharmacological treatment	6		24
No, I wouldn't be ready to test a new pharmacological treatment	7		28
No, I wouldn't be ready to test a new non-pharmacological treatment	14		56

Table B.2: Socio-demographic information and clinical status of patients included in the study (individual data). F = Female, M = Male, TBI = Traumatic Brain Injury, na = not applicable. Etiology: other includes chemical intoxication, cervical mishandling, and meningioma. Among the 36 patients who own a wheelchair, only 19 actively used it (53%).

ID	Sex	Time since injury (years)	Etiology	Tracheotomy	Gastrostomy	Verbal communication	Use of an alphabetic code	Use of a wheelchair	Survey completion
1	na	8	Stroke	Yes	Yes	No	No	Yes	n.s
2	na	21	Stroke	Yes	Yes	No	Yes	No	With a family member
3	na	26	Stroke	No	No	No	Yes	Yes	Alone
4	na	17	Other	No	Yes	No	Yes	No	na
5	na	20	Stroke	Yes	Yes	No	Yes	No	Alone
6	na	3	Stroke	No	No	No	No	No	With a family member
7	na	4	Stroke	Yes	Yes	No	Yes	No	With a ALIS member
8	na	20	Stroke	Yes	No	No	Yes	Yes	na
9	na	10	Stroke	No	No	No	No	Yes	na
10	na	19	Stroke	No	Yes	No	Yes	Yes	With a family member
11	na	27	Stroke	Yes	Yes	Yes	Yes	Yes	na

ID	Sex	Time since injury (years)	Etiology	Tracheotomy	Gastrostomy	Verbal communication	Use of an alphabetic code	Use of a wheelchair	Survey completion
12	na	6	Stroke	No	Yes	No	Yes	Yes	With a family member
13	na	20	TBI	Yes	Yes	No	Yes	Yes	Alone
14	na	20	Other	No	No	Yes	No	No	na
15	na	2	TBI	Yes	Yes	No	No	No	With a psychologist
16	M	18	Stroke	No	No	No	Yes	Yes	With an occupational therapist
17	F	6	Stroke	No	No	Yes	No	No	With an occupational therapist
18	M	6	Stroke	No	No	Yes	Yes	Yes	na
19	M	3	Stroke	Yes	Yes	No	Yes	Yes	With a nurse
20	M	7	Aneurysm	No	Yes	No	No	No	na
21	F	14	Stroke	No	No	No	No	Yes	na
22	F	13	Stroke	Yes	Yes	No	Yes	Yes	With a psychologist

ID	Sex	Time since injury (years)	Etiology	Tracheotomy	Gastrostomy	Verbal communication	Use of an alphabetic code	Use of a wheelchair	Survey completion
23	M	34	TBI	Yes	Yes	No	Yes	Yes	na
24	M	7	Stroke	No	Yes	No	Yes	Yes	na
25	M	9	Stroke	Yes	Yes	No	Yes	Yes	na
26	F	12	Infection	No	No	No	Yes	Yes	na
27	F	12	Stroke	No	Yes	No	Yes	Yes	Alone
28	F	36	Stroke	No	No	Yes	No	Yes	na
29	F	6	Stroke	No	Yes	No	Yes	Yes	na
30	F	7	Stroke	No	No	Yes	No	Yes	na
31	F	12	Stroke	No	No	Yes	Yes	No	Alone
32	F	16	Stroke	No	No	Yes	No	Yes	Alone
33	M	12	Stroke	Yes	Yes	No	Yes	Yes	na
34	M	4	Stroke	Yes	Yes	No	Yes	Yes	With a family member
35	F	20	Stroke	Yes	Yes	No	Yes	No	Alone

ID	Sex	Time since injury (years)	Etiology	Tracheotomy	Gastrostomy	Verbal communication	Use of an alphabetic code	Use of a wheelchair	Survey completion
36	M	1	Stroke	Yes	Yes	No	Yes	Yes	na
37	F	11	Infection	No	No	Yes	No	Yes	na
38	M	2	Stroke	Yes	Yes	No	Yes	Yes	With a family member
39	M	1	Stroke	Yes	Yes	No	Yes	Yes	With a family member
40	M	7	Stroke	Yes	Yes	No	Yes	Yes	na
41	F	13	Other	Yes	Yes	Yes	No	Yes	With an occupational therapist
42	M	6	Stroke	Yes	Yes	No	No	Yes	na
43	M	5	Stroke	Yes	Yes	No	No	Yes	na
44	F	2	Stroke	Yes	Yes	Yes	No	No	With a family member
45	F	2	Stroke	No	No	No	Yes	Yes	na
46	F	15	Stroke	No	No	Yes	Yes	Yes	na
47	M	22	TBI	Yes	Yes	No	Yes	No	na

ID	Sex	Time since injury (years)	Etiology	Tracheotomy	Gastrostomy	Verbal communication	Use of an alphabetic code	Use of a wheelchair	Survey completion
48	F	3	Stroke	Yes	Yes	No	Yes	Yes	With a social worker
49	M	6	Stroke	No	Yes	Yes	Yes	No	na
50	M	2	Stroke	Yes	Yes	No	Yes	Yes	With a family member
51	F	6	Other	No	Yes	No	Yes	No	With a family member

Table B.3: Main characteristics of pain in LIS patients included in the survey (WHO = World Health Organization) (individual data).

ID	Pain localisation	Pain features	Time since pain	Pain frequency	Pain communication	Pharmacological treatment (WHO classification)
2	Abdominals lower limb	Electrical shocks	> 10 years	> once a day	Communication code	Paracetamol (painkiller level 1), Rivotril (benzodiazepine)
3	Head, lower limb	No features	2 years	> once a day	Winces	Paracetamol (painkiller level 1)
5	Head, lower limb	Burn	3 years	>once a day	Winces	None
6	Lower limb	Electrical shocks	3 - 6 months	>once a day	Winces, Cries, look/blinking, Vocalizations, Communication code	Painkillers (level unknow), Anti-inflammatory
7	Lower limb	No features	> 1 year	Continue	Cries	None
10	Lower limb	No features	2 years	< once a day	Vocalizations, spasticity	Botox Injection (myorelaxant)
12	Head, lower limb	Burn	6 months - 1 year	> once a day	Communication code	Paracetamol (painkiller level 1)

ID	Pain localisation	Pain features	Time since pain	Pain frequency	Pain communication	Pharmacological treatment (WHO classification)
13	Lower limb	Burn, Electrical shocks	> 12 years	I do not know	Look/blinking, Communication code, no expression of pain	None
15	Head, upper limb, abdominals, lower limb, diffuse	No features	3 - 6 months	Continue	Winces, cries, vocalizations, Communication code	Paracetamol (painkiller level 1), Morphine (painkiller level 3), Carbamazepine (anti-epileptics),Pregabaline (anti-epileptics), Xylocaine (local anesthesic)
16	Upper and lower limb	Electrical shocks	16 years	Continue	No expression of pain	None
17	Back, abdominals	Electrical shocks	3 years	Once a day	No expression of pain	Lyrica (antiepileptic), Paracetamol (painkiller level 1)
19	Lower limb	No features	6 months - 1 year	> once a day	Winces, Look/blinking, Communication code	Paracetamol (painkiller level 1), Tramadol (painkiller level 2)
22	Abdominals	Painful cold sensation	3 years	> once a day	Winces, Vocalizations, Communication code	Paracetamol (painkiller level 1)
27	Back	No features	> 1 year	< once a day	Vocalizations, Communication code	Paracetamol (painkiller level 1)

ID	Pain localisation	Pain features	Time since pain	Pain frequency	Pain communication	Pharmacological treatment (WHO classification)
31	Head, upper and lower limb, back	Painful cold sensation, no features	1 - 3 months	I do not know	Winces, cries, vocalizations, Communication code	Paracetamol (painkiller level 1)
32	Back, lower limb	Electrical shocks	3 - 6 months	Once a day	No expression of pain	None
34	Head, lower limb, diffuse	Painful cold sensation, Electrical shocks	Since the beginning of the LIS	Continue	Winces, Look/blinking, Communication code	Paracetamol (painkiller level 1), Anti-depressant
35	Head, lower limb	Burn	5 years	> once a day	Winces	None
38	Upper and lower limb	Painful cold sensation, Electrical shocks	6 months - 1 year	I do not know	No expression of pain	None
39	Upper and lower limb	Electrical shocks	1 - 6 months	I do not know	Winces, Look/blinking	Do not know
41	Lower limb	No features	1 - 3 months	> once a day	Verbalization	None

ID	Pain localisation	Pain features	Time since pain	Pain frequency	Pain communication	Pharmacological treatment (WHO classification)
44	Upper limb	Burn, electrical shocks	6 months - 1 year	> once a day	Vocalizations	Anti-inflammatory patch
48	Lower limb	No features	3 - 6 months	Once a day	Communication code, no expression of pain	Paracetamol (painkiller level 1), Contramal (painkiller level 2)
50	Lower limb	No features	3 - 6 months	> once a day	Look/blinking, Communication code	None
51	Lower limb	Burn	2 years	> once a day	Winces, Look/blinking, Communication code	None

Table B.4: Bivariate analysis of the relationship between the use of pain treatments and the presence of sleep disturbance or cognitive disabilities, or pain intensity.

Variable, n (%)	Pharmacological				Non-pharmacological				
	Overall, N = 25	No, N = 10	Yes, N = 15	P-value*	Overall, N = 25	No, N = 21	Yes, N = 3	Do not know, N = 1	P-value*
Influence of sleep				0.33					0.40
No	13 (52%)	5 (20%)	8 (32%)		13 (52%)	10 (40%)	3 (12%)	0 (0%)	
Yes	10 (40%)	3 (12%)	7 (28%)		10 (40%)	9 (36%)	0 (0%)	1 (4%)	
Do not know	2 (8%)	2 (8%)	0 (0%)		2 (8%)	2 (8%)	0 (0%)	0 (0%)	
Decrease in concentration/attention				0.1					0.76
No	11 (44%)	2 (8%)	9 (36%)		11 (44%)	9 (36%)	1 (4%)	1 (4%)	
Yes	14 (56%)	8 (32%)	6 (24%)		14 (56%)	12 (48%)	2 (8%)	0 (0%)	
Increase in mood swings				0.69					0.70
No	16 (64%)	7 (28%)	9 (36%)		16 (64%)	14 (56%)	2 (8%)	0 (0%)	
Yes	9 (36%)	3 (12%)	6 (24%)		9 (36%)	7 (28%)	1 (4%)	1 (4%)	

Variable, n (%)	Overall, N = 25	Pharmacological		P-value*	Non-pharmacological				P-value*
		No, N = 10	Yes, N = 15		Overall, N = 25	No, N = 21	Yes, N = 3	Do not know, N = 1	
Decreased memory capacity				0.18					0.66
No	19 (76%)	6 (24%)	13 (52%)		19 (76%)	15 (60%)	3 (12%)	1 (4%)	
Yes	6 (24%)	4 (16%)	2 (8%)		6 (24%)	6 (24%)	0 (0%)	0 (0%)	
Tiredness				0.43					0.39
No	12 (48%)	6 (24%)	6 (24%)		12 (48%)	9 (36%)	2 (8%)	1 (4%)	
Yes	13 (52%)	4 (16%)	9 (36%)		13 (52%)	12 (48%)	1 (4%)	0 (0%)	
Depression				0.63					0.21
No	21 (84%)	9 (36%)	12 (48%)		21 (84%)	18 (72%)	3 (12%)	0 (0%)	
Yes	4 (16%)	1 (4%)	3 (12%)		4 (16%)	3 (12%)	0 (0%)	1 (4%)	
None				0.63					0.53
No	21 (84%)	9 (36%)	12 (48%)		21 (84%)	18 (72%)	2 (8%)	1 (4%)	
Yes	4 (16%)	1 (4%)	3 (12%)		4 (16%)	3 (12%)	1 (4%)	0 (0%)	

Variable, n (%)	Overall, N = 25	Pharmacological		P-value*	Overall, N = 25	Non-pharmacological			P-value*
		No, N = 10	Yes, N = 15			No, N = 21	Yes, N = 3	Do not know, N = 1	
Pain intensity				> 0.99					0.17
Greater or equal to 5	18 (72%)	7 (28%)	11 (44%)		18 (72%)	15 (60%)	3 (12%)	0 (0%)	
Lower than 5	7 (28%)	3 (12%)	4 (16%)		7 (28%)	6 (24%)	0 (0%)	1 (4%)	

* Fisher's exact test

Appendix C

Evaluation of the effect of analgesic treatment on signs of nociception-related behaviors during physiotherapy in patients with DoC: a pilot crossover randomized controlled trial

Table C.1: List of the medication of each patient during the week of hospitalization. (na = not applicable).

ID	Name	Actif principle	Dosage	Action
	Corsodyl	Chlorhexidine	200 mL 3 per day	Antibacterian
	Duratears	na	3,5g 3 per day	Ointment
	Terra-cortril	Hydrocortisone	5mL 1 per day	Ointment
	Acetylcysteine	Acetylcysteine	200mg 1 per day	Mucolytic
	Dafalgan	Paracetamol	1000mg (if pain/fever)	Antalgic
1	Keppra	Levetiracetam	500g and 1000mg 2 per day	Antiepileptic
	Nobiten	Nebivolol	5mg 1 per day	Antihypertensive
	Redoxvita	Vitamin C	5mg 1 per day	Vitamin
	Tradonal	Tramadol	50mg (if pain/fever)	Antalgic
	Trazolan	Trazodone	100mg 1 per day	Anxiolytic
	Zincotabs	Zinc	160mg 3 per day	na
	Clexane	Heparine	40mg 1 per day	Anticoagulant
	Combivent	Ipratropium, salbutamol	3 per day	Bronchodilator
	Corsodyl	Chlorhexidine	200mL 1 per day	Antiseptic
	Isobetadine	Polyvidone-iodine	1 per day	Ointment
	Visidic	Carbomere	10g 3 per day	Ointment
	Vitapantol	Vitamin A	2 per day	Nasal cicatrizer
	Scopolamine	Scopolamine	0,25mg/mL	Parasympatholytic
	Dafalgan	Paracetamol	10mg/mL (if pain/fever)	Antalgic

ID	Name	Actif principle	Dosage	Action
	Losec	Omeprazole	10mg 1 per day	Proton pump inhibitors
2	Oxynorm	Oxycodone	5mg 4 per day	Antalgic
	Lioresal	Baclofen	25mg 3 per day	Myorelaxant
	Prazepam	Prazepam	10 gtts 1 per day	Anxiolytic
	Movicol	Macrogol	13,7g (if constipation)	Laxative
	Moxonidine	Moxonidine	10mg (if hypertension)	Antihypertensive
	Rivotril	Clonazepam	4 gtts 1 per day	Antiepileptic
	Amantadine	Amantadine	100mg 2 per day	Pharmacologic stimulant
	Acetylcysteine	Acetylcysteine	600mg 1 per day	Mucolytic
	Clexane	Heparine	0,4mg 1 per day	Anticoagulant
	Durogesic	Fentanyl	25μg	Antalgic
	Alprazolam	Alprazolam	0,25mg 2 per day	Anxiolytic
	Dominal Forte	Prothipendyl hydrochloride monohydrate	80mg 1 per day	Antipsychotic
	Magnepamyl	Magnesium	1 per day	
3	Movicol	Macrogol	13,9g (if constipation)	Laxative
	Nexiam	Esomeprazol	20mg 1 per day	Gastric anti-secretory
	Temesta expidet	Lorazepam	2,5mg 1 per day (if crisis)	Anxiolytic
	Trazolan	Trazodone	100mg 1 per day	Anxiolytic
	Metformax	Metformine	850mg 2 per day	Antidiabetic

ID	Name	Actif principle	Dosage	Action
	Zanidip	Lercanidipine chlorhydrate	20mg 1 per day	Antihypertensive
	Bisoprolol	Bisoprolol	10mg 2 per day	Antihypertensive
4	Coversyl	Perindopril	5mg 1 per day	Angiotensin converting enzyme (ACE) inhibitors
	Amantadine	Amantadine	25mg 1 per day	Pharmacologic stimulant
	Desloratadine	Desloratadine	5mg 1 per day	Antihistaminic
	Nexiam	Esomeprazol	40mg 1 per day	Gastric anti-secretory
	Dafalgan	Paracetamol	1000mg (if pain/fever)	Antalgic
	D-Cure	Vitamin D	1 per week	Vitamin
	Novomix	Insulin aspartate	40 units/morning, 30units/midday	Antidiabetic
	Rilatine	Methylphenidate	10mg 1 per day	Psychostimulant
	Keppra	Levetiracetam	15mg 2 per day	Antiepileptic
	Sipralexa	Escitalopram	10mg 1 per day	Anxiolytic
5	D-cure	Vitamin D	1 per week	Vitamin
	Lioresal	Baclofen	25mg 3 per day	Myorelaxant
	Clexane	Heparine	100mg/mL 1 per day	Anticoagulant
	Dafalgan	Paracetamol	1000mg (if pain/fever)	Antalgic
	Inotyol	Ichthyolammonium	90g 1 per day	Ointment
	Isobetadine	Polyvidone-iodine	1 per day	Ointment

ID	Name	Actif principle	Dosage	Action
	Terra-cortril	Hydrocortisone	5mL 1 per day	Ointment
	Amlor	Amlodipine	5mg 1 per day	Antihypertensive
	Asaflow	Acetylsalicylic acid	80mg 1 per day	Antithrombotic
	Brilique	Ticagrelor	90mg 2 per day	Antithrombotic
	Coversyl	Perindopril	5mg 1 per day	Angiotensin converting enzyme (ACE) inhibitors
	Dafalgan	Paracetamol	1000mg 3 per day	Antalgic
	Durogesic	Fentanyl	25μg	Antalgic
6	Enterol	Saccharomyces boulardii	250 mg (if diarrhea)	Antidiarrheal
	Lioresal	Baclofen	25mg 3 per day	Myorelaxant
	Motilium	Domperidone	10 mg (if nausea)	Antiemetic
	Movicol	Macrogol	13,9g (if constipation)	Laxative
	Nexiam	Esomeprazol	20mg 1 per day	Gastric anti-secretory
	Prazepam	Prazepam	15mg/mL 3 per day	Anxiolytic
	Sipralexa	Escitalopram	10mg 1 per day	Anxiolytic
	Trazodone	Trazodone	100mg 1 per day	Anxiolytic
	Zolpidem	Zolpidem	10mg 1 per day	Hypnotic, sedative
	Clexane	Heparine	40mg/mL 1 per day	Anticoagulant
	Fraxiparine	Nadroparin calcium	0,4mg 1 per day	Anticoagulant
	D-Cure	Vitamin D	1 per week	Vitamin
	Dafalgan	Paracetamol	1000mg 3 per day	Antalgic

ID	Name	Actif principle	Dosage	Action
	Lioresal	Baclofen	25mg 3 per day	Myorelaxant
	Sipralexa	Escitalopram	10mg 1 per day	Anxiolytic
	Movicol	Macrogol	13,9g	Laxative
7	Nexiam	Esomeprazol	20mg 1 per day	Gastric anti-secretory
	Tradonal	Tramadol	50mg	Antalgic
	Trazolan	Trazodone	100mg 1 per day	Anxiolytic
	Combiven	Ipratropium salbutamol	3 per day	Bronchodilator
	Duratears	na	2 per day	Ointment
	Dulcolax	Bisacodyl	na	Laxative
	Inderal	Propranolol	10mg 2 per day	Sympatholytic
	Pulmicort	Budesonide	na	Bronchial anti-inflammatory
	Clexane	Heparine	40mg/mL 1 per day	Anticoagulant
	D-Cure	Vitamin D	1 per week	Vitamin
	Sipralexa	Escitalopram	10mg 1 per day	Anxiolytic
	Brilique	Ticagrelor	90mg 2 per day	Antithrombotic
	Lamictal	Lamotrigine	75mg 1 per day	Antiepileptic
	Losec	Omeprazole	10mg 1 per day	Proton pump inhibitors
	Amantadine	Amantadine	25mg 1 per day	Pharmacologic stimulant
8	Remergon	Mirtazapine	na	Antidepressant
	Aldactazine	Spironolactone	na	Antihypertensive

ID	Name	Actif principle	Dosage	Action
	Coveram	Perindopril, amlodipine	na	Antihypertensive
	Emconcor	Bisoprolol	5mg 1 per day	Antihypertensive
	L-Thyroxine	Levothyroxine	125mg 1 per day	Thyroid hormone
	Lipitor	Atorvastatine	40mg 1 per day	Hypocholesterolemic
	Lormetazepam	Lormetazepam	2mg 1 per day	Anxiolytic
	Quetiapine	Quetiapine	25mg 1 per day	Anxiolytic
	Lysomucil	Acetylcysteine	na	Mucolytic
	Depakine	Sodium valproate	500mg 1 per day	Anxiolytic
	Tradonal	Tramadol	50mg	Antalgic
9	Trazolan	Trazodone	100mg 1 per day	Anxiolytic
	Nexiam	Esomeprazol	40mg 1 per day	Gastric anti-secretory
	Diazepam	Diazepam	10mg 1 per day	Anxiolytic
	Insuman rapid stylo	Insulin	3mL 100unit/mL, 10 days	Antidiabetic
	Insuman basal stylo	Insulin	3mL 100unit/mL, 18 days	Antidiabetic
	Keppra	Levetiracetam	1000mg 2 per day	Antiepileptic
	Clexane	Heparine	40mg/mL 1 per day	Anticoagulant
	Amantadine	Amantadine	100mg 1/morning, 50mg/evening	Pharmacologic stimulant
10	Nexiam	Esomeprazol	40mg 1 per day	Gastric anti-secretory
	Amlor	Amlodipine	10mg 1 per day	Antihypertensive
	Coversyl	Perindopril	10mg 1 per day	Angiotensin converting enzyme (ACE) inhibitors

ID	Name	Actif principle	Dosage	Action
	Glucophage	Metformine	500mg 3 per day	Antidiabetic
	Spironolactone	Spironolactone	25mg 2 per day	Antihypertensive
	Emconcor	Bisoprolol	5mg 1 per day	Antihypertensive
	Simvastatine	Simvastatine	20mg 1 per day	Anticholesterolemic
	Dulcosoft	Macrogol	10g (if constipation)	Laxative
	Trazolan	Trazodone	100mg 1 per day	Anxiolytic
	Clexane	Heparine	40mg 1 per day	Anticoagulant
	Movicol	Macrogol	13,7g (if constipation)	Laxative
	Acetylcysteine	Acetylcysteine	600mg 1 per day	Mucolytic
11	Coversyl	Perindopril	5mg 1 per day	Angiotensin converting enzyme (ACE) inhibitors
	Dafalgan	Paracetamol	1000mg (if pain/fever)	Antalgic
	Terra-cortril	Hydrocortisone	5mL 1 per day	Ointment
	Isoten	Bisoprolol	5mg 1 per day	Antihypertensive
	Depakine	Sodium valproate	300mg 3 per day	Antiepileptic
	Emconcor	Bisoprolol	na	Antihypertensive
12	na	Omeprazole	10mg 1 per day	Proton pump inhibitors
	Cerazette	Desogestrel	75μg	Contraceptive
	Tradonal	Tramadol	50mg (if pain/fever)	Antalgic
	Prazepam	Prazepam	15mg/mL 2 per day	Anxiolytic
	Trazodone	Trazodone	100mg 1 per day	Anxiolytic

ID	Name	Actif principle	Dosage	Action
	Keppra	Levetiracetam	1000mg 2 per day	Antiepileptic
	Nexiam	Esomeprazol	20mg 1 per day	Gastric anti-secretory
13	Amlor	Amlodipine	5mg 1 per day	Antihypertensive
	Befact forte	Vitamin B complex	1 per day	Vitamin
	Melatonine	Melatonine	3mg 1 per day	Hypnotic, sedative
	Dulcosoft	Macrogol	1 per day	Laxative
	Clexane	Heparine	40mg 1 per day	Anticoagulant
	Isoten	Bisoprolol	10mg 1 per day	Antihypertensive
	Lioresal	Baclofen	10mg 3 x 2 per day	Myorelaxant
	Movicol	Macrogol	(if constipation)	Laxative
	Dafalgan	Paracetamol	1000mg 2 per day	Antalgic
14	Nexiam	Esomeprazol	20mg 1 per day	Gastric anti-secretory
	Clexane	Heparine	40mg 1 per day	Anticoagulant
	Stilnox	Zolpidem	10mg 1 per day	Hypnotic, sedative
	Actrapid penfill	Insulin	3mL 100unit/mL 3 per day	Antidiabetic
	Nobiten	Nebivolol	5mg 1 per day	Antihypertensive
	Clexane	Heparine	40mg 1 per day	Anticoagulant
	Movicol	Macrogol	13,7g (if constipation)	Laxative
	Alprazolam	Alprazolam	0,50mg 1 per day	Anxiolytic
15	D-Cure	Vitamin D	1 every 2 weeks	Vitamin
	Dafalgan	Paracetamol	1000mg (if pain/fever)	Antalgic

ID	Name	Actif principle	Dosage	Action
	Terra-cortril	Hydrocortisone	5mL 1 per day	Ointment
	Trazodone	Trazodone	100mg 1 per day	Anxiolytic
	Lioresal	Baclofen	10mg 3 per day	Myorelaxant
	Nexiam	Esomeprazol	40mg 1 per day	Gastric anti-secretory
	Fraxiparine	Nadroparin calcium	0,4mg 1 per day	Anticoagulant
	Amantadine	Amantadine	100mg 2 per day	Pharmacologic stimulant
	Emconcor	Bisoprolol	10mg 1 per day	Antihypertensive
	Movicol	Macrogol	(if constipation)	Laxative
	Amlor	Amlodipine	10mg 1 per day	Antihypertensive
16	Duovent	Ipratropium	na	Bronchodilator
	Laxoberon	Sodium picosulfate	7,5mg/mL, 3 per day	Laxative
	Motilium	Domperidone	10 mg (if nausea)	Antiemetic
	Losec	Omeprazole	10mg 1 per day	Proton pump inhibitors
	Moxon	Moxonidine	10mg 1 per day	Antihypertensive
	Morphine	Morphine	60mg 1 per day	Antalgic
	Midazolam	Midazolam	5mg per hour	Anxiolytic
	Valium	Diazepam	10mg/2mL 3 per day	Anxiolytic
	Zonegram	Zonisamide	300mg, 1 per day	Antiepileptic
	Pantoprazole	Pantoprazole	20mg, 1 per day	Gastric anti-secretory
	Normacol	Sodium dihydrogenophosphate	130mL	Laxative

ID	Name	Actif principle	Dosage	Action
17	Lovenox	Enoxaparine	0,4mL	Anticoagulant
	Propanolol	Propanolol	40mg 2 per day	Antihypertensive
	Sertraline	Sertraline	560mg 1 per day	Antidepressant
	Doliprane	Paracetamol	500mg (if pain/fever)	Antalgic
	Baclofen	Baclofen	na	Myorelaxant
	Circadin	Melatonine	2 per day	Hypnotic, sedative
	Tradonal	Tramadol	(if pain)	Antalgic
	Clexane	Heparine	na	Anticoagulant
	Alprazolam	Alprazolam	100mg 1 per day	Anxiolytic
	Movicol	Macrogol	(if constipation)	Laxative
	Losec	Omeprazole	10mg 1 per day	Proton pump inhibitors
18	Lovenox	Enoxaparine	0,4mL	Anticoagulant
	na	Paracetamol	(if pain/fever)	Antalgic
	Baclofen	Baclofen	15mg, 1 per day	Myorelaxant
	Keppra	Levetiracetam	500mg 1 per day	Antiepileptic
	Aspegic	Acetylsalicylic acid	100mg (if pain)	Antalgic
	Metoprolol	Metoprolol	100mg	Antihypertensive
	Escitalopram	Escitalopram	5mg 1 per day	Antidepressant

Table C.2: Procedure to follow regarding the choice of medication.

Level of medication	Active Agent	Dosage
Level 1	1) Paracetamol or Acetaminophene 2) Ibuprofen 3) Diclofenac	1g 600 mg 50mg
Level 2	1) Tramadol	50 mg
Level 3	1) Oxycodone 2) Morphine	5 mg 10 mg
Increase of a prior level 3 medication	1) 1/6 of the total dose of daily level 3 medication (e.g. the patient receives 30mg of oxycodone a day. He will be administered a single dose of 5 mg for the study), defined as a breakthrough dose in the management of chronic pain. To standardize the treatment, the dose of the prior level 3 medication will be converted to oxycodone. 2) If the oral intake or the gastrostomy use is not possible, the breakthrough dose will be administered by subcutaneous route. The dose will also be 1/6 of the daily dose and will be converted to injectable morphine.	

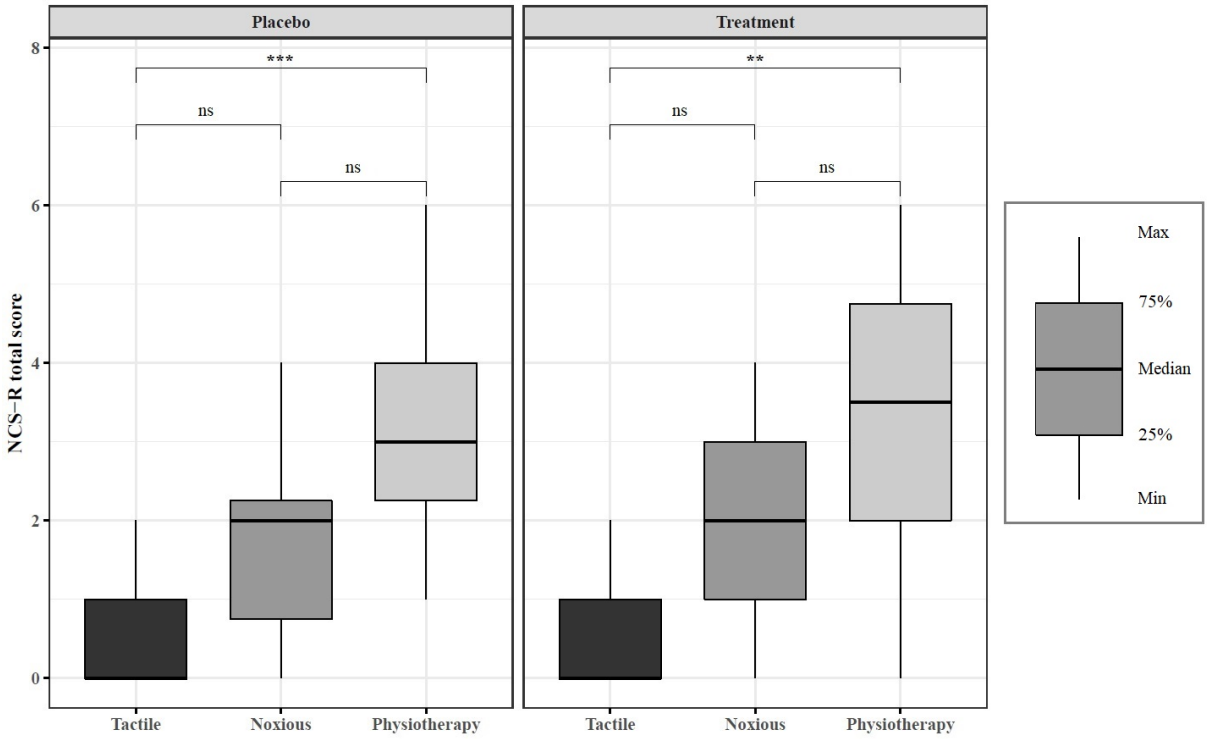

Figure C.1: Changes in the NCS-R facial expression subscore following tactile, noxious stimulation and physiotherapy during the clinical trial (n=10) after the placebo/treatment administration (NCS-R = Nociception Coma Scale-Revised, ns = no significance, * = $p < 0.05$, ** = $p < 0.01$).

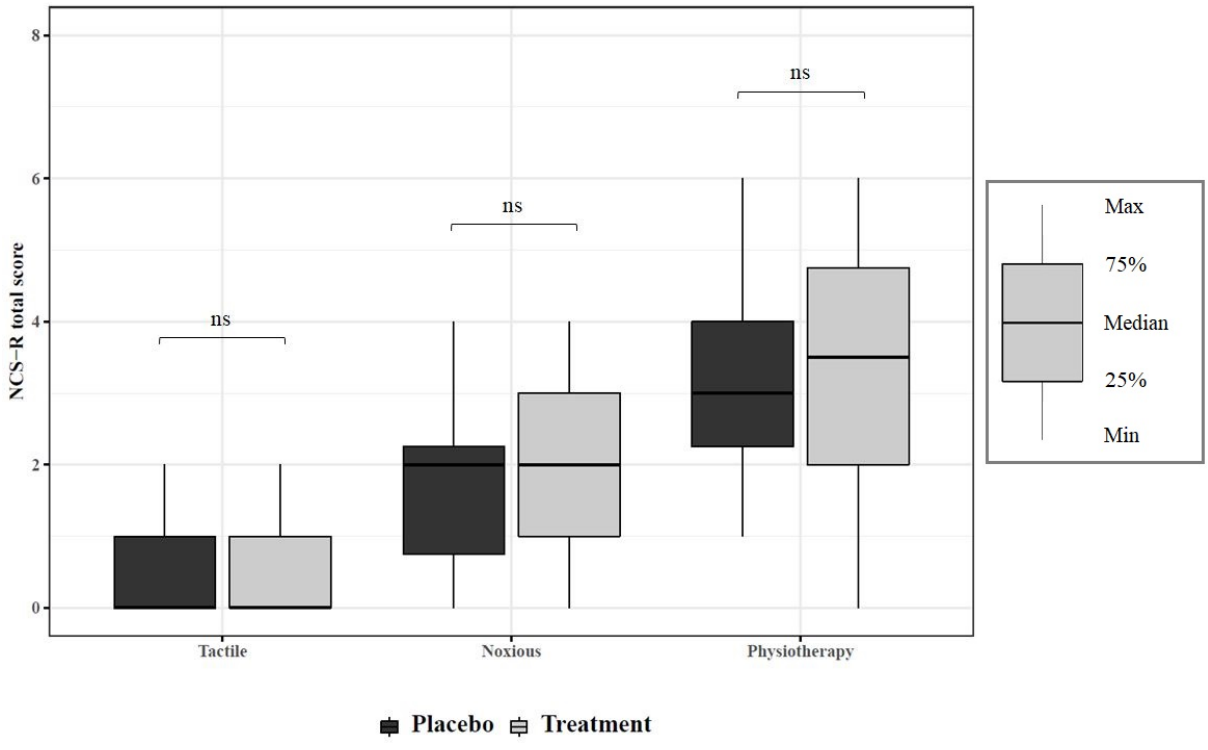

Figure C.2: Comparison of the NCS-R total scores after the placebo and treatment administration (n=10) following tactile, noxious stimulation and physiotherapy (NCS-R = Nociception Coma Scale-Revised, ns = no significance).

Table C.3: MAS score and NCS-R total score during physiotherapy before and after treatment/placebo administration in patients with potential pain (UL = Upper Limb, LL = Lower Limb, na = not applicable).

ID	Baseline			Placebo				Treatment			
	MAS score UL max	MAS score LL max	NCS-R score during PT	Inclusion phase 2	MAS score UL max	MAS score LL max	NCS-R score during PT	MAS score UL max	MAS score LL max	NCS-R score during PT
1	3	4	3	Yes	na	na	2	na	na	0
2	2	0	2	No	na	na	na	na	na	na
3	5	5	3	Yes	na	na	4	na	na	5
4	3	1	6	No	na	na	na	na	na	na
5	4	3	4	Yes	na	na	4	na	na	4
6	4	5	5	Yes	na	na	3	na	na	4
7	3	3	3	Yes	4	2	3	4	2	5
8	4	4	3	Yes	4	4	3	4	4	3
9	1	1	6	Yes	0	2	6	1	2	6
10	0	1	4	Yes	1	2	5	1	1	2
11	2	4	2	Yes	3	4	1	1	4	2

	Baseline				Placebo			Treatment		
ID	MAS score UL max	MAS score LL max	NCS-R score during PT	Inclusion phase 2	MAS score UL max	MAS score LL max	NCS-R score during PT	MAS score UL max	MAS score LL max	NCS-R score during PT
12	4	3	na	No	na	na	na	na	na	na
13	3	4	3	No	na	na	na	na	na	na
14	3	4	3	No	na	na	na	na	na	na
15	3	2	5	No	na	na	na	na	na	na
16	4	4	2	Yes	4	4	2	4	4	2
17	3	3	1	No	na	na	na	na	na	na
18	3	2	2	No	na	na	na	na	na	na

Appendix D

Pain and spastic features in chronic DoC patients: A cross-sectional retrospective study

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.rehab.2021.101566.