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Global structural integrity and effective connectivity in patients with disorders of consciousness

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ABSTRACT

Background: Previous studies have separately reported impaired functional, structural, and effective connectivity in patients with disorders of consciousness (DOC). The perturbational complexity index (PCI) is a transcranial magnetic stimulation (TMS) derived marker of effective connectivity. The global fractional anisotropy (FA) is a marker of structural integrity. Little is known about how these parameters are related to each other.

Objective: We aimed at testing the relationship between structural integrity and effective connectivity.

Methods: We assessed 23 patients with severe brain injury more than 4 weeks post-onset, leading to DOC or locked-in syndrome, and 14 healthy subjects. We calculated PCI using repeated single pulse TMS coupled with high-density electroencephalography, and used it as a surrogate of effective connectivity. Structural integrity was measured using the global FA, derived from diffusion weighted imaging. We used linear regression modelling to test our hypothesis, and computed the correlation between PCI and FA in different groups.

Results: Global FA could predict 74% of PCI variance in the whole sample and 56% in the patients' group. No other predictors (age, gender, time since onset, behavioural score) improved the models. FA and PCI were correlated in the whole population ($r = 0.86$, $p < 0.0001$), the patients, and the healthy subjects subgroups.

Conclusion: We here demonstrated that effective connectivity correlates with structural integrity in brain-injured patients. Increased structural damage level decreases effective connectivity, which could prevent the emergence of consciousness.

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Abbreviations

CRS-R	Coma recovery scale-revised
DWI	Diffusion weighted imaging
DMN	Default mode network
EMCS	Emergence from the minimally conscious state
FA	Fractional anisotropy
fMRI	Functional MRI
LIS	Locked-in syndrome
MCS	Minimally conscious state
PCI	Perturbational complexity index
TMS	Transcranial magnetic stimulation
TEP	Transcranial magnetic stimulation evoked potential
UWS	Unresponsive wakefulness syndrome

Introduction

Understanding the emergence or loss of consciousness in severely brain-injured patients is an ongoing challenge for neuroscientists [1]. The nosology of the resulting disorders of consciousness (DOC) is expanding [2,3]. DOC usually arise after a period of coma, a transient state characterized by the total lack of arousal and awareness [4,5]. Patients can recover wakefulness, as assessed by the presence of eye opening, without awareness, as they only show reflexive behaviour: this characterizes the unresponsive wakefulness syndrome (UWS) [6], previously called the vegetative state [7,8]. The recovery of minor and fluctuating signs of consciousness, such as visual tracking, localization to noxious stimulation, contextual emotional responses, or reproducible movement to commands, indicates the transition to the minimally conscious state (MCS) [9]. MCS has been further broke down into MCS- and MCS+, the latter showing direct or indirect hints of preserved language processing [10,11]. By definition, all these patients are non-communicating: when they recover functional communication, or functional use of objects, they are said to have emerged from the MCS (EMCS) [12]. On the other hand, some patients can lose any ability to move or speak but they in fact are fully conscious: these patients suffer from locked-in syndrome (LIS) but can be erroneously diagnosed as DOC from behavioural clinical assessment [13,14]. The differential diagnosis of these conditions is currently mainly behavioural, based on standardized scales such as the Coma Recovery Scale-Revised (CRS-R [15]).

However, the underlying physiopathology of DOC remains poorly understood [16]. In the attempts to identify the neural correlates of consciousness, functional, structural, and more recently effective connectivity patterns and impairments have been studied in patients with DOC (e.g., refs. [17–21]). Briefly, functional connectivity is a statistical measure of correlation between neuronal activities that has been tremendously used with functional MRI (fMRI) but also with EEG. Structural connectivity reflects the anatomical connections between neurons, which can for example be assessed using diffusion tractography imaging. Effective connectivity is defined as the causal link between neurophysiological events [22], and can be measured with transcranial magnetic stimulation coupled with high density EEG (TMS-EEG). Individually, each kind of connectivity is altered in DOC, but the relation between them remains largely unknown.

Diffusion weighted imaging (DWI) is an MRI sequence assessing the direction and amplitude of water molecules movements, which are impeded by structures such as axonal tracts. The fractional anisotropy (FA) is a measure of the degree of anisotropy of these movements, and it reflects the white matter integrity [23–25]. DWI features can differentiate groups of UWS and MCS patients [24], and can show structural damage in the tracts connecting the precuneus with the anterior forebrain [26], as well as the tracts connecting the thalamus to the posterior cingulate, whose integrity was correlated with the residual level of consciousness in DOC [27]. More generally, the thalamo-cortical structural connectivity has been shown to be the most affected in UWS patients, while MCS+ exhibited preserved connections with the temporal lobe and the premotor areas, as compared to MCS- patients [28].

TMS–EEG is a non-invasive brain stimulation technique that can record the effects of a perturbation on the ongoing electrical activity of the brain. This technique can assess the effects of a direct cortical stimulation on distant areas and thus provides a measure of effective connectivity. This connectivity can be modulated in UWS patients, using repetitive TMS [29] or transcranial direct current stimulation [30]. The TMS evoked potentials (TEPs) are slow, localized at the stimulation site, and short lasting in UWS patients, while they are more complex, with faster oscillations spreading away from the stimulation target, in MCS patients [31,32]. The perturbational complexity index (PCI) was designed to summarize the capacity of the brain to sustain complex interaction after a perturbation, hence its global effective connectivity [33]. The sensitivity of this index to distinguish unconscious from –minimally– conscious conditions at the single patient level was validated on a large benchmark population [21]. We subsequently demonstrated its specificity using cerebral 18-fluorodeoxyglucose positron emission tomography (FDG-PET) [34]. In healthy subjects, PCI is low during physiological (NREM sleep) [35] and pharmacological (general anaesthesia) unconsciousness [36,37]. Effective connectivity was also demonstrated to be altered in UWS patients using EEG data and dynamic causal modelling [38] or partial direct coherence [39]. Dynamic causal modelling assessment of effective connectivity was also performed in fMRI, demonstrating an altered connectivity of the posterior cingulate within the default mode network (DMN) of UWS more than of MCS patients as compared to healthy controls [20].

Little is known about how structural integrity allows the emergence of functional or effective connectivity in severely brain-injured patients. A better understanding of the relationship between these connectivities may contribute to unravel the neural correlates of consciousness, and of brain physiology in general. For example, we do not know to what extent TEPs complexity (i.e. the signal spatiotemporal spreading after the perturbation) is related to measurable global structural connectivity. Multimodal approaches, studying different kinds of connectivity, are unfortunately scarce in this population. Such an approach was used to study two DOC patients with functional hemispherectomy [40], showing that the same functional, metabolic, and electrophysiological dysfunction can be underlined by different structural damage (major loss of tracts versus relatively preserved architecture) and lead to different disorders of consciousness (UWS versus MCS). Annen et al. demonstrated, using FDG-PET and DWI, that there was a correlation between functional and structural connectivity in the DMN of DOC patients, which was even stronger in the thalamus of those who emerged from the MCS [41]. Multimodal without integrative approaches, using structural and functional connectivity [42], or using functional connectivity, metabolic and structural data were also reported in patients with DOC [43], but lacked any insight into the structure-function relationship. The interest of effective-structural connectivity relationship has been studied in other populations. In a multimodal TMS, fMRI, and DWI study on schizophrenia, impaired effective connectivity between thalamus and insula and between thalamus and superior frontal gyrus was found. This deficit was not associated with impaired structural connectivity,

and functional connectivity was also preserved [44]. The authors suggest that the underlying pathology might be located within the thalamus itself, thus not accessible using DWI and fMRI. This illustrates the added value of multimodal imaging studies in such complex disease, as some information can be accessible only by one of the techniques. While studying language processing in healthy subjects, using Granger causality on fMRI data, effective connections between the primary auditory cortex and the lateral planum temporale and anterior superior temporal gyrus, and between the lateral planum temporale and the posterior superior temporal gyrus were detected. This lead to the discovery of fibre tracts structurally connecting these regions, once again underlying the potential of multimodal neuroimaging [45].

Our hypothesis is that the complexity of TEPs, hence of the PCI, is dependent on the underlying structure. Indeed, the PCI is high if the TEPs are complex, if they spread in time and space in a non-stereotypical way. To be able to do so, a minimum amount of anatomical connections between distant brain areas are necessary. The FA can be used to assess the structural integrity, and is high in a normal brain, as there are many tracts going in different directions. Our aims in this study are to non-invasively investigate the link between global structural integrity (global FA) and effective connectivity (PCI) in patients with DOC.

Materials and methods

Population

Thirty-nine non-acute patients were assessed using TMS–EEG and DWI. The PCI of 24 were reported in previous TMS–EEG studies [21,33,34]. All patients suffered from an acquired brain injury leading to a period of coma, then to various levels of impaired consciousness or to LIS. All patients were included more than four weeks after the injury, when deemed medically stable. They were excluded if they had prior neurological, neurosurgical or psychiatric disorders, or if they had any contraindication to TMS–EEG and MRI (i.e., active epilepsy, electronic implanted devices, external ventricular drain). We also recruited 14 healthy subjects as a control population, with similar exclusion criteria. All participants or their legal surrogates gave their informed consent to take part to the study. The Ethics Committee of the Medical School of the University of Liege approved the study.

Behavioural assessments

Behavioural diagnosis was established after repeated CRS-R assessments, including the day of MRI and TMS–EEG [15,46]. The CRS-R is a standardized and validated scale to study the residual level of consciousness of brain-injured patients. It consists of six subscales (auditory, motor, visual, oromotor/verbal, communication, and arousal), each comprising items of increasing complexity, allowing to detect subtle signs of consciousness (MCS) or of functional communication or object use (EMCS) [12]. MCS was further divided between MCS+ when the patients were able to respond to command, and MCS- when they showed other signs of minimal consciousness [10]. LIS diagnosis was performed prior to the inclusion in this study, and was confirmed by the ability of these patients to communicate using eye-movements [13].

TMS–EEG

Single pulse TMS–EEG was performed and recorded similarly to our previous studies [21,31,33]. We used a figure-of-eight coil driven by a mobile stimulator (Nexstim Ltd., Finland) to stimulate the left or right superior parietal lobule and superior frontal lobule, avoiding obvious structural lesion as detected on the subjects' MRI [47]. These two targets were identified with a neuronavigation system (Nexstim Ltd., Finland) using infrared camera and a software aiming device preventing any stimulation more than 2 mm away from the target. We recorded around 200 to 300 trials on each site for healthy subjects, and around 400 trials for most of the patients (to preserve sufficient data quality despite the expected artefacts in this population). The exact number of trials varied, and is reported for the session with the best PCI in Table 1. The intensity was adjusted for optimal response amplitude while avoiding TMS or muscle artefacts (evoked electric field of 100–150 V/m). By using a 60 channel sample and hold amplifier (Eximia, Nexstim Ltd., Finland), we recorded EEG while avoiding the large artefact evoked by the TMS pulse. Auditory evoked potentials were also prevented using a white noise masking throughout the stimulation sessions. Data were pre-processed on MATLAB 2007 (Matworks, Natick, MA). All the artefacted trials (e.g., electrode movement, eye movement, overwhelming muscle activity) were visually identified and discarded. At least 150 good trials per session were kept for further analysis. Isoelectric channels, or channels with constant or major artefacted activity were also visually selected and discarded. Independent component analysis was sometimes used to remove further artefacts, such as 50 Hz line noise, muscle activity, or blinks. The EEG signal was transposed from the scalp to the cortical level using source reconstruction (based on a 3-spheres BERG method and weighted minimum norm constraint). Sources significantly activated by the TMS pulse were isolated using bootstrap statistics. A matrix of significant sources against time was then created, and compressed as in Casali et al. [33] to compute the PCI. The best PCI of each subject was kept for analysis (PCI max).

MRI

MRI was acquired with a 3T MRI scanner (Allegra, Siemens, DWI: 64 non-collinear directions using a b-value = 1000 s/mm^2, two $b = 0$, $TR = 5700$ ms, $TE = 87$ ms, matrix size = 128x128, 45 slices, slice thickness = 3 mm, gap = 0.3 mm; and T1 3D MPRAGE). Light sedation was required to obtain movement artefact free data in 15 patients with DOC. We used typical pre-processing steps [48,49] to analyse DWI data, employing eddy current distortion correction [50] utilizing FSL diffusion toolbox 2.0 (FSL 5.0, FMRIBs Software Library, http://www.fmrib.ox.ac.uk/fsl, Oxford, UK). We applied the same rotations to diffusion-weighted volumes and their corresponding gradient directions. We then stripped the skull and used a mask to isolate the white matter with the brain extraction tool [51]. We used weighted linear least squares fitted to the log-transformed data to estimate the FA image for each subject. If needed, after visual inspection, we removed any vibration artefact by excluding the volumes with the highest FA values (diffusion gradient in the x direction greater than 0.8) [52]. Finally, we computed the tensor eigenvalue maps for each subject. The global FA value for each subject was obtained by averaging the FA values of the voxels in the white matter mask, using FSL maths [53], as in Ref. [25].

Statistics

Difference in FA values between healthy subjects and patients was tested using independent sample t-test. The Levene's test was used to assess the homogeneity of variance. Linear correlation between FA and PCI, in the whole group, in the patients, and in the healthy subjects was tested using one-tailed Pearson's correlation, as we expected a positive relationship. We used a linear regression model with a single predictor to test if structural integrity, approached with global FA, could predict effective connectivity,

Table 1
Demographical data and neuroimaging results.
Demography (gender, age, diagnosis, aetiology of the brain injury, and time since onset in weeks), and results of max PCI (and the corresponding session's number of trials) and global FA of our population. *F: Female; M: male; HS: Healthy subject; -: Not applicable; TBI: Traumatic brain injury.*

Subject	Gender	Age	Best diagnosis	Best CRS-R	Aetiology	Onset(Weeks)	PCI max	Total n of trials	FA
HS1	F	24	HS	–	–	–	0.495	278	0.344
HS2	F	26	HS	–	–	–	0.606	253	0.356
HS3	M	20	HS	–	–	–	0.648	217	0.352
HS4	M	23	HS	–	–	–	0.608	242	0.369
HS5	M	27	HS	–	–	–	0.576	250	0.345
HS6	F	30	HS	–	–	–	0.569	225	0.347
HS7	F	24	HS	–	–	–	0.487	217	0.342
HS8	M	32	HS	–	–	–	0.510	268	0.353
HS9	F	25	HS	–	–	–	0.660	295	0.373
HS10	F	22	HS	–	–	–	0.553	196	0.358
HS11	M	28	HS	–	–	–	0.621	381	0.363
HS12	F	24	HS	–	–	–	0.511	248	0.364
HS13	F	22	HS	–	–	–	0.551	244	0.369
HS14	F	20	HS	–	–	–	0.667	316	0.362
Pat1	F	35	LIS	22	Ischemic	163	0.475	253	0.355
Pat2	M	45	LIS	15	Ischemic	6	0.584	235	0.372
Pat3	M	23	EMCS	23	TBI	60	0.502	252	0.342
Pat4	F	60	EMCS	16	Haemorrhage	7	0.523	264	0.337
Pat5	M	51	EMCS	22	Ischemic	21	0.452	400	0.347
Pat6	F	32	MCS+	11	TBI	200	0.434	399	0.315
Pat7	F	26	MCS+	11	TBI	145	0.380	400	0.297
Pat8	M	39	MCS+	17	Haemorrhage	37	0.432	401	0.303
Pat9	M	40	MCS+	5	TBI	45	0.491	400	0.316
Pat10	M	20	MCS+	11	TBI	190	0.380	274	0.301
Pat11	M	27	MCS+	19	Anoxic	5	0.379	302	0.335
Pat12	M	46	MCS+	12	Mixed	1371	0.438	321	0.316
Pat13	M	31	MCS-	7	TBI	207	0.409	328	0.297
Pat14	F	50	MCS-	7	Mixed	33	0.400	400	0.289
Pat15	F	25	MCS-	5	TBI	33	0.368	399	0.296
Pat16	F	38	MCS-	10	TBI	15	0.491	291	0.326
Pat17	F	27	MCS-	5	Haemorrhage	13	0.378	400	0.297
Pat18	M	54	MCS-	13	Anoxic	23	0.390	400	0.306
Pat19	F	19	MCS-	10	TBI	188	0.223	400	0.286
Pat20	M	26	MCS-	10	TBI	630	0.413	400	0.289
Pat21	F	44	UWS	6	Anoxic	14	0.267	452	0.304
Pat22	M	81	UWS	6	Ischemic	5	0.238	170	0.293
Pat23	M	21	UWS	7	TBI	24	0.249	194	0.283

represented by PCI. To verify the effect of gender and age as potential predictors, we created a second model with a hierarchical entry design. To check that the results were not only driven by healthy subjects, we performed the same analysis again using only the patients group. In this model, we introduced in a hierarchical entry the CRS-R total score, the time since injury, age, and gender as potential co-predictors. We assessed the assumption of errors independence using the Durbin-Watson statistics, and checked the assumption of no multicollinearity. All analyses were performed using SPSS 20 (IBM Corp., Armonk, N.Y., USA). Results were considered significant at $p < 0.05$.

Results

Out of the 39 patients enrolled, we had to exclude 16 of them because patients moved too much and we aborted the TMS–EEG session (n = 7), or because we could not compute the global FA (n = 5) (when the patients moved too much, and one had an extremely deformed brain), or the PCI (n = 7) (when the signal-to-noise ratio was too low). Some patients had multiple issues and neither the global FA nor the PCI could be obtained. For the following analyses, we used the remaining sample of 23 adult patients (13 males, 11 traumatic brain injuries, median time since injury 33 weeks (5–1371), mean age 37 ± 15 years) and 14 healthy subjects (five males, mean age 25 ± 4 years old) for a total of 37 participants. Based on behavioural assessments, five patients could communicate (two LIS and three EMCS, grouped for analysis as E-LIS). Seven patients were MCS+, eight were MCS-, and three were UWS. Healthy subjects and patients did not significantly differ by gender ($\chi^2(1) = 1.508$, $p = 0.187$), but the controls were significantly younger (mean = 24.8, standard error (SE) = 0.94) than patients (mean = 37.8, SE = 3.16) ($t(26) = -3.83$, $p = 0.001$). Four patients (3 UWS and 1 MCS-) had a PCI max under 0.31, meaning that all but one MCS patients had a PCI max in the same range than healthy subjects. FA was significantly lower in patients than in healthy subjects (mean = 0.31, SE = 0.01 and mean = 0.36, SE = 0.003 respectively) ($t(32) = 7.6$, $p < 0.001$). In other words, FA was 14% lower in the patients group. Demographical data and neuroimaging results (PCI and FA) are reported in Table 1.

Our linear regression model showed that FA could significantly predict 74% of PCI max value in the whole population ($F(1,35) = 100.45$ $p < 0.001$; $R^2 = 0.74$). PCI was significantly correlated with FA ($r = 0.86$, $p < 0.0001$) (Fig. 1). In the patients' subpopulation, the model was still significant ($F(1,21) = 27.17$ $p < 0.001$) and FA predicted 56% of PCI max ($R^2 = 0.56$). There was a significant relationship between PCI and FA in this subgroup ($r = 0.75$, $p < 0.0001$). Neither age, gender, total CRS-R, nor time since injury did have any significant effects on the models (Table 2). A significant, although less strong, positive correlation between PCI and FA was also found in the control group ($r = 0.5$, $p = 0.035$).

Structural integrity and TEPs complexity (their spatio-temporal spreading and the frequency of the oscillations) decrease in a

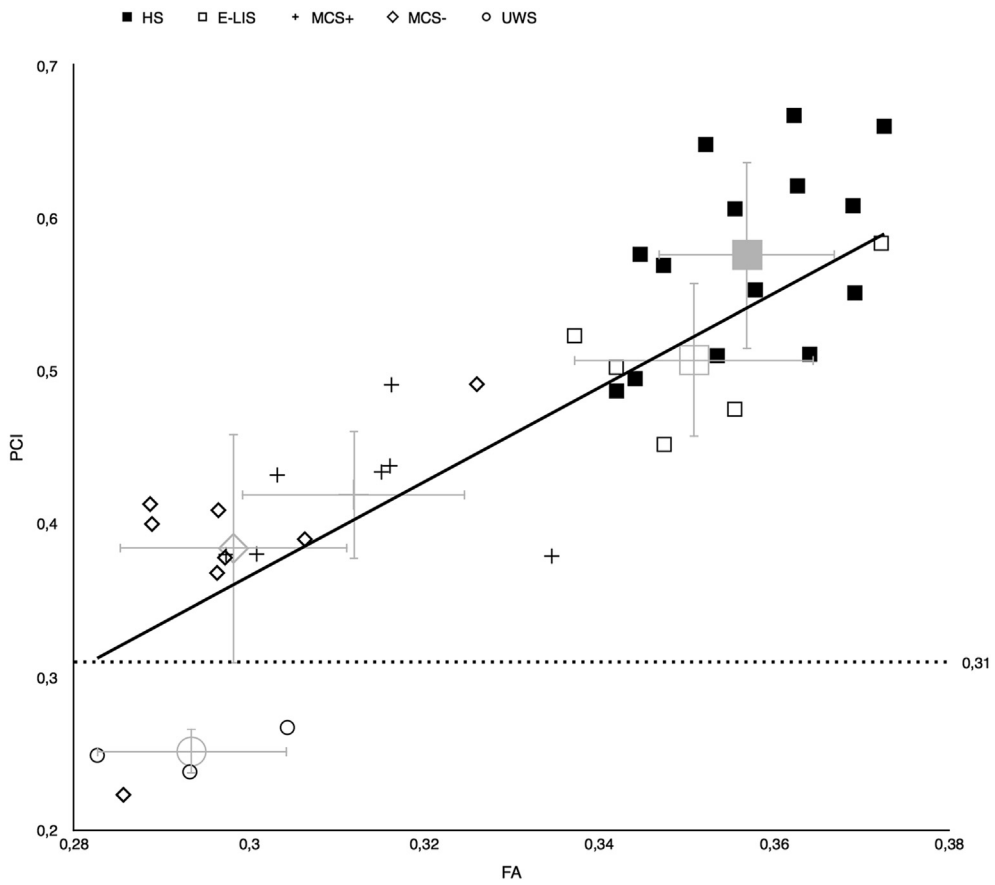

Fig. 1. PCI over global FA in subjects and subgroups.
This scatter plot illustrates the positive linear relationship between FA and PCI in patients and controls ($r = 0.86$ $p < 0.0001$, R2 = 0.74). Subjects are plotted with a different symbol according to their diagnosis (circle for UWS, diamond-shape for MCS-, plus for MCS+, empty square for EMCS and LIS, and black square for healthy subjects). The mean and standard deviation for each subgroup are plotted using lighter grey. Dot line represents the threshold for PCI (horizontal, 0.31). *HS: Healthy subjects; E-LIS: EMCS and LIS group.*

parallel fashion from normal in healthy controls and LIS to very impaired in UWS, with intermediate aspect in the MCS (Fig. 2). In summary, we found that the global FA, our surrogate marker of structural integrity, was linearly and positively correlated with the PCI, reflecting the brain's ability to sustain complex responses to a stimulation, in a population of brain-injured and healthy subjects.

Discussion

In this study, our aim was to investigate the link between structural integrity and effective connectivity at the global level in patients with DOC. With 23 patients and 14 healthy subjects, we demonstrated that structural integrity, approached with global FA, could explain 74% of the effective connectivity variability, represented by PCI. In other words, during wake condition, brain's causal interactions are strongly dependant on structure at the global level. When considering only the patients subgroup, PCI max variance was still mainly explained by FA. Interestingly, we found that adding the time since onset and behavioural assessment (CRS-R best score) did not improve the model. Although patients with better diagnosis have better structural and effective connectivity, we found no effect of behavioural scores alone. This suggests that both better effective connectivity and better CRS-R scores are linked to preserved brain structures. Although wallerian degeneration after structural damage can lead to drastic changes in FA over time [54], we could not find an effect of the time period between the brain injury and the examination. This might be due to the wide range of type and severity of structural damage in our population, including traumatic, anoxic, ischemic, haemorrhagic and mixed brain injuries, ranging from limited pons stroke to diffuse cortical and subcortical contusions or major diffuse anoxic lesions, for example.

The fact that there is a structure-function relationship might seem trivial. Indeed, clinical neurology has for a long time viewed the brain as a sum of functional areas anatomically delimitated (e.g., [55]). Networks are now the centre of much more attention, (e.g., [22]), which partly explains the amount of studies on brain connectivity in various conditions, including (un)consciousness. However, the structural-effective connectivity relationship has not yet been demonstrated in the brain-injured population. Indeed, the level of structural damage, and the potential inherent deformations, may hinder appropriate measure of structural integrity. That is also the reason why we approached it with a global index (FA), as opposed to tractography. Nevertheless, we still had to exclude one patient as her brain was too deformed and segmentation failed. Similarly, PCI requires dedicated TMS-EEG equipment that is not widely available, and it can be tricky to assess non-collaborating patients, who can present lots of artefacts (e.g., involuntary eye movements, head movements, perspiration) and limited number of target areas to stimulate (areas median enough to avoid muscle artefacts can be severely damaged, or inaccessible due to the presence of shunts, for example). Nonetheless, we here demonstrate that PCI, thus the perturbational effective connectivity, which is the most straightforward causal link between brain

Table 2
Linear regression models.
The unstandardized coefficients B and their standard error for the constant and the predictor, as well as the standardized coefficient beta for the predictor are reported here. R square and significance level is also reported for each model.
First model applied to the whole sample, including patients and controls. FA significantly predicted PCI variance ($R^2 = 0.74$). Age and gender did not significantly improve the model (change in $R^2 = 0.01$, $p = 0.48$). In the second model using patients' data only, FA still significantly predicted PCI variance ($R^2 = 0.56$). We here used a hierarchical approach on the patients' data to check for behavioural and time since onset effects, and checked again for potential effect of age and gender in this subgroup. These co-predictors did not have a significant influence on the model (change in $R^2 = 0.03$, $p = 0.84$).

Whole group	B	SE B	β
Step 1			
Constant	−0.66	0.11	
FA	3.43	0.34	0.86*
Step 2			
Constant	−0.6	0.13	
FA	3.35	0.36	0.84*
Age	0	0	-0.11$^{N.S.}$
Gender	-0.01	0.02	-0.05$^{N.S.}$
Patients	**B**	**SE B**	**β**
Step 1			
Constant	−0.49	0.17	
FA	2.85	0.55	0.75*
Step 2			
Constant	−0.63	0.5	
FA	3.45	0.91	0.91*
Age	0	0	-0.08$^{N.S.}$
CRS-R total score	0	0	-0.18$^{N.S.}$
Time since injury	0	0	0.14$^{N.S.}$
Gender	0	0.03	-0.02$^{N.S.}$

Note: R^2 for step 1 = 0.74. *$p < 0.001$. ΔR^2 for step 2 = 0.01 ($p = 0.48$).
Note: R^2 for step 1 = 0.56. ΔR^2 for step 2 = 0.03 ($p = 0.84$) *$p < 0.001$. N.S.: not significant.

areas, strongly correlates with the brain structural integrity in the whole population. We show that the level of structural damage parallels the level of effective connectivity impairment. When considering only the patient's group, there was still a moderate relationship.

Structural integrity accounts for more than half of the effective connectivity variance, but not all of it. There are several potential explanations for that. Some neural tracts might be damaged but remain functional. The opposite might also occur, with fully preserved but disconnected or not functional structures, which prevents them to contribute to effective connectivity [56,57]. Other factors might negatively influence effective connectivity in the presence of a preserved brain structure. Some neurons, or brain areas, might be incapable to react to stimulations due to prolonged hyperpolarization [58]. Neurotransmitters depletion may also impair function despite preserved structure [59], and might be approached using magnetic resonance spectroscopy [60]. This underlines the necessity to use multimodal imaging in this challenging population, and to preferably combine techniques able to study structure, function, and effective connectivity, when trying to unveil the complex neurophysiology of DOCs.

The positive relationship between PCI and global FA was found in a population of brain-injured patients. The consciousness of these patients was altered after a severe brain insult, which left at least some degree of structural damage. This correlation would not have been true in physiological or pharmacological alterations of consciousness. Indeed, in the sleeping or anaesthetized healthy subjects, the PCI would drop rapidly, as the TEPs would become slow, localized, and stereotypical under the influence of the modified brain physiology. However, the brain structure, hence the global FA, would not be altered in these situations. There would be a total lack of correlation between these global parameters. This might not be true using a more regional approach. Indeed, Barttfeld and colleagues demonstrated that the correlation between structural and functional connectivity in monkeys was maximal under deep sedation, while it was actually quite limited during wakefulness [61]. Using implanted electrodes in patients suffering from refractory epilepsy, a modest ($\rho = 0.21$) correlation between structure and effective connectivity was found at the local level [62]. Previous studies have underlined the importance of preserved structure in specific networks such as the DMN [27],

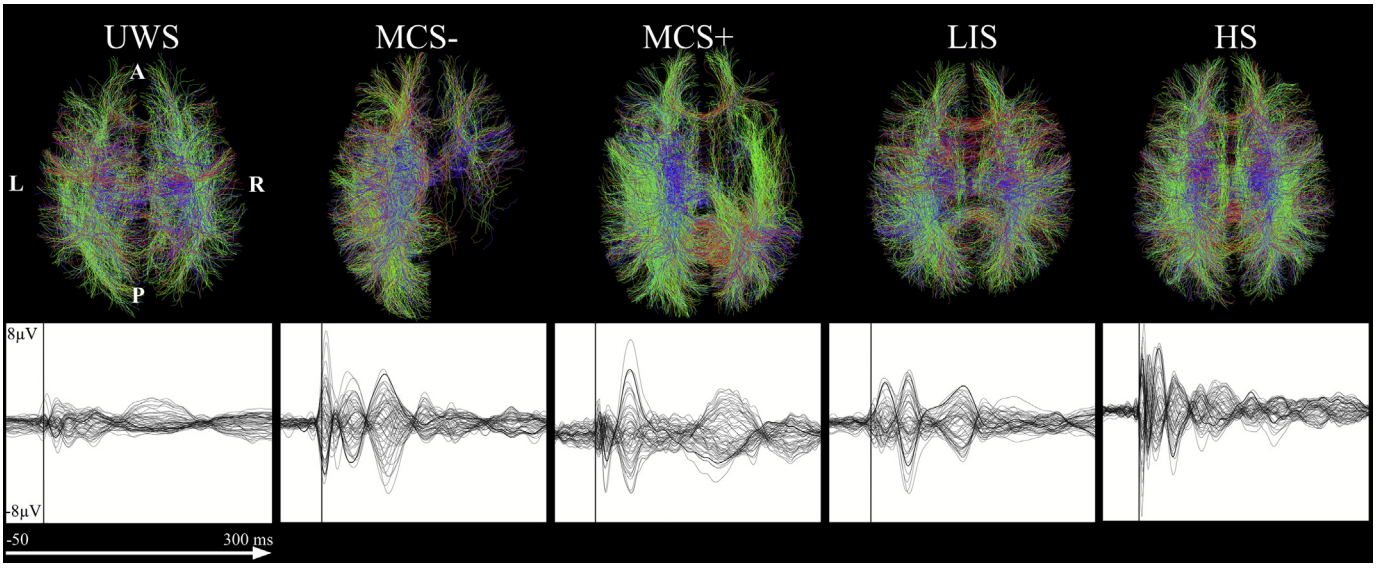

Fig. 2. Tractography image and TMS evoked potential in UWS, MCS-, MCS+, LIS, and healthy subject.
Illustration of the tractography image (top view, minimal tract length 50 mm) and TMS evoked potential of subjects in each category. Although there is a large variability in the aetiology and severity of brain injuries, there is a clear increase in track density from UWS to controls. The TMS evoked potentials are almost flat in this particular UWS subject, and become more complex both in shape and times as we progress to MCS, LIS, and healthy subjects. *A: Anterior; P: Posterior; L: Left; R: Right; UWS: Unresponsive Wakefulness Syndrome; MCS: Minimally Conscious State; LIS: Locked-in Syndrome; HS: Healthy Subject.*

or between the anterior forebrain mesocircuit and the DMN [26]. It is thus possible that a good structural global index might have to take into account the relative preservation of these specific regions. It is also not known whether sufficient complexity can be reached only through the activation of fronto-parietal networks, or any other network supported by a relatively preserved structure.

Future studies should thus further investigate this combined structural-effective connectivity approach with local, rather than global, values. Indeed, building a structural-effective connectome would allow exploring the networks that matters for consciousness, and the underlying structure that would be necessary to do so. Doing so in healthy subjects under anaesthesia would allow studying the dynamics of effective connectivity modifications on a stable structural connectome. This would shed light on important mechanisms behind the loss and recovery of consciousness in a healthy brain.

Our study has some limitations. The first one is the sample size, and especially the low number of UWS. Despite the exclusion criteria, the limited number of patients with chronic DOC, and the difficulty to perform TMS–EEG in this challenging population, we managed to include 39 patients and to compute PCI and global FA in 23 of them. Increasing that number might have increased the number of the UWS subgroup, but without guarantee. Indeed, UWS has a very poor prognosis, even when compared to other DOC [63], and are thus less represented in the chronic DOC population. Another limitation of our study is the significant age difference between our control and the patients' groups. However, effective connectivity as measured with TMS–EEG does not change significantly with physiological aging as demonstrated by Casarotto et al. [64]. There is an age-related modification of global FA, as reported in refs. [65,66], but the change is small, and unlikely to drive the association we found between global FA and PCI.

Conclusion

Despite a vast literature on the importance of structural integrity and effective connectivity in patients with DOC, no study explored how the brain anatomy is related to effective connectivity. Here we demonstrated that the majority of effective connectivity variance is explained by structure, as approached by PCI and FA, respectively. This result underlines that both structural and effective connections need to be relatively preserved for consciousness to emerge. Future studies will have to determine if there is a minimal amount of structural connectivity below which no consciousness can be observed, and if there are specific networks that need to be preserved, identified in a structural-effective connectome.

Conflicts of interest

The authors do not report any conflict of interest.

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