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Coma and vegetative states: state of the art and proposal of a novel approach combining existing coma scales

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Abstract

Brain damage of various aetiologies can lead to different disorders of consciousness (DOC), varying from coma to vegetative, to minimally conscious states. Each state is characterised by a different degree of wakefulness, awareness, pain sensitivity and is differentially handled with respect to treatment, ethical considerations and end-of-life decisions. Thus, its correct identification is crucial while devising or modulating appropriate treatment strategies. Actually, the main coma scales cannot always accurately determine the state of consciousness of an individual, while other tools (*e.g.* imaging techniques) present a certain degree of uncertainty. A complementary approach may be constituted by a 24-hour observation of patients, for a sufficient period of days, using an *ad hoc* behavioural scale, further correlated with physiological and pharmacological parameters measured on patients. The method herein described might help recognising the presence of consciousness of the different DOC patients, and thus discerning a vegetative from a minimally conscious state.

Key words
- coma
- vegetative state
- minimally conscious state
- Glasgow Coma Scale

DEFINITION OF CONSCIOUSNESS

Among the various causes of brain damage, the most common are of traumatic, hemorrhagic-ischemic and metabolic nature (drugs, excess of insulin, diabetes, ethanol) [1]. Before partial or full recovery from severe brain damage, survivors classically go through different clinical entities, generally referred to as disorders of consciousness (DOC) [2, 3]. Defining consciousness and its implications, however, is a difficult task.

The concept of consciousness has been approached from numerous disciplines, ranging from philosophy to psychology to medicine and neuroscience. These differential approaches have resulted in numerous definitions and considerations. According to Laborit [4], consciousness arises from the interaction among multiple factors, such as imagination (which is a mechanism of escape from the problems of reality), autonomic nervous system automatisms and the unconscious part of the mind. From a cognitive neuroscience point of view, consciousness recalls notions like awareness, wakefulness and attention. A simple definition of consciousness often states that consciousness is the ability to be aware of themselves and surroundings [1]. Again, consciousness is defined resting upon its capability of allowing the discrimination between self and external objects and events. Consciousness has, obviously, a neural counterpart, *i.e.* neural pathways involving several brain regions (neural basis of consciousness). From a neural point of view, consciousness might be regarded as an emergent property depending on complex interactions between cortex/forebrain, limbic system and brainstem [5].

A number of neuroanatomical and neurophysiological considerations indicate where conscience may reside. For instance, various cell groups in the brainstem modulate wakefulness by ascending projections to the cerebral cortex [6]. Also, there are presumably glutaminergic projections from the classical reticular ponto-mesencephalic nuclei to the intralaminar nuclei of the thalamus, which in turn project to large areas of the cerebral cortex. As a rule, the ascending reticular activating system is considered one of the crucial

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systems involved in the arousal and wakefulness state *(Figure 1)* [6].

Plum and Posner, who along with Jennet first described the locked-in syndrome and the vegetative state during the 1960s and the 1970s [7, 8], consider consciousness as a state of constant awareness of themselves and their environment, with preserved responsiveness to external stimuli. As a result, consciousness constitutes a distinct entity compared to vigilance/alert/arousal. Specifically, an individual may be alerted, awake, with eyes open, but not conscious, unaware (in whole or in part) of his surroundings, and unable to react appropriately to stimuli received from the environment [9].

Thus, consciousness is not an all-or-nothing phenomenon and its clinical assessment relies on inferences made from responses to external stimuli that are observed at the time of the examination [10]. Current tools do not allow measuring consciousness objectively and unequivocally [11].

NOSOGRAPHY AND IDENTIFICATION OF COMA, VEGETATIVE AND MINIMALLY CONSCIOUS STATES

Disorders of consciousness (DOC) have been divided into different nosological categories/states [12]. Coma is typically characterised by the absence of arousal (and thereby consciousness) and often referred to as unarousable unresponsiveness *(Figure 2)*.

The recovery of spontaneous or elicited eye-opening, in the absence of voluntary motor activity, marks the transition from coma to vegetative state (VS). VS is characterised by wakefulness without awareness, no interaction with the environment and it typically follows a coma; after one month the term persistent vegetative state is used; after three months for a non-traumatic insult or one year for a traumatic insult some authors use the term permanent vegetative state, which implies no chance of recovery *(Figure 3)*.

Signs of voluntary motor activity are indicative of a minimally conscious state (MCS). The transition from VS to MCS is characterised by the appearance of voluntary reproducible behaviours, such as performing simple commands, "yes/no" verbal or gestural answering (regardless of accuracy), understandable verbalization, motor activity that occur in relation to emotionally relevant stimuli (including eye-movement research or continue monitoring with the eye/eyes). To be minimally conscious, patients have to show limited but clear evidence of awareness of themselves and/or their environment.

To emerge from the MCS, a functional communication or the use of objects is necessary [8]. The term functional communication means that the language plays a useful function for the person using it and that it allows changing the social environment in a predictable and controllable way. MCS, for which no generally accepted standards of care have been proposed [8], is one of the most sensitive categories to recognise, whereby clinical studies [11] have shown how difficult it is to distinguish between reflex/automatic movements and voluntary movements [13]. This difficulty results in an

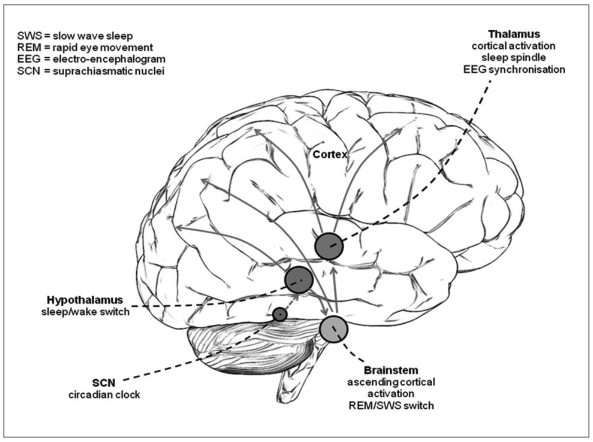

Figure 1
The ascending reticular activating system is responsible for arousal and wakefulness. Adapted from [6].

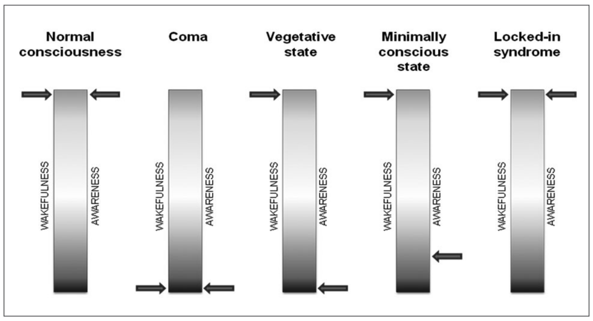

Figure 2
Awareness and wakefulness can be considered the two principal components of consciousness. Normal individuals show a positive correlation between awareness and wakefulness in all their physiological states, contrarily to what happens in the different DOC states. Adapted from [10].

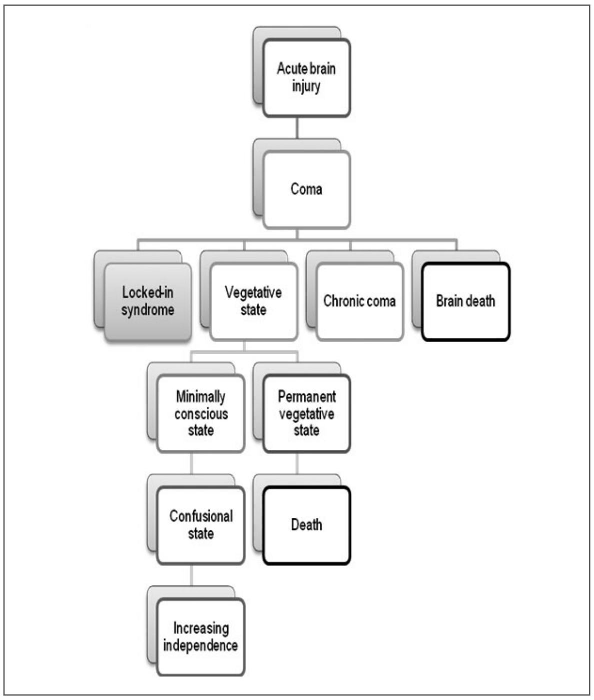

Figure 3
Flow chart of the course of a brain injury. Adapted from [10].

underestimation of behavioural signs of consciousness and hence misdiagnosis, which is estimated to occur in about one-third to nearly half of chronically vegetative patients [8]. Clinical testing for the absence of consciousness in VS is much more ambiguous then testing for absence of wakefulness in coma. VS is one end of a spectrum of awareness and the subtle differential diagnosis with MCS necessitates repeated evaluations by skilled examiners.

COMA SCALES: ADVANTAGES AND LIMITATIONS

The improvement of medical techniques of reanimation, from 1960s onwards, resulted in an increased number of patients surviving traumatic or hypoxic-ischemic brain injuries. As a consequence, various scales for identification of the state of DOC have been developed, providing a valuable diagnostic tool. Use of these scales often relies upon the ability of the observer to accurately and consistently assess the patient. The number of existing scales is remarkably high and a detailed description of all of them would extend beyond the scopes of the present review. We therefore decided to focus on a subset of scales frequently used in hospital settings.

Some scales were developed for a specific use or scope, while others are more general. In general, scales describe the symptoms of the patients (clinical approach, used by the clinical staff). Examples of symptoms are the verbalisation, the vegetative functions or the amount of damage linked to the trauma. Each scale is specific to one or more particular symptoms. Along with the clinical approach, there is also a behavioural approach, whose aim is to study the phenomenon as a whole. Behavioural analysis contemplates the state of DOC patients with a descriptive approach, not necessarily targeted toward a specific symptom but approaching it from a more general perspective.

Currently, there is no unique scale universally adopted for all the various stages of coma and the problems related to them, and patients have to be stimulated to determine their category of coma.

The most widespread coma scale in Europe is the Glasgow Coma Scale (GCS), a nosographic and neurological scale assessing the functional status of the central nervous system, along with the consciousness of a person in a DOC state. This scale, published in 1974 by Teasdale and Jennett [14], was initially developed to assess level of consciousness after traumatic head injury. Its score is obtained from three different tests, assessing eye, verbal and motor responses, and it varies between 3 (deep coma or death) and 14 (or 15, in its revised version, for a fully awake person). The GCS is often paired to a similar American scale, the Rancho Los Amigos Levels of Cognitive Functioning Scale (LOCF), whose score ranges from 1 (non-responsive cognitive functioning) to 8 (normal cognitive functioning).

Another important tool, the Wessex Head Injury Matrix (WHIM), was developed by Shiel *et al.* to monitor all stages of recovery from coma to rehabilitation [15] and consists in a 62 items scale, ordered in a hierarchical way (following the order of appearance observed during recovery) and divided into 6 subscales (communication, attention, social behaviour, concentration, visual awareness, and cognition). A validation study, conducted by Majerus *et al.* [16], evidenced the effectiveness of this scale in monitoring subtle changes in patients emerging from the VS and those in a MCS. Compared to the GCS, which has proven extremely useful in the very acute stages of coma, the WHIM seems characterized by a higher degree of flexibility. On the other hand, the validation study also evidenced the limits of the sequence of recovery proposed by Shiel *et al.*, as the proposed order of recovery could not be replicated for all items of the scale [16].

Another important scale was specifically developed to identify patients emerging from MCS and differentiate them from those in VS: the Coma Recovery Scale Revised (CRS-R). The latter has a structure similar to the GCS, with very detailed subscales assessing arousal, auditory, visual, motor and oromotor capabilities, and verbal communication, with a score ranging from 0 (worst) to 23 (best). Such level of detail allows the identification of subtle signs of recovery of consciousness [2]; yet, its use is heavily time consuming (due to the clinical nature of the assessment) and therefore of limited feasibility in the intensive care setting [17].

Wijdicks *et al.* proposed an alternative to the GCS [18]: the full outline of unresponsiveness (FOUR) scale, which tests four components (eye, motor, brainstem reflexes and respiratory function), with a score ranging from 0 to 4 (a 0 score in all the four categories meaning brain death). The FOUR score is able to provide greater neurological details than the GCS [18]. In particular, the scale tests induced eye movements and for this reason it can be employed in the early recognition of a locked-in syndrome (LIS). Moreover, the FOUR is intended to address eye tracking of a moving object, one of the most important signs indicating the transition to a MCS [11]. Nevertheless, it should be noted that the evaluation of brainstem reflexes, by testing pupil, cornea and cough reflexes, as well as the evaluation of respiratory function, may result complex for untrained hospital personnel [3].

Beside these general scales, a number of other scales were already in use or were developed, in order to cover more specific aspects or to try and complement their limitations. Below we report a brief description of some of these scales. The Hunt and Hess scale [19] was introduced in 1968 to classify the severity of a subarachnoid haemorrhage (SAH) based on the patient's clinical condition but it is now used less frequently and has generally been replaced by the World Federation of Neurosurgical Societies classification (WFNS SAH Grading Scale), which uses the GCS and focal neurological deficit to assess severity of symptoms of SAH [20]. The Fisher Grading Scale on Relation of SAH to Vasospasm uses the computed tomography (CT) to classify the appearance of subarachnoid haemorrhage [21]. This scale has been further modified by Claassen *et al.* to take into account the effects of intraventricular or intracerebral hemorrhages [22]. Haemorrhages may also be caused by abnormalities of the vascular structures or arteriovenous malformations (AVM), which can occur in the brain or along the spinal cord. The pressure of the blood flow can also lead to the formation of aneurysms. The Spetzler Martin Grading Scale evaluates the risk of surgery for a patient with AVM. The scale gives a score between 1 and 6, a grade 1 AVM being considered as low risk for surgery, while grade 6 AVM is considered as not operable [23]. In general, brain injured patients at higher risk of deterioration or mortality are identified using CT scans. One of the most widely reported CT grading systems is the Marshall classification [24].

The Sensory Modality Assessment and Rehabilitation Technique (SMART) is a tool to measure levels of sensory, motor and communicative responses, in order to assess patient's awareness [25]. As for acute stroke patients, the National Institutes of Health Stroke Scale (NIHSS) was developed to provide a quantitative evaluation of their neurological status. Using a 15-item neurologic examination stroke scale, which can be performed in less than 10 minutes, a trained observer evaluates parameters such as level of consciousness, language, extraocular movements and visual-field loss, motor strength, ataxia, dysarthria and sensory loss [26]. The scale represents several different neurological deficits upon a single axis, valued by a score from 0 to 30, higher scores indicating a large stroke and lower scores indicating a small stroke. The Western neuro sensory stimulation profile (WNSSP) is another tool developed to assess cognitive functions in severely impaired head-injured patients and a prognostic tool for slow-to-recover patients. It consists of 32 items whose scores are sufficiently broad to demonstrate patients' improvement in arousal/attention, expressive communication, and in the response to auditory, visual, tactile, and olfactory stimulation [27].

The first scale to be defined for sedated patients is the Ramsay Sedation Scale (RSS), a scale designed to define the grade of arousal of the patients, consisting of six different levels of sedation [28]. An additional scoring system available to intensive care units (ICU) is the Acute Physiology and Chronic Health Evaluation II (APACHE II), a classical tool developed to rate the severity of disease. It is usually applied within 24 hours of admission of adult patients to an ICU and its score varies from 0 to 71, higher scores corresponding to more severe diseases/higher risk of death [29]. More recently, the APACHE II has been replaced by the APACHE III and the Simplified Acute Physiology Score II (SAPS II), whose outcome provides a score ranging from 0 to 163 and a predicted mortality between 0% and 100% [30]. The Injury Severity Score (ISS) is another evaluation tool for trauma severity, based upon the Abbreviated Injury Scale (AIS), a system that classifies each injury in nine different body regions, ranging from 1 to 6 depending on the extent of injury. The ISS identifies six different body regions and its score, ranging from 1 to 75, is calculated from the highest AIS severity code in each of the three most severely injured of these body regions. If any of the three AIS scores is a 6 (a not survivable injury), the ISS score is automatically set at 75. A major trauma is defined as the ISS being greater than 15 [31].

Still regarding traumas, the revised trauma score (RTS) is an efficient tool, objective and relatively fast to perform, with minimal equipment. It consists of a combination of results from three categories, Glasgow Coma Scale (GCS), systolic blood pressure (SBP) and respiratory rate (RR). Each category has a score ranging from 0 to 4, so that the total score ranges from 0 to 12. Such a score correlates well with the probability of survival of the patient. The score indicates the need for a more or less rapid intervention [32]. The trauma score – injury severity score (TRISS), instead, is a logistic regression model for evaluating the outcome of trauma care, quantifying probability of survival as related to severity of injury, by analyzing the anatomical and physiological features and the age of patients [33]. This score comprises several parameters, including the ISS and the RTS. Recently, Nakahara *et al.* developed a simplified alternative logistic regression model to predict survivability of traumatic injuries [34]. The American Spinal Injury Association (ASIA) developed, during the 1980s, a classification of spinal cord injury (SCI) severity (first published in 1982; for a recent revision, see [35], modified from the previous Frankel classification [36]). The classification has a sensory and a motor component. The sensory component has 28 levels, evaluated through a 3-point score, while the motor component has 10 levels, assessed through a 6-point score. The resultant ASIA impairment scale ranges from the complete impairment to the no impairment state.

A NOVEL APPROACH: BEHAVIOURAL AND PHYSIOLOGICAL CONTINUOUS OBSERVATION OF PATIENTS WITH DISORDERS OF CONSCIOUSNESS

One of the priorities occurring after a brain damage is to assess the degree of dysfunction of the central nervous system (CNS). The best measure of the overall brain dysfunction is the level of consciousness as assessed clinically [37]. Thereafter, in order to support the clinical evaluation, neuroimaging techniques (such as the magnetic resonance imaging) and a number of coma scales [38] constitute a remarkable diagnostic tool. Moreover, behavioural and neuro-radiologic protocols – aimed at improving outcome predictions [38] – are constantly being developed to complement classical procedures (such as GCS, magnetic resonance imaging and electroencephalography) used to assess brain dysfunction. The level of consciousness itself also gives an early indication of the potential outcome (as stated by the Glasgow Outcome Scale, GOS). In general, prognosis for DOC patients may lead to three main different outcomes: recovery of consciousness, functional recovery and mortality (see *Table 1*).

In this paragraph, we will focus on the results currently obtained through clinical assessment, neuroimaging techniques and various coma scales. We will then argue that, notwithstanding the fundamental information provided by these techniques, a novel, integrated approach, to be applied to the study of DOC patients, may beget considerable advancements.

As for clinical assessment, there are still no standardised evaluation procedures for the clinical bedside examination of patients with impaired consciousness. Most clinicians rely on systematic evaluations of arousal/wakefulness and behavioural responses to various forms of stimulation. However, such methods have proven inadequate in the detection of minimal signs of responsiveness [38]. The bedside neurological examination of DOC patients should focus on the assessment of the integrity of the CNS (in particular, brainstem pathways and other subcortical structures, mediating pupillary responses, ocular movements, oculovestibular reflexes, breathing patterns) and on the presence of (higher level) cortical functions (such as voluntary behaviour). More in the detail, cortical functioning is examined through the observation of spontaneous activity and the responses to external stimulation. The aim is to distinguish automatic or reflexive behaviours (reliant on spinal or subcortical pathways) from cortically-mediated behaviours (representing a certain degree of awareness). This can be challenging, in particular in case of relatively simple behaviours, such as a finger movement, and requires a certain degree of expertise (specialized training is essential for staff members responsible for providing assessment and treatment services to DOC patients), along with optimal environmental conditions. In general, the more a behaviour is complex, the fewer its occurrence is needed to demonstrate consciousness. For this reason, since most of the behaviours observed are simple, single bedside examinations are often inadequate to conclusively establish level of unconsciousness. As evidence of this, misdiagnosis is still unfortunately frequent (data collected in 2006 evidenced that misdiagnoses ranged from 15% to 43%), regardless of the constant efforts sustained by medical staff [38, 39].

Along with the clinical assessment of the state of arousal and awareness of consciousness in patients with severe brain damage, measurements of cerebral metabolism and brain activations in response to sensory stimuli with positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and electrophysiological methods can provide information on the presence, degree, and location of any residual brain function. MRI is very important and can reveal residual functionalities (isolated cerebral networks may remain active in rare cases). Overall cerebral metabolism in MCS is decreased to values comparable to those observed in the VS [10]. fMRI and ^{15}O-PET and can be combined with traditional structural MRI and electroencephalography (EEG) or magnetoencephalography (MEG), to offer an integrative view of the damaged brain [38]. Brain metabolism can also be quantified in neuroimaging studies using fluorodeoxyglucose PET (FDG-PET) imaging, a measure of cerebral glucose metabolism rate. These studies revealed that overall cerebral metabolism is reduced by more than 50% in VS patients [38]. Laureys *et al.* also demonstrated that there is a general loss of distributed network processing and a loss of brain activation outside primary sensory cortices for elementary auditory and somatosensory stimuli in the VS [40]. In general, cortical functions are compromised in VS cases. Only a few studies have addressed patterns of brain activation in MCS patients. fMRI studies on MCS patients [41] evidenced a widespread activation of the language network when exposed to auditory stimuli provided by family members, but FDG-PET showed a marked reduction of resting metabolic rates near to those presented by VS cases. fMRI can also track emotional processing associated to the activity of the amygdala and the subcortical structures related to emotion, stimulated by a recording of the patients' mother's voice [38]. However, although metabolic and molecular studies might eventually provide useful correlates of the differences between MCS and VS and represent a promising avenue in the assessment of patients with severe brain damage, at present they can only identify functionality at the most general level and cannot be used to advance diagnostic or prognostic distinctions. Moreover, imaging studies entail issues of expense and accessibility, are methodologically complex and need careful quantitative analysis and interpretation. In addition, as for all PET studies in human beings, issues of radiation exposure must be considered and may preclude longitudinal or follow-up studies of these patients [10]. For these reasons, research is currently focusing on more economical and viable solutions, like the interesting model of EEG involving motor imagery to detect command-following tasks, recently developed by Cruse *et al.* [39].

Table 1
Prognosis and functional outcome according to the Glasgow Outcome Scale (GOS) at 1 year after prolonged unconsciousness in adults with traumatic brain injury (TBI) or non-traumatic brain injury (Non-TBI)

	TBI (%)	Non-TBI (%)
Unconscious at least 1 month		
Death	33	53
VS	15	32
SD	28	11
MD	17	3
GR	7	1
Unconscious at least 3 months		
Death	35	46
VS	30	47
SD	19	6
MD/GR	16	1
Unconscious at least 6 months		
Death	32	28
VS	52	72
SD	12	0
MD/GR	4	0

VS: vegetative state; SD: severe disability; MD: moderate disability; GR: good recovery. Adapted from [38].

As discussed in detail in the previous paragraph, a number of standardised neurobehavioural assessment scales were designed to provide a comprehensive overview of neurobehavioural functions and to measure the different levels of consciousness, as long as to detect subtle clinical changes during the rehabilitation of DOC patients [38]. Notwithstanding their validity and reliability (for instance, the CRS-R helped considerably in distinguishing features of VS and MCS), the standardised measuring methods of these techniques do not

Table 2
Sample of behavioural items shared by the principal coma scales currently in use

Spontaneous (non-induced) behaviours	Induced behaviours
Eye opening	Eye response
Eye movements	Eye tracking
Oral reflexive movements	Object recognition
Vocalisation/oral movements	Verbal response
Intelligible verbalisation	Non-functional communication: intentional
Non-functional communication: intentional	Functional communication: accurate
Functional communication: accurate	Motor/limb response
Motor/limb movements	Object manipulation
Abnormal posture	Postural response

Table 3
Sample of physiological parameters monitored and pharmacological infusion by ICU. The ICU measures biomedical parameters (mean arterial pressure, heart rate, central venous pressure, intracranial pressure, end-tidal CO_2, oxygen saturation values obtained from pulse oximetry, temperature t_1 and t_2) and concentration, volume, type of medication and diffusion rates of drugs infusion

Physiological parameters	Pharmacological treatment
Arterial blood pressure (BP)	First analgesic, *e.g.* Fentanest (Fentanyl)
Intracranial pressure (ICP)	Second analgesic, *e.g.* Ultiva
Heart rate (HR)	Third analgesic, *e.g.* Diprivan
End-tidal carbon dioxide (CO_2)	Curare, muscle relaxant
Temperature	

allow case-specific questions to be addressed. Most of the standardised scales currently in use distinguish behavioural items between two categories, spontaneous and induced (a stimulus specifically provided to provoke a response). In general, the limited number of observations collected does not allow differentiating random movements from voluntary low frequency movements; thus, a longer behavioural observation could help recognizing between voluntary and involuntary movements. A standardised approach is able to help recognizing the integrity of neurological processes, but an individualized approach would be needed in order to diagnose the subtle differences between the VS and the MCS [38]. Although current research is focusing on facilitating recovery of consciousness, and elucidating the pathophysiology underlying DOC, little is known about the neurophysiologic substrate. These goals could be achieved favouring collaborative partnerships across rehabilitation centres and disciplines, such as neuroscience, biophysics, neurosurgery, neurology and bioethics [38].

The aim of most of the scales currently in use is to study some selective aspects of DOC patients at a given time, but no system is designed to take into account the condition of patients throughout the entire day. One possibility is to develop a scale whose behavioural items are derived from the main coma scales previously exposed and represents the most informative parameters expressed by DOC patients (see *Table 2*). Such a scale could be used for the observation of the patient over 24 hours (through video recording), for several days, both in the presence and absence of induced behaviours.

One of the issues related to spontaneous (non-induced) behaviours is that a behaviour (shown for instance by a potential MCS patient) might be a response unintentionally induced by any cause not easily/immediately recognisable (*e.g.*, a particular pitch of the voice, a nurse opening the window in the room of the patient, and so forth). The presence of such "hidden stimulations" cannot be avoided, but increasing the time of observation can lower the weight of these biases, for the number of spontaneous data collected may help limiting the risk of overestimating the relevance of hidden induced behaviours in the analysis. Spontaneous and induced behaviours can also be statistically compared between them and the prolonged time of observation can lead to the identification of the presence of voluntary behaviours, a signal indicating the transition from VS to MCS. Moreover, a frequent sampling may entail the possibility of recognising the occurrence of rare behavioural events, whose importance should be evaluated by an experienced behaviourist, since the level of statistical significance does not directly measure the magnitude or scientific importance of the observed result [42, 43].

Spontaneous behavioural data can be used to plot a circadian rhythm of the activities of the patient. Also, it is possible to correlate such behavioural scores with some of the biomedical/physiological parameters and pharmacological infusion measured by the apparatuses linked to the bed of the intensive care unit (ICU) *(Table 3)*, and to adopt Cox proportional hazard model (CPHM) and Markov chain Monte Carlo (MCMC) methods [44] to analyse the behavioural/biomedical patterns found, so as to recognise the presence of behavioural/biomedical sequences and possibly associate them to (and consequently consider them predictive of) a particular state of coma (VS or MCS) (see statistical analysis paragraph below). To this purpose, a dedicated software and hardware system has already been developed (Monitoring of Vital Data Mo.Vi.Da.12, PRS Italia, Rome, Italy), capable of reading, extracting and processing the parameters collected by the ICU.

It is important to mention that, even if behavioural/biomedical sequences generated by MCMC methods may be difficult to interpret, any relevant association found could be a further diagnostic method for the identification of the subtle differences that might differentiate the MCS from the VS, implementing the current clinical assessment, neurobehavioural scales and neuroimaging techniques and, by constructing a personal circadian rhythm of the DOC patient, helping to address case-specific issues.

Statistical analysis

Overall, behaviours include all the processes used to respond to internal factors and to stimuli from the physical and social environments. Reactions to changes in internal conditions, as well as to stimuli from the physical and social environments, may be expressed as simple behavioural items as well as elaborate behavioural patterns. Usually, latencies, frequencies and durations of the behavioural items are scored and then analysed by applying parametric and non-parametric analyses of variance (ANOVA). Also, the sequences of items, and the time-points at which they occur, are frequently recorded. Nevertheless, by applying only ANOVA, information about the temporal structure of behaviour is often neglected and many results are missed [45]. An analysis of the temporal sequence of behaviour, revealing the behavioural items occurring in sequence, could supply additional information about the mechanisms and functions of behaviour.

When data consist of sequences of events and the time points at which they occur, the time structure of a behavioural pattern may be analysed by models based on the Continuous Time Markov Chain (CTMC) and its generalisations [44], a stochastic process in which several states are successively visited, with the "velocity" of switching between the states entirely described by a set of transition rates. The transition rate from behaviour to another provides distinct and additional information to that obtained from the frequencies and/or durations of those items. Anyway, behaviour is even better described by generalised models (semi-Markov models), because the CTMC model relies on the Markov assumption (the independency of the transmissions between behavioural categories from both the previous behaviour and the time a behavioural category has already lasted), which is frequently violated in behavioural studies.

Among the generalised models, the Cox proportional hazard model is particularly useful. The hazard function is the chance per unit time of occurrence of a failure, which in a behavioural setting corresponds to the lengths of the behavioural item, while the hazard function itself is the termination rate of a behavioural item or the transition rate from an item to another. The relative transition rate expresses the speed of switching from a behavioural item to another. A multilevel approach to the CTMC models, the Markov Chain Monte Carlo (MCMC) methods, permits the detection of the effects of treatments under investigation with a small number of individuals (due to the detailed information provided by the time-structure of behaviour), which is often the case of DOC patients.

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