SUPPLEMENTARY ONLINE MATERIAL

for

Intrinsic functional connectivity differentiates minimally conscious from unresponsive patients

Athena Demertzi*[1], Georgios Antonopoulos*[1], Lizette Heine[1], Henning U. Voss[2], Julia Sophia Crone[3,4,5], Martin Kronbichler[3,4], Eugen Trinka[5], Carlo de Los Angeles[6], Mohamed Ali Bahri[7], Christophe Phillips[7], Carol Di Perri[1], Francisco Gomez[8], Luaba Tshibanda[9], Andrea Soddu[10], Audrey Vanhaudenhuyse[11], Vanessa Charland-Verville[1], Nicholas D Schiff[12,13], Susan Whitfield-Gabrieli[6]* and Steven Laureys[1]* (*equal contribution)

Corresponding author

Demertzi Athena

Coma Science Group, Cyclotron Research Center

Allée du 6 août n° 8, Sart Tilman B30

Université de Liège

4000 Liège, Belgium

Tel: +32 (0)4 3662303

email: a.demertzi@ulg.ac.be

Supplementary Material 1

Peak coordinates of the seed regions of interest which were selected according to the literature for the replication of the fMRI resting state patterns of the six studies intrinsic connectivity networks. The regions were defined as 10mm- (for cortical areas) and 4mm-radius* (for subcortical structures) spheres around peak x,y,z coordinates.

Intrinsic connectivity network	**Brodmann area [centered at x, y, z]**	**Reference**
Default mode network		
Posterior cingulate cortex/precuneus	31 [0 -52 27]	(Raichle, 2011)
Medial prefrontal cortex	9 [-1 54 27]	
Lateral parietal cortex [left] [right]	39 [-46 -66 30] [49 -63 33]	
Inferior temporal cortex	21 [-61 -24 -9] [58 -24 -9]	
Cerebellum	[-25 -81 -33] [25 -81 -33]	
Thalamus*	[0 -12 9]	
Brainstem*	[12 -24 -24]	(Boveroux et al., 2010)
Frontoparietal network		
Dorsolateral prefrontal cortex [left] [right]	9 [-43 22 34] [43 22 34]	(Fair et al., 2009)
Inferior parietal lobule [left] [right]	40 [-51 -51 36] [51 -47 42]	
Premotor cortex left [left] [right]	6 [-41 3 36] [41 3 36]	
Midcingulate cortex	23 [0 -29 30]	
Angular gyrus [left] [right]	39 [-31 -59 42] [30 -61 39]	
Precuneus [left] [right]	7 [-9 -72 37] [10 -69 39]	
Brainstem*	[12 -24 -24]	(Boveroux et al., 2010)
Cerebellum	[-4 -56 -40]	
Thalamus [left] [right]*	[-4 -12 0] [4 -12 0]	
Salience		
Orbital frontoinsula [left] [right]	12 [-40 18 -12] [42 10 -12]	(Seeley et al., 2007)
Temporal pole [left] [right]	38 [-52 16 -14] [52 20 -18]	
Paracingulate	32 [0 44 28]	
Dorsal anterior cingulate [left] [right]	24 [-6 18 30] [6 22 30]	
Supplementary motor area [left] [right]	6 [-4 14 48] [4 14 48]	
Superior temporal gyrus [left] [right]	22 [-62 -16 8] [64 -38 6]	
Parietal operculum [left] [right]	40 [-60 -40 40] [58 -40 30]	
Ventrolateral prefrontal cortex	47 [42 46 0]	
Dorsolateral prefrontal cortex [left] [right]	46 [-38 52 10] [30 48 22]	
Thalamus*	[-12 -18 6] [12 -18 6]	
Hypothalamus* [left] [right]	[-10 -14 -8] [6 -16 -6]	
Periaqueductal grey*	[-4 -24 -2]	

Intrinsic connectivity network	**Brodmann area [ROI centered at x, y, z]**	**Reference**
Ventral tegmental area * [left] [right]	[8 -8 -14] [-10 -14 -10]	
Auditory		
Superior transverse temporal gyrus [left] [right]	41/42 [-44 -6 11] [44 -6 11]	(Maudoux et al., 2012)
Precentral gyrus [left] [right]	6 [-53 -6 8] [58 -6 11]	
Anterior cingulate cortex	24 [6 -7 43]	
Visual cortex	19 [-6 -88 37] [6 -88 37]	
Sensorimotor		
Primary motor cortex	3 [-39 -26 51] [38 -26 48]	(Raichle, 2011)
Supplementary motor area	[0 -21 48]	
Visual		
Primary visual cortex	17 [-13 -85 6] [8 -82 6]	(Thomason et al., 2011)
Secondary visual cortex	18 [-6 -78 -3] [6 -78 -3]	(De Luca et al., 2006)
Associative visual cortex	19 [-30 -89 20] [30 -89 20]	

References:

Boveroux P, Vanhaudenhuyse A, Bruno M-A, Noirhomme Q, Lauwick S, Luxen A, et al. Breakdown of within- and between-network resting state functional magnetic resonance imaging connectivity during propofol-induced loss of consciousness. Anesthesiology 2010; 113: 1038-53.

De Luca M, Beckmann CF, De Stefano N, Matthews PM, Smith SM. fMRI resting state networks define distinct modes of long-distance interactions in the human brain. Neuroimage 2006; 29: 1359-67.

Fair DA, Cohen AL, Power JD, Dosenbach NU, Church JA, Miezin FM, et al. Functional brain networks develop from a "local to distributed" organization. PLoS Comput Biol 2009; 5: e1000381.

Maudoux A, Lefebvre P, Cabay JE, Demertzi A, Vanhaudenhuyse A, Laureys S, et al. Auditory resting-state network connectivity in tinnitus: a functional MRI study. PLoS One 2012; 7: e36222.

Raichle ME. The restless brain. Brain Connect 2011; 1: 3-12.

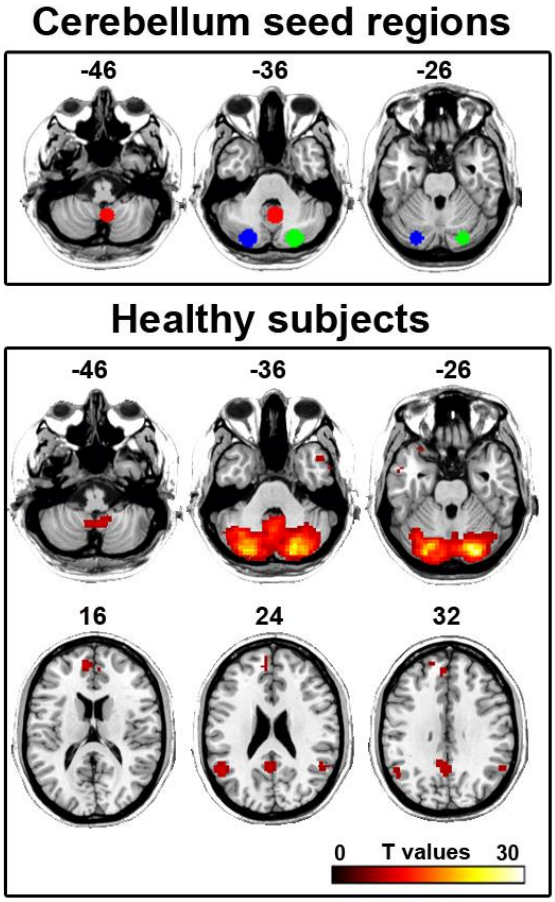
Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. J Neurosci 2007; 27: 2349-56.

Thomason ME, Dennis EL, Joshi AA, Joshi SH, Dinov ID, Chang C, et al. Resting-state fMRI can reliably map neural networks in children. Neuroimage 2011; 55: 165-75.

Supplementary Material 2

The cerebellar network was utilized as a control to the other intrinsic connectivity networks' contribution to the level of consciousness. The cerebellar network was replicated by utilizing seed regions on the posterior lobe bilaterally [-25 -81 -33] [25 -81 -33] (Raichle, 2011) and on the inferior semi-lunar lobule [-4 -56 -40] (Boveroux et al., 2010). The upper panel illustrates the positions of the seed regions. The lower panel summarizes the results of functional connectivity as estimated across the group of healthy controls (n=21). The statistical map is thresholded at FWE $p<0.05$ (whole-brain level) and rendered on a T1 template (transverse view, neurological convention). The numbers on top of the map indicate the number of slice.

Of note is that when patients' Coma Recovery Scale-Revised total scores were used as regressors on connectivity in the cerebellum, no areas showed correlating activity with the behavioural scores.



References:

Raichle ME. The restless brain. Brain Connect 2011; 1: 3-12.

Supplementary Material 3

With the aim to describe which of the studied networks can best differentiate between patients in minimally conscious state (MCS) and vegetative state/unresponsive wakefulness syndrome (VS/UWS), a feature ranking methodology was used (see also *Network ranking and selection* in the *Methods* section of the main manuscript). To that end, we relied on the clinical labels of VS/UWS and MCS which derived from behavioural assessment with the Coma Recovery Scale-Revised and which was also congruent with Positron Emission Tomography scan.

For the feature ranking, as a confirmatory step a linear kernel support vector machine classifier was utilized (Burges, 1998). A leave-one-out cross-validation scheme tested the classifier with a set of 45 feature vectors extracted from patients in MCS (n=26) and in VS/UWS (n=19; Liège dataset). The feature vectors were individual connectivity values extracted from each patient by means of binary masks representing areas which showed higher connectivity in MCS compared to VS/UWS (see *Group-level connectivity analysis* in the *Methods* section of the main manuscript). The estimated classification accuracy was performed with normalization using the L2 norm (Graf et al., 2003) and without normalization.

All networks showed acceptable accuracy in single-patient separation. The auditory network was the most highly ranked system with the ability to classify congruently most of the studied patients in MCS (n =26) and in VS/UWS (n=19; Liège dataset).

	Feature selection criterion (t-test)			**Single-feature classification**		
Network	**t value**	**Rank**	**p value**	**True positives (MCS)**	**True negatives (VS/UWS)**	**Performance accuracy**
Auditory	8.32	1	<.001	25	18	43/45
Visual	7.79	2	<.001	23	15	38/45
Default mode	6.95	3	<.001	23	15	38/45
Frontoparietal	6.82	4	<.001	23	15	38/45
Salience	6.21	5	<.001	24	15	39/45
Sensorimotor	5.87	6	<.001	24	13	37/45

References:

Burges C. A tutorial on support vector machines for pattern recognition. Data Min. Knowl. Discov 1998; 2: 121-67.

Graf AA, Smola AJ, Borer S. Classification in a normalized feature space using support vector machines. IEEE transactions on neural networks / a publication of the IEEE Neural Networks Council 2003; 14: 597-605.

Phillips CL, Bruno M-A, Maquet P, Boly M, Noirhomme Q, Schnakers C, et al. "Relevance vector machine" consciousness classifier applied to cerebral metabolism of vegetative and locked-in patients. Neuroimage 2011; 56: 797-808.

Supplementary Material 4

Demographic and clinical characteristics of patients. Using the Coma Recovery Scale-Revised (Giacino et al., 2004), patients were diagnosed as in a vegetative state/unresponsive wakefulness syndrome (VS/UWS) according to the following criteria (The Multi-Society Task Force on PVS, 1994): 1. no evidence of awareness of self or environment and an inability to interact with others; 2. no evidence of sustained, reproducible, purposeful, or voluntary behavioural responses to visual, auditory, tactile, or noxious stimuli; 3. no evidence of language comprehension or expression; 4. presence of eyes-open eyes-closed periods; 5. sufficiently preserved hypothalamic and brainstem autonomic functions to permit survival with medical and nursing care; 6. bowel and bladder incontinence; 6. variably preserved cranial-nerve and spinal reflexes.

Patients were diagnosed as in a minimally conscious state (MCS) when they demonstrated discernible evidence of awareness of self or environment, on a reproducible or sustained basis, by at least one of the following behaviours (Giacino et al., 2002): 1. purposeful behaviour (including movements or affective behaviour that occur in relation to relevant environment stimuli and are not due to reflexive activity), such as visual pursuit or sustained fixation occurring in direct response to moving or salient stimuli, smiling or crying in response to verbal or visual emotional (but not neutral) stimuli, reaching for objects demonstrating a relationship between object location and direction of reach, touching or holding objects in a manner that accommodates the size and shape of the object, vocalizations or gestures occurring in direct response to the linguistic content of questions; 2. command following; 3. gestural or verbal yes/no response (regardless of accuracy); 4. intelligible verbalization. Emergence from the MCS is signalled by the return of functional communication and/or object use.

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	1	f	34	TBI	3034	Reproducible movement to command	Visual pursuit	Flexion withdrawal	Vocalization/oral movement	None	Without stimulation	12	MCS		DAI, mainly in L thalamus and fronto-temporal regions, L brainstem & cerebellum atrophy	Irregular theta

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	2	m	62	Stroke	13	None	Visual pursuit	Flexion withdrawal	Oral reflex	None	With stimulation	7	MCS		Porencephalic post-ischemic cavity in R sylvian area. pontine & supratentorial diffuse hyperintense ischemic lesions, R fronto-parietal and Bilateral hippocampal atrophy	BR 7 Hz irregular
1	3	f	59	SAH	21	Auditory startle	Visual pursuit	Flexion withdrawal	None	None	Without stimulation	8	MCS		Supra-callosal hemorrhage. Bifrontal subcortical hyperintense lesions	BR theta, delta range, non-reactive
1	4	m	30	TBI	246	Reproducible movement to command	Fixation	Flexion withdrawal	Vocalization/oral movement	None	With stimulation	10	MCS		DAI, mainly in frontal lobes, mid-corpus callosum, mesencephalon, pons and R thalamus	BR 13 Hz reactive
1	5	m	83	Hematom a	13	Reproducible movement to command	None	Flexion withdrawal	Oral reflex	None	None	6	MCS		Vast hemorrhagic lesion involving R fronto-temporal-parietal regions, R thalamus, R basal ganglia, R mesencephalon Intraventricular hemorrhage, midline shift	BR delta Hz

Center	# Patient1	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	6	m	34	SAH	1077	Reproducible movement to command	Object recognition	Flexion withdrawal	None	Non-Functional: Intentional	With stimulation	12	MCS		Bilateral frontal parenchymal damage, hypertensive hydrocephalus	BR theta range, reactive
1	7	m	50	TBI	257	Reproducible movement to command	Visual pursuit	Flexion withdrawal	Oral reflex	None	Without stimulation	11	MCS		Hyperintense lesions in brainstem, L thalamus, R frontal lobe and hippocampi. Cortical and subcortical atrophy	Moderate diffuse brain damage
1	8	m	52	Seizure	20	Reproducible movement to command	Visual pursuit	Flexion withdrawal	Vocalization/oral movement	Non-Functional: Intentional	Without stimulation	13	MCS		R cortico-subcortical fronto - temporal hyperintense lesions	BR theta, non-reactive symmetrical
1	9	m	47	TBI	533	Reproducible movement to command	Object recognition	Flexion withdrawal	Oral reflex	None	Without stimulation	13	MCS		Bilateral leucoencephalopathy. Hyperintense lesions in anterior pons, midbrain & frontal lobe, cortical and subcortical atrophy. Hippocampal atrophy R>L	BR diffuse theta

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	10	m	38	Stroke	1854	Auditory startle	Visual pursuit	Flexion withdrawal	Vocalization/oral movement	None	With stimulation	9	MCS		Hyperintense lesions in L thalamus & posterior limb of L internal capsule, extending to the L cerebral peduncle & corpus callosum, ventricular enlargement L>R	BR 5-6 Hz irregular reactive
1	11	m	29	TBI & anoxia	64	Auditory startle	Visual pursuit	Flexion withdrawal	Oral reflex	None	Without stimulation	9	MCS		No lesions on CT	BR 7 Hz, irregular non-reactive, symmetrical
1	12	m	41	Anoxia	9900	Reproducible movement to command	Visual pursuit	Flexion withdrawal	Vocalization/oral movement	None	Without stimulation	12	MCS		Periventricular leucoencephalopathy, hyperintese lesions in bilateral frontoparietal convexity & external capsule, cortical and subcortical atrophy L>R	BR 6-7 Hz, symmetrical
1	13	m	23	TBI	301	Auditory startle	Visual pursuit	Flexion withdrawal	Oral reflex	None	Without stimulation	9	MCS		Parenchymal damage extensively involving the left hemisphere, compensatory enlargement of the ventricles L>R	BR theta, delta range (R>L)
1	14	m	53	Anoxia	1241	None	Visual pursuit	Flexion withdrawal	Vocalization/oral movement	None	Without stimulation	9	MCS		Diffuse cortical and subcortical atrophy	BR 8 Hz, symmetrical

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	15	f	46	Subdural-hematoma	242	Auditory startle	Visual pursuit	Flexion withdrawal	Oral reflex	None	With stimulation	7	MCS		Diffuse periventricular leukoencephalopaty, lesion in L mesencephalon, diffuse cortical atrophy	BR 8 Hz posterior, 4-6 Hz other regions
1	16	m	60	TBI	15	Reproducible movement to command	Object recognition	Automatic motor reaction	Intelligible verbalization	Non-Functional: Intentional	With stimulation	18	MCS		Post-contusion lesions in R mesencephalon & corpus callosum, DAI mainly in R frontal hemisphere	BR theta
1	17	m	25	TBI	1157	Reproducible movement to command	Object recognition	Automatic motor reaction	Oral reflex	None	With stimulation	16	MCS		Post-contusion lesions in bifrontal, L temporal, L thalamus and R midbrain	8 Hz, non-reactive
1	18	m	61	TBI	135	Reproducible movement to command	Object localization	Flexion withdrawal	Oral reflex	None	Without stimulation	12	MCS		DAI in semioval centers, in L mesencephalon peduncle, in corpus callosum and juxtacortex of both hemispheres. Intraventricular hemorrhage. Diffuse white matter damage in rolandic regions & semioval centers, associated with wallerian degeneration. Moderate diffuse cortical and subcortical atrophy	BR 6-7 Hz, reactive

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	19	m	68	TBI	360	None	Visual pursuit	Flexion withdrawal	None	None	Without stimulation	4	MCS		L anterior frontal post-traumatic hemorrhagic lesion. R nucleocapsular hematoma extending up to the R centrum semiovale, bilateral subdural hematoma	BR 6 Hz, symmetrical, non-reactive
1	20	m	35	TBI	1331	Reproducible movement to command	None	Flexion withdrawal	Oral reflex	None	Without stimulation	8	MCS		Cortico-subcortical hyperintese lesions in bilateral frontal and R temporo-occipito-parietal regions. Cortical atrophy, ventricular enlargement L>R	BR 4 Hz symmetrical, low voltage, non-reactive
1	21	f	48	TBI	291	Localisation of sounds	Visual startle	Flexion withdrawal	Vocalization/oral movement	None	With stimulation	9	MCS		DAI in brainstem, in frontal, temporal and right parietal lobes, corpus callosum, R thalamus and R basal ganglia, hyperintense fronto-parietal lesions	BR rhythm 8-9 Hz
1	22	m	73	SAH	35	None	Fixation	None	Oral reflex	None	With stimulation	4	MCS		Hemorrhagic intraparenchymal and cortical lesions in R temporal and L orbitofrontal lobes, bilateral occipital subdural hemorrhage	BR irregular theta/delta range

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	23	m	23	TBI	645	Reproducible movement to command	Visual pursuit	None	Oral reflex	None	Without stimulation	9	MCS		DAI in brainstem, corpus callosum, hippocampi, frontal, parietal - temporal (R>L) and L occipital regions. Biemispheric subdural hygroma. Cortical atrophy in R hemisphere and R cerebellum. Ventricular enlargement R>L	BR theta and delta, L posterior 7-9 Hz , R posterior 3-4 Hz
1	24	m	11	Anoxia	1482	Reproducible movement to command	Object localization:reaching	Flexion withdrawal	Vocalization/oral movement	Non-Functional: Intentional	Without stimulation	13	MCS		Cortical (mainly fronto-parietal) and subcortical atrophy, brainstem atrophy	BR non-reactive delta-theta
1	25	f	48	SAH	215	Reproducible movement to command	None	Automatic motor reaction	Intelligible verbalization	None	With stimulation	12	MCS+		Hyperintense lesions in L fronto-parietal and occipital lobes	BR 6-7 Hz (R>L)
1	26	m	66	TBI	674	Reproducible movement to command	None	Abnormal posturing	Vocalization/oral movement	None	With stimulation	7	MCS		Hyperintense lesions in R brainstem and cerebellum. Hypertensive hydrocephalus	BR non-reactive delta range, diffuse theta dysrhytmia
1	27	f	52	TBI & anoxia	283	Auditory startle	None	Flexion withdrawal	Vocalization/oral movement	None	With stimulation	5	VS/UWS		Diffuse leucoencephalopathy, mainly in rolandic & medial occipital areas. Diffuse cortical and subcortical atrophy	Left anterior paroxistic activity, very low voltage

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	28	m	36	Anoxia	6709	Auditory startle	None	Flexion withdrawal	Oral reflex	None	Without stimulation	6	VS/UWS		Hyperintense lesions in brainstem, cerebellum, basal ganglia, thalami (L>R) posterior cingulate & posterior parietal regions. Diffuse cortical & subcortical atrophy	BR theta, symmetrical, non-reactive
1	29	m	30	Anoxia	743	Auditory startle	None	Flexion withdrawal	Oral reflex	None	Without stimulation	6	VS/UWS		Severe cortical and subcortical atrophy	No cortical activity (artifactual recording)
1	30	m	74	Anoxia	92	Auditory startle	None	Abnormal posturing	Oral reflex	None	With stimulation	4	VS/UWS		Diffuse leucoencephalopathy. Diffuse cortical & subcortical atrophy, most pronounced in rolandic & medial occipital regions. Hyperintense lesions in basal ganglia	BR unstructured non-reactive theta
1	31	f	44	Anoxia	8	None	None	Abnormal posturing	None	None	With stimulation	2	VS/UWS		Diffuse hyperintese anoxic cortical lesions, mainly involving the parieto-temporo-occipital junctions. Hyperintense lesion in caudates	BR diffuse non-reactive low-voltage delta

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	32	m	67	Hemorrhage	43	Auditory startle	None	Flexion withdrawal	Oral reflex	None	With stimulation	5	VS/UWS		Intraparenchimal hemorrhagic lesion involving L temporal, thalamus & basal ganglia	BR 6 Hz, diffuse, symmetrical, non-reacive
1	33	m	63	Stroke	30	Auditory startle	Visual startle	Flexion withdrawal	Vocalization	None	Without stimulation	8	VS/UWS		Bihemispheric ischemic hyperintense lesions, mainly in posterior parietal & occipital lobes	BR unstructured delta
1	34	m	31	TBI	849	Auditory startle	Visual startle	Flexion withdrawal	Oral reflex	None	Without stimulation	7	VS/UWS		DAI in fronto-parietal iuxtacortex and brainstem. Diffuse leucoencephalopathy. Severe cortical-subcortical atrophy	BR delta, diffuse
1	35	m	87	SAH	7	None	None	Flexion withdrawal	Oral reflex	None	With stimulation	4	VS/UWS		Left intraparenchymal temporal hemorrhagic lesion. Perimesencephalic & subarachnoid bioccipital hemorrhage	BR diffuse theta
1	36	m	29	TBI & anoxia	72	None	Visual startle	Flexion withdrawal	Oral reflex	None	With stimulation	6	VS/UWS		Diffuse cortical damage and leucoencephalopathy, more pronounced in rolandic & temporo-occipital regions. Basal ganglia damage. Wallerian degeneration	BR theta/delta, low voltage

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	37	f	41	Anoxia	1572	Auditory startle	None	Abnormal posturing	Oral reflex	None	Without stimulation	5	VS/UWS		Severe cortical & subcortical atrophy. Atrophy of midbrain. Diffuse leucoencephalopathy	BR unstructured theta
1	38	f	50	Anoxia	38	None	None	None	Vocalization/oral movement	None	With stimulation	3	VS/UWS		Small acute vascular lesion in R mesencephalon, multiple hyperintese lesions in both cerebellar hemispheres, damage in R rolandic area, precuneus & medial occipital regions	BR 4-5 Hz, diffuse, symmetrical, non-reactive
1	39	m	44	Anoxia	27	Auditory startle	None	Abnormal posturing	None	None	Without stimulation	4	VS/UWS		Diffuse cortical & basal ganglia (caudates) damage. Subcortical fronto-parieto-temporal hyperintense lesions	BR 6 Hz, irregular, non-reactive, symmetrical
1	40	f	16	Anoxia	27	Auditory startle	None	Abnormal posturing	Oral reflex	None	With stimulation	4	VS/UWS		Periventricular leucoencephalopathy, hyperintense lesions in basal ganglia, semioval centers & fronto-parietal regions, diffuse cortical & subcortical atrophy, brainstem atrophy	BR delta

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	41	f	49	Stroke	129	Auditory startle	None	None	Oral reflex	None	Without stimulation	4	VS/UWS		Hyperintese lesions in pons & external capsule. Diffuse leucoencephalopathy	BR diffuse unstructured non-reactive delta
1	42	m	36	Anoxia	2031	Auditory startle	None	Abnormal posturing	Vocalization/oral movement	None	Without stimulation	6	VS/UWS		Bilateral periventricular leucoencephalopathy, supratentorial cortical & subcortical atrophy, cerebellum atrophy	BR 7 Hz, symmetrical
1	43	m	34	Anoxia	7814	Auditory startle	None	Abnormal posturing	Oral reflex	None	Without stimulation	5	VS/UWS		Diffuse severe cortical- subcortical & cerebellar atrophy. Brainstem atrophy.	BR 6 Hz, symmetrical, non-reactive
1	44	f	49	Anoxia	277	Auditory startle	None	Flexion withdrawal	Vocalization/oral movement	None	With stimulation	6	VS/UWS		Diffuse leucoencephalopathy. Diffuse cortical & subcortical atrophy	BR delta, symmetrical, non-reactive
1	45	m	65	SAH	26	None	None	Abnormal posturing	None	None	With stimulation	2	VS/UWS		Vast L temporo-parieto-occipital hemorrhagic lesion with intraventricular bleeding & midline shift	BR delta

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	46	f	52	TBI	18	None	None	Abnormal posturing	Oral reflex	None	None	2	coma		Ventral-paramedian mesencephalon hemorrhage, R extradural hematoma, L temporal intraparenchymal & subdural hematoma, temporo-parietal laminar necrosis	BR theta and delta, symmetrical
1	47	f	61	SAH	38	None	None	Flexion withdrawal	Oral reflex	None	None	3	coma		Hyperintense lesions in R pons and mesencephalon, bilateral frontal regions & middle corpus callosum. Peri-cerebellar subdural collection	BR 6.5 Hz theta
1	48	m	73	SAH	7	None	None	Flexion withdrawal	None	None	None	2	coma		L frontoparietal intraparenchimal hemorrhage. Subfalcine herniation	BR 6 Hz irregular non-reactive, R lateralized theta
1	49	m	70	SAH	2	None	None	Abnormal posturing	None	None	None	1	coma		Hemorrhagic lesion in R lentiform nucleus, thalamus & R cerebral peduncle	BR irregular delta
1	50	m	78	TBI	14	None	None	None	None	None	None	0	coma		(CT scan) lesions in L frontoparietal & R occipital	BR 4 Hz

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
1	51	f	64	Stroke	7	None	None	None	None	None	None	0	coma		Bilateral brainstem, midbrain & cerebellar non-hemorrhagic ischemic lesions	BR 8Hz, irregular, symmetrical
2	1	f	32	Stroke	916	Reproducible movement to command	None	Flexion withdrawal	Vocalization/oral movement	Non-Functional: Intentional	With stimulation	9	MCS	1	Hyperintense mainly involving somatosensory areas & formatio reticularis	BR 8-10 Hz diffuse theta
2	2	f	56	SAH	590	Auditory startle	Visual pursuit	Abnormal posturing	Oral reflexive movement	None	Without stimulation	8	MCS	1	Hyperintense lesions in corpus callosum, R temporo-parietal & bilateral frontal regions. Diffuse cortical & subcortical atrophy	BR 5-7 Hz sporadic epileptiform potentials
2	3	m	66	TBI & SAH	82	None	Visual startle	Flexion withdrawal	None	None	Without stimulation	5	VS/UWS	0	L frontal cortex and anterior corpus callosum hyperintense lesions, L temporal lobe prolaps; Waller's degeneration in the L brainstem; secondary hydrocephalus	BR 2-5 Hz, symmetrical, diffuse beta activity
2	4	f	36	Anoxia	66	Auditory startle	None	Abnormal posturing	Oral reflexive movement	None	Without stimulation	5	VS/UWS	0	Widespread biemispheric cortical damage	BR 5-10 Hz, symmetrical, sporadic beta activity

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
2	5	f	28	Encephalitis	148	None	Visual pursuit	Flexion withdrawal	Oral reflexive movement	None	Without stimulation	8	MCS	1	Periventricular leucoencephalopathy. Hyperintense lesions in bilateral frontal lobes, basal ganglia, insula and hippocampi	BR 1.5-2 Hz, symmetrical,sporadic beta activity
2	6	f	62	SAH	74	Localisation of sounds	Visual pursuit	Flexion withdrawal	Oral reflexive movement	None	Without stimulation	10	MCS	1	Hemorrhage in R frontal lobe extending to the horns of lateral ventricles. Ventricular enlargement	BR 6-8 Hz, epileptiform potentials
2	7	m	55	Anoxia	82	None	Visual startle	None	Oral reflexive movement	None	With stimulation	3	VS/UWS	0	Hyperintense lesions in bilateral rolandic and paramedian occipital regions	Weak 6-8 Hz activity, bilateral epileptiform potentials, sporadic beta-activity
2	8	m	46	Stroke	41	Auditory startle	Visual startle	Automatic Motor Response	Oral reflexive movement	None	With stimulation	9	MCS	1	Hyperintense lesions in fronto-parietal, L temporal, dorsal occipital regions and L thalamus. Cortical bilateral fronto-parietal atrophy	BR 4-6 Hz, sporadic beta, diffuse delta
2	9	m	54	Anoxia	77	Auditory startle	Visual startle	None	None	None	With stimulation	3	VS/UWS	0	Diffuse cerebral cortex and basal ganglia damage. Diffuse cortical atrophy	BR 4-6 Hz, diffuse delta-theta

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
2	10	m	65	Anoxia	85	Localisation of sounds	Fixation	Object Manipulation	Oral reflexive movement	None	Without stimulation	11	MCS	1	Haemorrhage in L thalamus and putamen, extending to the L insula; cortical and subcortical micro-haemorrhage lesions in both hemispheres	BR 6-8 Hz, diffuse delta-theta
2	11	f	35	Status epilepticus	49	None	None	Flexion withdrawal	Oral reflexive movement	None	Without stimulation	5	VS/UWS	1	Hyperintense lesions in thalamus and caudates. Bilateral Hippocampal atrophy	BR 5-6 Hz, diffuse theta & delta-theta activity
2	12	m	31	TBI	66	None	None	Localization to Noxious Stimulation	Oral reflexive movement	None	With stimulation	5	MCS	1	Hyperintense lesions in the insula, hippocampus, caudal thalamus, midbrain and brainstem. Extended parenchymal damage in L temporal lobe &hippocampus	BR 5-6 Hz, diffuse delta-theta
2	13	m	52	SAH	146	Localisation of sounds	Visual startle	Localization to Noxious Stimulation	Oral reflexive movement	None	Without stimulation	9	MCS	0	Acute intraparenchymal hemorrhage in R fronto-temporal regions and in cortical sulci of both hemispheres. Cortical bifrontal atrophy.	BR 6-7 Hz, diffuse theta

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
2	14	f	71	SAH	355	Auditory startle	Visual pursuit	Flexion withdrawal	Oral reflexive movement	None	Without stimulation	9	MCS	1	No evident parenchymal alterations	BR 6-8 Hz, diffuse delta-theta
2	15	m	61	Stroke	75	None	Visual pursuit	Localization to Noxious Stimulation	Oral reflexive movement	None	With stimulation	8	MCS	1	Severe haemorrhage in R basal ganglia, insula and L thalamus. Haemorrhage in the R gyrus frontalis superior with compression of the R lateral ventricle	BR 4-8 Hz symmetrical, diffuse delta-theta activity, sporadic epileptiform potentials
3	1	f	28	Stroke	2393	Localisation of sounds	Visual pursuit	None	None	None	Without stimulation	7	MCS	1	Bilateral central thalamic infarction, R lateral geniculate nucleus infarct, left entorhinal atrophy, R calcarine cortex infarct, extensive ventral pontine infarction.	8-9 Hz PDR, slightly asymmetric, R>L slowing
3	2	m	39	Anoxia	1203	Consistent movement to command	Visual pursuit	Flexion withdrawal	Intelligible Verbalization	Non-Functional: Intentional	Without stimulation	15	MCS	1	DAI L>R with extensive left sided leucomalacia	Very asymmetric, rarely up to 8 Hz PDR on the L, none on R, otherwise R>L slowing dominated by mostly 4-6 Hz frequencies

Center	# Patient	Gender	Age	Etiology	Interval (days)	Auditory function	Visual function	Motor function	Oromotor/Verbal function	Commu-nication	Arousal	Total score	Diagnosis	Classification (1=MCS, 0=UWS)	Structural lesions on MRI	EEG basic rhythm
3	3	m	27	TBI	3815	Auditory startle	Visual startle	Localization to Noxious Stimulation	Vocalization/Oral Movement	None	Without stimulation	9	MCS	1	Diffuse DAI. Ventricular enlargement mark with marked dilation of third ventricle	PDR 6-8 Hz b/l, slightly slower on L
3	4	m	29	TBI	2318	Consistent movement to command	Object Recognition	Functional Object Use	Intelligible Verbalization	Functional: accurate	Attention	23	EMCS	1	Radial pattern DAI. Brainstem atrophy. moderate ventricular enlargement	bilateral 8-9 Hz PDR, intermittent b/l slowing at times
3	5	f	23	TBI & anoxia	3856	Localisation of sounds	Visual pursuit	Localization to Noxious Stimulation	Vocalization/Oral Movement	None	Without stimulation	12	MCS	1	DAI, diffuse cortical atrophy	9-11 Hz PDR bilaterally, symmetric, excess beta
3	6	m	23	TBI	1588	Consistent movement to command	NT	None	None	Non-Functional: Intentional	None	5	MCS	1	DAI, right orbitofrontal infarction and encephalomalacia, bilateral thalamic Duret hemorrhages (L> R), moderate dilation of third ventricle	Alpha, R>L theta
3	7	f	22	TBI	2412	Localisation of sounds	None	Flexion withdrawal	Oral reflexive movement	None	Without stimulation	7	VS/UWS	0	Post-traumatic lesions in bilateral frontal lobes. R thalamus atrophy	Theta disorganized

Center 1: Liège, Center 2: Salzburg, Center 3: New York
SAH: subarachnoid hemorrhage; TBI: traumatic brain injury; DAI: Diffuse axonal injury
L: left, R: right, F: frontal, T: temporal, NA: not applicable, BR: basic rhythm

References:

Giacino JT, Ashwal S, Childs N, Cranford R, Jennett B, Katz DI, et al. The minimally conscious state: Definition and diagnostic criteria. Neurology 2002; 58: 349-53.

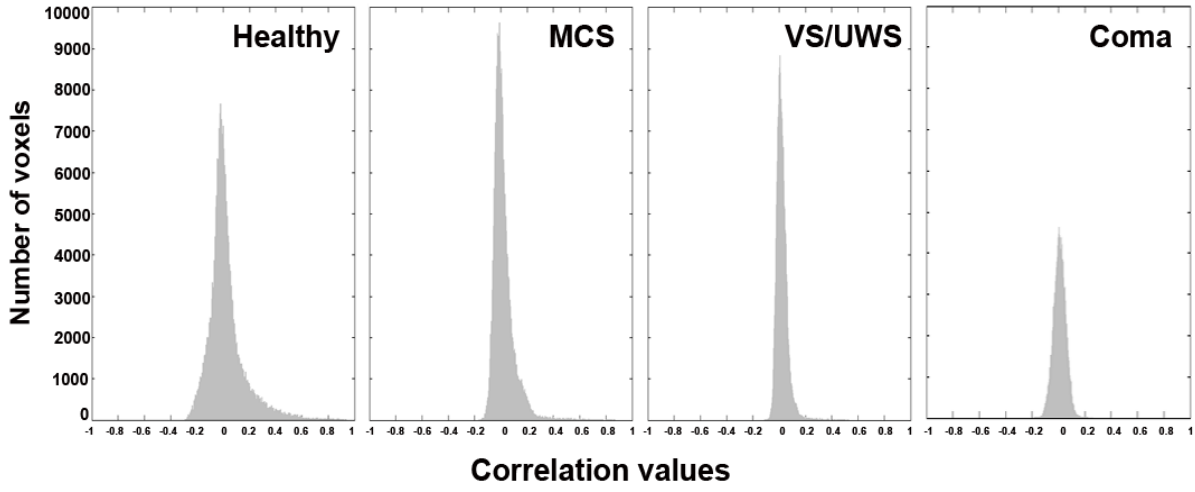
Giacino JT, Kalmar K, Whyte J. The JFK Coma Recovery Scale-Revised: measurement characteristics and diagnostic utility. Arch Phys Med Rehabi 2004; 85: 2020-9.

The Multi-Society Task Force on PVS. Medical aspects of the persistent vegetative state (1). N Engl J Med 1994; 330: 1499-508.

Supplementary Material 5

Functional MRI signal-based denoising has been suggested as a successful strategy for an accurate resting state functional connectivity assessment than mere regression of motion parameters (Power et al., 2015). In our data analysis, the denoising procedure encompassed: 1) motion artifact detection using the artifact detection toolbox (ART); 2) regressing out the realignment parameters, their derivatives and the ART-detected motion outliers, and 3) an anatomical component-based noise correction method (aCompCor) which models the influence of noise as a voxel-specific linear combination of multiple empirically estimated noise sources, such as white matter, gray matter and cerebrospinal fluid (see Fig. 1 and *Methods* in main manuscript for details).

Such a denoising procedure is known to lead to a distribution of the seed-to-voxel correlation values around zero (Chai et al., 2012). The effects of the here employed denoising steps were tested with the same methodology. Specifically, seed-to-voxel functional connectivity was estimated for a seed region placed on the medial prefrontal cortex [10mm sphere around the coordinates (−1, 49, −2)] for the group of healthy controls and each patient group. As in Chai et al (2012), the resulting seed-to-voxel correlation values were distributed around zero for all tested groups (figure below).



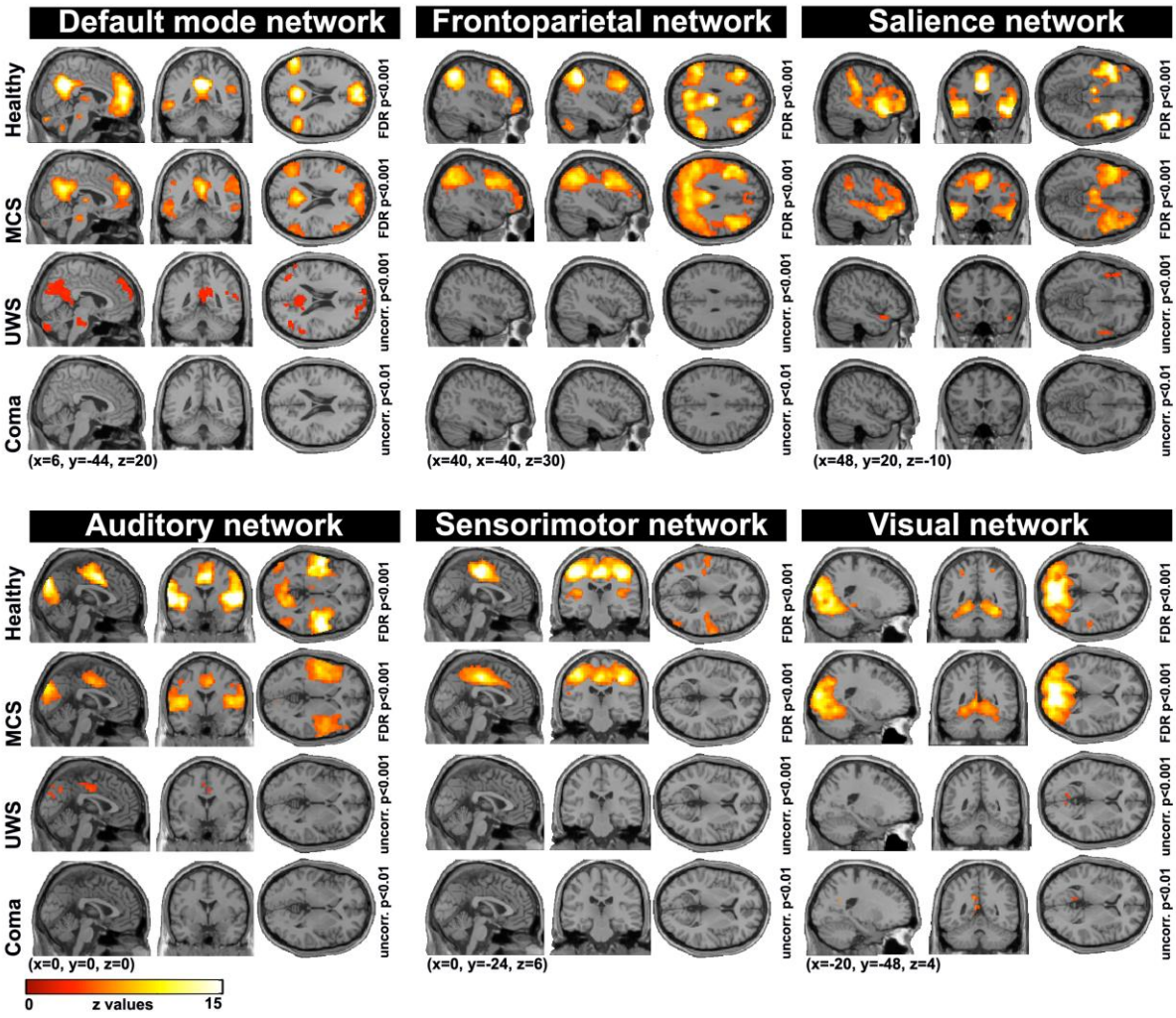
References:

Chai XJ, Castanon AN, Ongur D, Whitfield-Gabrieli S. Anticorrelations in resting state networks without global signal regression. Neuroimage 2012; 59: 1420-8.

Power JD, Schlaggar BL, Petersen SE. Recent progress and outstanding issues in motion correction in resting state fMRI. Neuroimage 2015; 105: 536-51.

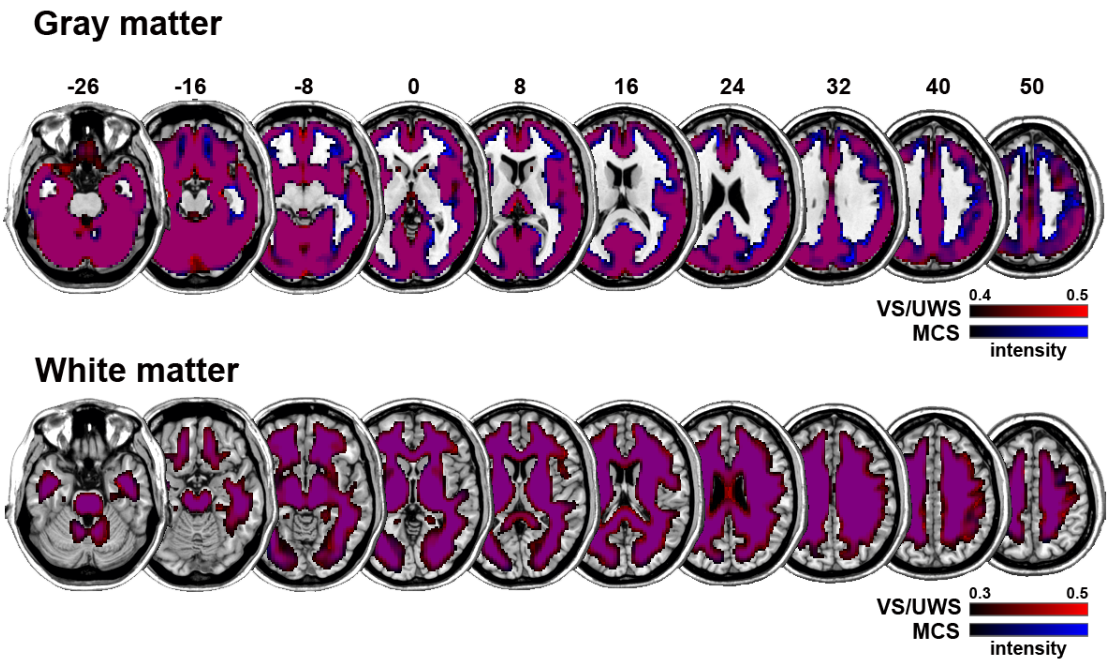
Supplementary Material 6

The studied intrinsic connectivity networks across healthy controls (n=21), patients in minimally conscious state (MCS, n=26), unresponsive wakefulness syndrome (VS/UWS, n=19) and coma (n=6). The spatial patterns represent average correlation maps of the selected seed regions for each network per group. Statistical maps are rendered on a structural T1 magnetic resonance template (x, y and z values indicate Montreal Neurological Institute coordinates of represented sections, neurological convention).



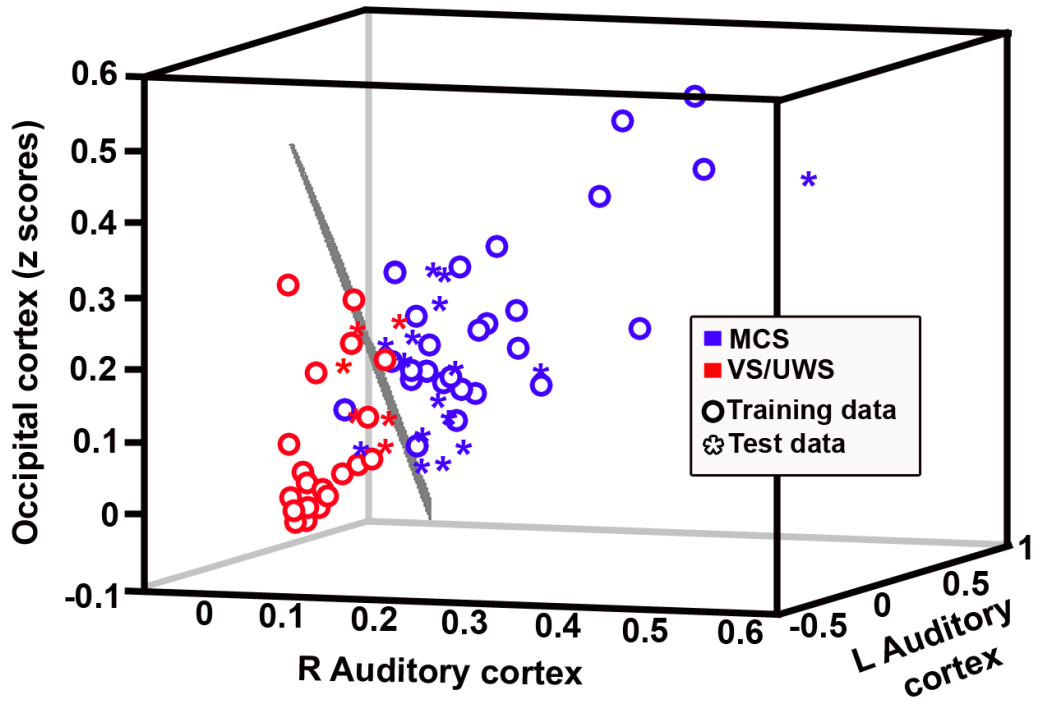
Supplementary Material 7

Average maps of gray and white matter volumes for the group of patients in vegetative state/unresponsive wakefulness syndrome (VS/UWS, red overlay) and minimally conscious state (MCS, blue overlay). The overlays are transparent over each other at 50% (magenta signifies common areas) and are rendered on a T1 template (transverse view, neurological convention). The numbers on top of the maps indicate the number of slice.



Supplementary Material 8

The auditory-visual crossmodal functional connectivity discriminates single patients in minimally conscious state (MCS) from patients in vegetative state/unresponsive wakefulness syndrome (VS/UWS). The figure summarizes the results of the support vector machine classifier showing connectivity values (mean Fisher's z scores) in a 3-dimensional feature space representing bilateral auditory and occipital cortices. The classifier was trained on data obtained in Liège (n=45 patients, circles) and validated on independently assessed patients (asterisks) assessed in Salzburg (n=15) and New York (n=7). Based on this three-feature crossmodal interaction, 20 out of the 22 independently assessed patients were classified congruently, namely the behavioral diagnosis matched the classification outcome. The plane (in grey) represents the decision boundary between the two classes.



Supplementary Material 9

The auditory-visual crossmodal functional connectivity discriminates single patients in minimally conscious state (MCS, blue) from patients in vegetative state/unresponsive wakefulness syndrome (VS/UWS, red). The three-dimensional space indicating connectivity between left auditory, right auditory and occipital cortex (Supplementary Material 8) has been compressed into two dimensions to represent the distance of each patient from the decision plane (arbitrary values).

To test the robustness of the classifier, we evaluated whether the same classifier generalized to healthy control subjects scanned in Liège and Salzburg (n=39; no healthy control data were available for the New York centre). The upper panel shows that the majority of healthy controls (37 of 39, 95%) were classified as MCS (left-hand side of the line). The lower panel summarizes the classifier's decision on the validation dataset (in asterisks) including patients independently assessed in Salzburg (n=15) and New York (n=7).