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Resting-state networks distinguish locked-in from vegetative state patients☆

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ABSTRACT

Purpose: Locked-in syndrome and vegetative state are distinct outcomes from coma. Despite their differences, they are clinically difficult to distinguish at the early stage and current diagnostic tools remain insufficient. Since some brain functions are preserved in locked-in syndrome, we postulated that networks of spontaneously co-activated brain areas might be present in locked-in patients, similar to healthy controls, but not in patients in a vegetative state.

Methods: Five patients with locked-in syndrome, 12 patients in a vegetative state and 19 healthy controls underwent a resting-state fMRI scan. Individual spatial independent component analysis was used to separate spontaneous brain co-activations from noise. These co-activity maps were selected and then classified by two raters as either one of eight resting-state networks commonly shared across subjects or as specific to a subject.

Results: The numbers of spontaneous co-activity maps, total resting-state networks, and resting-state networks underlying high-level cognitive activity were shown to differentiate controls and locked-in patients from patients in a vegetative state. Analyses of each common resting-state network revealed that the default mode network accurately distinguished locked-in from vegetative-state patients. The frontoparietal network also had maximum specificity but more limited sensitivity.

Conclusions: This study reinforces previous reports on the preservation of the default mode network in locked-in syndrome in contrast to vegetative state but extends them by suggesting that other networks might be relevant to the diagnosis of locked-in syndrome. The aforementioned analysis of fMRI brain activity at rest might be a step in the development of a diagnostic biomarker to distinguish locked-in syndrome from vegetative state.

1. Introduction

Coma is a transient state that could progress toward death or different levels of consciousness impairments ranging from vegetative state (VS) or unresponsive wakefulness to minimally conscious state to full consciousness, with or without aftermaths (Giacino et al., 2014). An uncommon outcome from coma is locked-in syndrome (LIS) which is difficult to clinically differentiate from VS. Both conditions share non-responsiveness, but VS patients are awake although still unaware of themselves or their environment, whereas LIS patients demonstrate preserved awareness, aphonia, quadriplegia and a "fail-soft" communication mode that only uses eye movements or blinking (Plum and Posner, 1983). These patients have a disruption of all supranuclear motor pathways except those that control eye movements, usually secondary to a lesion of the ventral part of the pons.

Due to preserved awareness, LIS is not a disorder of consciousness but can be mistaken for one. While this is not a problem for patients who suffer from acute motor tracts lesions without coma, other LIS patients are initially in coma before evolving to LIS. In this case, it is important to diagnose this transition as early as possible, in order to account for these patients' subjective experience and to introduce an eye/eyelid movement code to communicate (Bernat, 2006). However, the arousal level fluctuates and eye movements may be inconsistent during this transition period, making it difficult for caregivers to distinguish LIS from VS. The diagnosis is indeed often delayed, made by the patient's relatives rather than the caregivers, and takes over 2.5 months on average (Laureys et al., 2005).

☆ All authors declare that they have no conflicts of interest.

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To reduce this delay, different diagnostic tools have been tested, among which electrophysiology (event-related potentials) (Perrin et al., 2006; Schnakers et al., 2009), fluorodeoxyglucose positron emission tomography (Giacino et al., 2014; Phillips et al., 2011) and task-dependent functional MRI (Bardin et al., 2011, 2012; Moreno et al., 2011), which demonstrated nearly the same level of consciousness in locked-in patients as in healthy volunteers and helped to distinguish them from vegetative patients. However, none of these methods is a perfect diagnostic tool: event-related potentials are sensitive to noise and are examiner-dependent, positron emission tomography lacks reliable criteria and task-dependent functional MRI is neither practical nor reproducible. Consequently, the need for a diagnostic tool remains unmet.

More recently, the study of slow fluctuations of the fMRI BOLD signal at rest, i.e. in an awake but non-stimulated state, has revealed spontaneous co-active regions, or map (spontaneous co-active map, SAM). Some of them are consistent across subjects, in either healthy controls (Beckmann et al., 1995; Damoiseaux et al., 2006; Smith et al., 2009; Kalcher et al., 2012) or patients (Rotarska-Jagiela et al., 2010; Zhou et al., 2010; Heine et al., 2012; Demertzi et al., 2014, 2015; Qin et al., 2015). They are called resting-state networks (RSN). Some of them might support low-level cognitive activity given that they involve primary and/or secondary cortices, whereas others, which involve tertiary cortices, probably support high-level cognitive activity. One of the most extensively studied RSNs is the default mode network (DMN) (Raichle et al., 2001; Buckner et al., 2008). It is a high-level cognitive RSN, initially defined as the regions which were more active at rest than in any goal-oriented cognitive activity (Raichle et al., 2001). Its putative involvement in self-orientated awareness makes it an attractive candidate to assess the disorders of consciousness (Vanhaudenhuyse et al., 2010). Indeed, its disorganisation is observed in sleep (Horovitz et al., 2008, 2009; Larson-Prior et al., 2009; Koike et al., 2011; Wu et al., 2012; Uehara et al., 2013), pharmacologically induced loss of consciousness (Greicius et al., 2008; Boveroux et al., 2010; Stamatakis et al., 2010; Martuzzi et al., 2011; Schrouff et al., 2011) and pathological disorders of consciousness (Vanhaudenhuyse et al., 2010; Boly et al., 2009; Vanhaudenhuyse et al., 2011; Norton et al., 2012; Soddu et al., 2012). However, other RSNs exist (Beckmann et al., 1995; Damoiseaux et al., 2006; Kalcher et al., 2012; De Luca et al., 2006) and some of them have also been reported to be modified in sleep (Larson-Prior et al., 2009; Martuzzi et al., 2011; Wu et al., 2012; Sämann et al., 2011; Spoormaker et al., 2012), pharmacologically induced loss of consciousness (Greicius et al., 2008; Boveroux et al., 2010; Schrouff et al., 2011; Guldenmund et al., 2013) and disorders of consciousness (Demertzi et al., 2014, 2015; Qin et al., 2015). Their value in the differential diagnosis between VS and LIS remains to be assessed.

Seed-based approaches are the simplest methods to study brain connectivity. However, they are more sensitive to noise than multivariate analyses and since they need spatial a priori they are not suitable for studying injured brains, which potentially present functional reorganisations if not disturbed by significant anatomical deformations. Accordingly, we assessed brain connectivity based on spatial independent component analysis (spatial ICA) which is adapted to single-subject analysis. Although mostly used in group analysis, ICA makes it possible to separate networks of co-activated regions from noise at the single-subject level (McKeown et al., 1998), based on validated operational criteria (Roquet et al., 2014). These spontaneous co-activity maps will be further referred to as SAMs. Some of these SAMs are shared among different subjects and are called RSNs (as above). These correspond to the networks provided by group ICA. The other SAMs are idiosyncratic networks, i.e. SAMs that can only be seen in one or a few subjects or in a given session, sometime related to an abnormal activity such as epileptic seizures or hallucinations.

The aim of this study was to assess the sensitivity and the specificity of SAMs and RSNs in distinguishing LIS from VS regarding their distribution in a normal control population (CTRL). Since LIS patients are conscious with most high-order functions preserved, we hypothesised that they might differ from VS in their numbers of SAMs, RSNs, RSNs dedicated to high-level cognitive processing and in the presence of a DMN. Alternatively, LIS patients are expected to be undistinguishable from CTRLs based on the RSNs dedicated to high-level cognitive processing and the presence of a DMN. Last, as an exploratory analysis, sensitivity and specificity were also assessed for the other RSNs. This is one step in the progress toward a diagnostic tool.

2. Material

2.1. Participants

Twenty-five patients consecutively admitted to the Strasbourg's medical intensive care unit were screened for their participation and all were included in the study. Eight patients were removed from analysis: five patients did not satisfy the following diagnosis criteria for VS (three patients in minimal conscious state, two patients in coma) or LIS (one patient in coma with pontine lesion), and two did not satisfy MRI criteria (one had excessive head motion during the MRI session (>1.5° or millimeters), and one had excessive artefactual MRI signals due to artificial breathing assistance), leaving 12 VS patients and 5 LIS patients. The inclusion period was extended for LIS in order to increase the cohort of LIS patients from three to five. All of the VS patients but one suffered from diffuse brain injuries. This patient with a focal injury (labelled VSF) actually suffered from a limited brainstem lesion, like the LIS patients, although his diagnosis was definitely VS with no sign of consciousness. Because of his cortex was preserved, this patient was considered separately. Therefore, 12 patients in VS with diffuse lesions (mean age, 54.2 years; range, 21–87 years; seven females), 1 VSF patient (age, 40 years; male), 5 patients in LIS (mean age, 49.0 years; range, 37–70; one female, and 19 healthy control (CTRL) participants (mean age, 30.9 years; range, 19–51; five females) were included in the study. Controls had no history of neurological or psychiatric disorders. Demographic and clinical data are presented in Table 1. A LIS diagnosis required concordant assessment of preserved consciousness between the rehabilitation unit staff and the patient's relatives. All patients in LIS suffered from brainstem lesion and were initially in coma. A LIS diagnosis in this study refers to classic LIS (Bauer et al., 1979), and does not relate to functional LIS diagnosis (also known as complete or total LIS (Schnakers et al., 2009; Bruno et al., 2011)), which is entirely based on paraclinical assessments such as fMRI or evoked potentials due to the extreme behavioural motor dysfunction in these patients including paralysis of eye motility. Before MRI acquisition, patients were clinically examined using the Wessex Head Injury Matrix scale (WHIM) (Shiel et al., 2000). According to Turner-Stokes et al. (2015), a score between 1 and 9 corresponds to a VS state (for non-LIS patients), because item 10 was not validated by VS patients (visual pursuit is compatible with VS for the Working Party of the Royal College of Physician (2003) and Turner-Stokes et al. (2015). Among the five patients who did not satisfy the VS criteria and were therefore removed from analysis, three had a WHIM score higher than 9, respectively 13, 14 and 15, consistent with minimal conscious state). VS patients did not show either goal-directed behaviour or responsiveness to verbal orders or signs of communication. All patients were assessed using the Glasgow Coma Scale. This study was approved by the local ethics committee. Controls and patients' representatives gave written informed consent.

2.2. Data acquisition

Four hundred and five whole-brain T2*-weighted echo planar images were acquired interleaved on a Siemens Magnetom® Avanto 1.5T (Siemens, Erlangen, Germany) with the following session parameters: TR = 3 s; flip angle = 90°; TE = 43 ms; FOV = 256 mm × 256 mm × 128 mm; Imaging matrix = 64 × 64 × 32; 4-mm^3 isotropic voxels, with fat saturation preparation, leading to a

Table 1
Patients' demographic, clinical and imaging data.
LIS, locked-in syndrome; VS, vegetative state. Ages at the MRI acquisition are given in years; time of MRI in days after the injury. Wessex Head Injury Matrix scale (WHIM) scores correspond to the last completed item on the scale. The Glasgow Coma Scale (GCS) was performed at admission.

Patient	Gender	Age	Aetiology	Time of MRI	WHIM at MRI	GCS	Outcome at 6 months
LIS 1	Male	37	Trauma	76	3	3	LIS
LIS 2	Female	70	Anoxia	107	3	7	Dead
LIS 3	Male	39	Trauma	75	2	4	LIS
LIS 4	Male	47	Ischemia	12	2	4	Dead
LIS 5	Male	52	Hematoma	180	3	6	Dead
VS 1	Male	54	Anoxia	10	2	4	Dead
VS 2	Female	32	Anoxia	3	1	3	Dead
VS 3	Female	21	Anoxia	5	1	3	Dead
VS 4	Male	87	Septic shock	7	1	3	Dead
VS 5	Male	44	Hypoglycaemia	32	2	5	Dead
VS 6	Male	53	Anoxia	3	9	5	VS
VS 7	Male	73	Anoxia	3	1	3	Dead
VS 8	Male	53	Anoxia	5	1	3	Dead
VS 9	Female	59	Anaphylactic shock	15	3	3	VS
VS 10	Male	71	Anoxia	16	1	3	Dead
VS 11	Female	49	Hypoglycaemia	18	1	5	VS
VSF	Male	40	Anoxia	11	3	3	Dead

total acquisition time lasting about 20 min. A 3D MPRAGE T1-weighted image was also acquired at the same session (1-mm^3 isotropic voxels).

2.3. Data preprocessing

After conversion to Nifti format, the images were preprocessed using Statistical Parametric Mapping software v8 (Welcome Department of Cognitive Neurology, London, UK) working on Matlab R2012b (The MathWorks, Inc., Sherborn, MA, USA). For each participant, the first five images were removed to account for T1 partial saturation and the 400 remaining images were then motion corrected. One participant had translation or rotation >1.5 mm or 1.5° and was consequently removed from the analysis.

2.4. Connectivity analysis

For each participant, a single-subject ICA was performed using FMRLAB software 2.3 (Swartz Center for Computational Neuroscience, University of San Diego, San Diego, CA, USA), modified to work on Nifti format, with an implementation of the INFOMAX algorithm (Bell and Sejnowski, 1995). Dimensions were reduced from 400 to 250 by principal component analysis before running ICA. From the whole set of 250 independent components (each one is a z-score 3D map, thresholded at ± 1.5 for display purposes), the SAMs were manually selected by an expert (DR) according to validated operationalised criteria (Roquet et al., 2014).

All SAMs were further classified according to a simplified version of the Kalcher et al. proposal (Kalcher et al., 2012) based on the individual ICAs of 1000 healthy controls. The 8 RSNs consisted in the default mode network (DMN), the precuneal and posterior cingulate network (PPCN), the anterior cingulate and fronto-polar network (ACFPN) sometimes referred to as the salience network, the fronto-parietal network (FPN, right and left were considered as a whole) also called the executive control network, the external temporal network (ETN), the occipito-parieto-frontal network (OPFN) or dorsal attentional network, the occipital network (ON) or visual network and the central network (CN) (Fig. 1), also known as the sensorimotor network. The DMN, PPCN, ACFPN and FPN were considered as networks underlying high-level cognitive activity, whereas ETN, OPFN, CN and ON were considered as sub-serving low-level cognitive activity based on their main involvement of primary and secondary cortices. Modifications from Kalcher et al. consisted in merging C.01 and C.02 into one ON, C.07 (left-FPN) and C.09 (right-FPN) into one FPN, as well as C.03 and C.06 into one DMN. A manual classification was preferred since it has been reported to be more reliable than a template-matching procedure (Franco et al., 2009). Two raters (DR, JF, blinded to the diagnosis) classified each SAM as one of eight common RSNs or as idiosyncratic (uncommon networks, see supplementary material). Inter-rater agreements for the distinction between RSN and idiosyncratic SAMs, and for the classification as a DMN were assessed using Cohen's kappa coefficient (Cohen, 1960). In case of discrepancy, the final classification was made by consensus.

For each network, sensitivity was assessed for the three groups. Specificity was also evaluated for LIS relative to VS.

2.5. Statistical analysis

Age was compared between groups using ANOVA at $p < 0.05$. Time from injury to scanning was compared between LIS and VS patients using the two-sample Student *t*-test. Gender was compared between LIS, VS and CTRL subjects by the two-sample independent chi-square test. Regarding measurements of connectivity, LIS was compared to VS and CTRL groups using the two-sample independent chi-squared tests on the following measures: the presence of SAMs, RSNs, high-level RSNs and each RSN separately. Statistical between-group voxel-wise analyses of RSN images were not performed due the limited number of patients in the LIS groups.

3. Results

3.1. Clinical data

CTRLs were significantly younger than LIS and VS patients ($p < 0.001$) (mean ± standard deviation, CTRL: 30.9 ± 8.1; LIS: 49.0 ± 13.2; VS: 54.2 ± 18.6). Due to fluctuating states at the early stages after injury, patients in LIS were scanned significantly later than patients in VS in order to ensure the diagnosis ($p < 0.001$). However, the delay of 90 days after injury is in agreement with the average delay to ascertain a LIS diagnosis (Laureys et al., 2005). Gender did not differ between LIS and CTRL nor LIS and VS subjects.

3.2. Inter-rater agreement for RSN classification

In all three groups of participants, 232 SAMs were selected. They were then classified as RSNs or as idiosyncratic networks (i.e., a 9 category classification). Moderate agreement was found between the two raters for labelling one SAM as a RSN or as an idiosyncratic network, with a kappa coefficient of $\kappa = 0.56$. The agreement for classifying a SAM as a high-level or as a low-level RSN was excellent ($\kappa = 0.90$). The agreement on the DMN classification among all the SAMs was excellent ($\kappa = 0.88$). The kappa for PPCN, ACFPN, FPN, ETN, OPFN, CN and ON ranged from good to excellent, with $\kappa = 0.77$, 0.71, 0.83, 0.89, 0.62, 0.90 and 0.93, respectively.

3.3. Spontaneous co-activity maps

The CTRL group presented between three and 20 SAMs. Means and standard-deviations of the number of SAMs are reported in the first column of Table 2. LIS had from one to 12 SAMs. Only one out of 11 patients in VS (VS11) presented at least one SAM (one SAM in this case), leading to a very low mean for VS, whereas the VSF patient had 14 SAMs. Statistical analyses revealed no differences between the LIS and CTRL groups in having SAMs (versus having no SAMs), whereas the LIS group significantly differed from the VS group ($p < 0.001$). The presence or absence of SAMs was a sensitive test for a LIS diagnosis (sensitivity, 100%), and its specificity was 91% relative to VS (Table 3).

3.4. Resting-state networks

The mean image of each RSN is displayed in Fig. 1. Among the SAMs observed in the CTRL group, 78.1% were classified as RSNs, leading to an average of 7.3 ± 3.2 RSNs per subject, while the others were considered as idiosyncratic (Table 2, second column). Since two SAMs in a given subject could sometimes be labelled as the same RSN, on average CTRL subjects presented 4.7 (± 1.4) out of the eight RSNs. As for LIS patients, 71.8% of the SAMs corresponded to RSNs, leading to a mean 5.3 ± 3.1 RSNs per subject. These corresponded to 4.6 ± 0.9 out of the eight RSNs. Regarding the VS patient providing signs of connectivity, his only SAM was classified as RSN a (therefore 0.1 ± 0.3 per VS subject were RSNs). Eight of the VSF's SAMs were labelled as RSNs in 4 of the 8 reference classes. Statistical analyses of the presence of RSNs revealed a difference between LIS and VS patients ($p < 0.001$) but not between LIS and CTRL subjects. This was a sensitive test for a LIS diagnosis, similar to those reported for the SAMs (sensitivity, 100%; specificity, 91%) (Table 3).

3.5. High-cognitive-level resting-state networks

The CTRL and LIS groups presented on average 2.9 ± 0.8 and 2.4 ± 0.9 high-cognitive-level RSNs, respectively (Table 2), whereas the RSN of patient VS11 was not of a high-order. Regarding VSF, one out of four RSNs was a high-cognitive-level RSN (FPN). Having versus not having high-cognitive-level RSNs was significantly different between LIS and VS patients ($p < 0.0001$), but again not between LIS and CTRL subjects. This was a sensitive and specific test for a LIS diagnosis (both 100%, Table 3).

3.6. Default mode network

The DMN was the only network that was observed in every healthy participant (Table 3) and LIS patient. In contrast, none of the VS patients had a DMN, nor did the VSF patient. Accordingly, the difference between the LIS and VS groups was very significant ($p < 0.0001$), whereas the LIS group did not differ from the CTRL group. Presence versus absence of a DMN was highly sensitive for LIS diagnosis (sensitivity, 100%) and highly specific relative to VS (specificity, 100%; see Table 3). Slices of the DMN of each LIS patients are available in the supplementary material.

3.7. Other RSNs

The non-DMN high-cognitive-level networks were not as regularly present in CTRLs, except for the FPN which was absent in only one healthy participant. The PPCN was observed in about half of the subjects, whereas the ACFPN was observed in an even smaller percentage (Table 3). In LIS, all high-order RSNs were found in at least one subject, although only the FPN and DMN reached sufficient consistency (Table 3). No statistical difference occurred in any high-order RSNs between LIS and CTRL subjects. Patients in VS did not show high-order RSNs, making the DMN and FPN statistically different between LIS and VS patients ($p < 0.0001$ and $p < 0.01$, respectively). The VSF patient showed a FPN, but not a DMN, PPCN or ACFPN.

Concerning the low-cognitive-level networks, all three groups presented an ON. The difference between the LIS and VS patients was still significant ($p < 0.05$) and its specificity for LIS was high (91%). Only one LIS patient showed the somatomotor-related CN, resulting in no difference between LIS and VS patients but a clear tendency to significance between LIS and CTRL ($p = 0.051$). OPFNs were present only in CTRL and LIS subjects, the latter significantly differing from VS patients ($p < 0.01$), with maximum sensitivity (100%). No VS patient had an ETN, but since only a few CTRL and LIS subjects presented an ETN, no difference was noted between groups. VSF presented an ETN, ON and CN but not an OPFN.

4. Discussion

This study was designed to assess the sensitivity and specificity of the presence of SAMs, RSNs, high-level RSNs and the DMN in distinguishing LIS from VS patients and LIS from a healthy control population (CTRL). SAMs or RSNs were either absent or scarce in VS in contrast to LIS patients. LIS patients were undistinguishable from CTRL subjects. Focusing on the RSNs supporting high-cognitive-level activities (defined as not involving primary or secondary cortices) markedly increased specificity.

Most of the patients in VS did not show any signs of connectivity, including idiosyncratic networks. This lack of connectivity may be explained by the diffuse brain injuries making them likely to have strong functional connectivity disorders. In contrast, brainstem insults would entail limited connectivity disturbances. The VSF patient in VS suffering from a focal brainstem injury actually showed many SAMs, including two high-order networks (FPN and ETN). Accordingly, spontaneous connectivity might be preserved in some way as long as the telencephalon and the diencephalon remain intact. The fact that most of the VS patients had diffuse brain injuries might limit the reach of the present results to this condition. Our observations need to be replicated on VS patients with a focal brainstem lesion (like the VSF patient in the present study) before generalising them to the whole VS group.

It might be argued that the failure of brain co-activities in VS patients could be due to the wakefulness state, i.e. these patients would have fallen asleep during the scanning. As we did not assess the level of arousal during the MRI session, we cannot ascertain that this did not occur more frequently in VS than in LIS. Previous studies on connectivity during sleep reported that RSNs including the DMN and FPN can be modified and even disappeared when the subject fell asleep (Sämann et al., 2011). However, the absence of RSN during sleep does not mean an absence of SAM: brain areas still co-activate during sleep such that SAMs should be observed, although different from RSN (Picchioni et al., 2013). Accordingly, the almost total absence of SAM in VS suggests a more profound brain disorganisation of functional connectivity rather than a simple difference in arousal level.

The DMN has already been reported to mediate awareness of self (Vanhaudenhuyse et al., 2010). Accordingly, it has been reported to be absent in VS patients and altered in the minimal conscious state (Demertzi et al., 2014, 2015; Vanhaudenhuyse et al., 2011). The present results support these previous observations and they extend them by giving sensitivity and specificity values and the favourable reliability of the test, which has a high kappa value. Therefore, not only is the DMN specific to LIS, but its high sensitivity in the LIS and CTRL groups potentially makes it a reliable diagnostic tool. This observation of the preserved DMN in LIS fully agrees with a single-case observation from Vanhaudenhuyse et al. (2010). However, this perfect sensitivity in both the CTRL and LIS groups was perhaps a fluke: since the cognitive state was not constrained during the fMRI acquisition, the DMN may not be observed in some participants, as suggested by its absence in some sessions in healthy subjects (Kalcher et al., 2012; Demertzi et al., 2014). This could explain, together with methodological differences and the type of lesion, the discrepancy with Demertzi et al. (2014) who reported an altered DMN in close to one-third of their patients in VS.

The FPN could also be taken into account to refine the differential diagnosis between LIS and VS. The FPN on its own is not present in a sufficient number of LIS subjects to be highly sensitive. However, it is highly specific. Accordingly, further studies might test the possibility that the presence of either the DMN or the FPN is sufficient for suspecting LIS. This could limit the false-negative result of a DMN-based procedure if its sensitivity turns out to be lower than estimated in the present study. FPN has been reported to be involved in externally orientated awareness (awareness of environment) (Vanhaudenhuyse et al., 2011), whereas the DMN would support

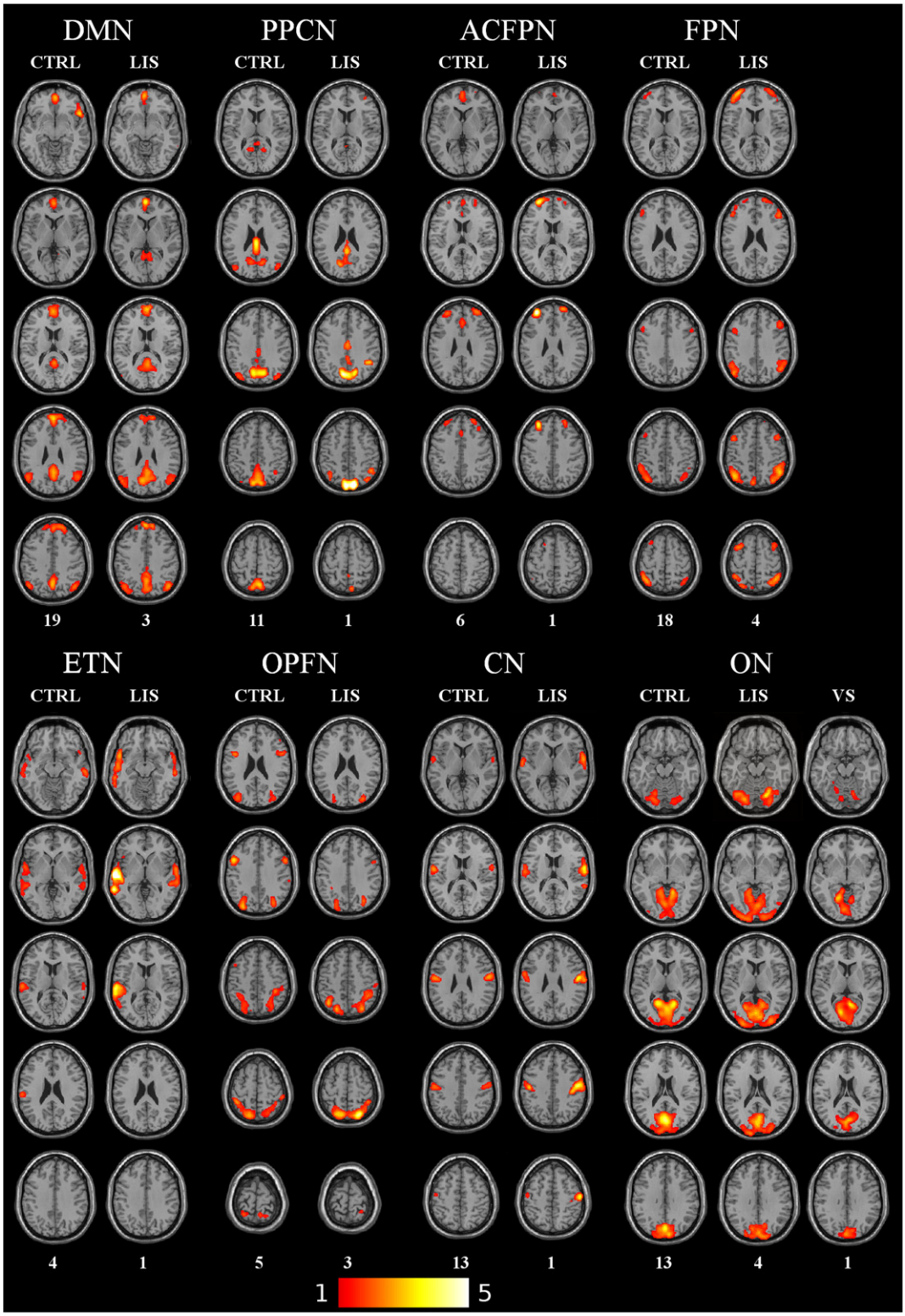

Fig. 1. Mean images of each resting-state network (RSN). All networks are constructed from normalised, resliced (2-mm^3 isotropic voxels), smoothed (FWHM = 8 mm) and thresholded images (z-score > 1.0). Slices are displayed with a 12 mm gap in the z-direction starting from the z-coordinate indicated below the first slice. Left is left side of the brain (neurological orientation). DMN: default mode network; PPCN: precuneal and posterior cingulate network; ACFPN: anterior cingulate and fronto-polar network; FPN: the fronto-parietal network; ETN: external temporal network; OPFN: occipito-parieto-frontal network; CN: central network; ON: occipital network. CTRL, LIS and VS refer to groups of healthy participants, locked-in syndrome and vegetative-state patients, respectively. Numbers below images correspond to the number of subjects per group presenting each RSN.

internally orientated awareness. Therefore, these results could be interpreted as an alteration of both internally and externally orientated networks in loss of consciousness (Boveroux et al., 2010; Vanhaudenhuyse et al., 2011).

The low-order ON is assumed to underlie visual processing. We observed that it could be preserved in a patient in VS. This result is in accordance with previous reports of altered high-order but preserved low-order RSNs during sedation (Boveroux et al., 2010; Martuzzi et al.,

Table 2
Number of SAMs, RSNs and high-level RSNs.
CTRL, LIS and VS refer to groups of healthy participants, locked-in syndrome and vegetative-state patients, respectively. The mean numbers of SAMs and among them the numbers of RSNs and high-order RSNs are expressed as the mean (standard deviation). The presence of these three observations in LIS patients was compared to CTRL and VS subjects separately, using chi-square tests. a for $p < 0.001$; b for $p < 0.0001$.

	SAM		RSN		High order RSN	
CTRL	9.4	(4.4)	7.3	(3.2)	2.9	(0.8)
LIS	7.8	(4.9)	5.3	(3.1)	2.4	(0.9)
VS	0.1[a]	(0.3)	0.1[a]	(0.3)	0[b]	(0)

2011). In contrast, only one of the LIS patients presented a CN, possibly in line with their absence of sensory processing or intentional motor activity.

The strong difference of connectivity between VS patients and the VSF patient suggests that the type of lesion strongly influences the presence of the networks. Still, the DMN was absent in this patient and reinforced the role of the DMN in distinguishing LIS from all VS patients. However, we suggest replicating our results on VS patients with a focal brainstem lesion before generalising them to the entire VS group.

The ultimate aim of this study was to assess the SAMs as a diagnostic tool to distinguish LIS from VS patients. The method was designed to be easily transferred to the clinical setting (Soddu et al., 2011). First, the acquisition is task-free, i.e. the MRI acquisition at rest does not require collaborative patients, which makes fMRI investigations easier than task-based MRI in the context of disorders of consciousness. Second, using the data-driven ICA in place of the seed-based approach, no spatial a priori about the networks' patterns was required and artefacts such as physiological noise and head-motion are separated from the neuronal signal, making the method highly sensitive. Third, to avoid spatial transformations of the brain into a standardised space, which would not work properly on injured brains, the method is based on individual spatial ICA in the patient space. The manual selection of SAMs and their classification as RSNs turned out to be a reliable procedure, especially for the DMN. Still, manual selection requires expertise and is time-consuming. A reliable and sensitive pre-selection system has been developed in parallel to this study to help and shorten SAM selection (Sourty et al., 2015), but an automatic classifier remains to be developed.

Although the LIS population was the largest reported to date, these promising results with good sensitivity and specificity remain to be replicated on a larger sample to ensure they can be generalised. Such assessments would be particularly interesting when family and caregivers have a feeling there is a reaction, whereas nothing emerges from clinical examinations, particularly when patients are unable to communicate using eye movements. In this case, such a biomarker may provide help to objectivise what frequently could remain an intuition during the early stages after awakening, and may shorten the time to diagnosis. Here, LIS patients were acquired once the diagnosis was ensured. Accordingly, RSNs can only be considered here as "potential" biomarkers. The study which could ascertain their relevance at the early stage would require assessing patients for which the diagnosis is still doubtful, in a longitudinal perspective. In such case some difficulties might emerge as the fluctuations of arousal would probably affect the probability to observe high-cognitive-level networks. Last, although the presence of the DMN might be interpreted as the presence of internally oriented cognition, its absence cannot be interpreted as the absence of awareness as it is sometimes absent in some healthy controls or can be modified when the subject fall asleep (Sämann et al., 2011).

5. Conclusions

This study provides evidence that SAMs are promising in assessing awareness. All our hypotheses were confirmed: the total number of SAMs, the total number of RSNs, and the number of RSNs underlying high-level cognitive activities turned out to be significantly greater in LIS than in VS patients and did not differ between the LIS and CTRL groups. The presence of a DMN and other high-order networks reaches the high sensitivity and specificity required to distinguish LIS from VS at the individual level. The DMN may only underlie part of the consciousness, i.e. self-consciousness, and this study raises the possibility that other networks might also be considered, e.g. the FPN.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nicl.2016.06.003.

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Table 3
Sensitivity and specificity of the resting-state networks.
CTRL, LIS and VS refer to groups of healthy participants, locked-in syndrome and vegetative-state patients, respectively. Specificity values correspond to LIS relative to VS. The presence of the networks in LIS patients was compared to CTRL and VS subjects separately, using chi-squared tests. a for $p < 0.05$; b for $p < 0.01$; c for $p < 0.001$; d for $p < 0.0001$.

Sensitivity (%)				High order RSN				Low order RSN			
	SAM	RSN	High-order RSN	DMN	PPCN	ACFPN	FPN	ETN	OPFN	CN	ON
CTRL	100	100	100	100	58	32	95	21	67	68	68
LIS	100	100	100	100	20	20	80	20	67	20	80
VS	9[c]	9[c]	0[b]	0[d]	0	0	0[b]	0	0[b]	0	9[a]
Specificity (%)											
	91	91	100	100	100	100	100	100	100	100	91

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