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ORIGINAL ARTICLE

Functional connectivity and metabolic brain alterations in sleepwalkers

Greta Mainieri[1,2] | Magali Jane Rochat[3] | Elena Cantoni[3] | Giovanni Sighinolfi[3] | Micaela Mitolo[3] | Giuseppe Loddo[4] | Francesco Mignani[2] | Susanna Mondini[2] | Raffaele Lodi[1,3] | Federica Provini[1,2] | Caterina Tonon[1,3]

[1]Department of Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy

[2]IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy

[3]Functional and Molecular Neuroimaging Unit, IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy

[4]Department of Primary Care, Azienda AUSL di Bologna, Bologna, Italy

Correspondence
Federica Provini, IRCCS, Istituto delle Scienze Neurologiche di Bologna, Bellaria Hospital, Via Altura, 3, 40139 Bologna, Italy.
Email: federica.provini@unibo.it

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Abstract

Objective: Disorders of arousal (DoA) are characterized by an intermediate state between wakefulness and deep sleep, leading to incomplete awakenings from NREM sleep. Multimodal studies have shown subtle neurophysiologic alterations even during wakefulness in DoA. The aim of this study was to explore the brain functional connectivity in DoA and the metabolic profile of the anterior and posterior cingulate cortex, given its pivotal role in cognitive and emotional processing.

Methods: Fifteen consecutive patients with DoA (9 males, mean age 26.3 ± 7.7) and 15 age- and sex-matched healthy controls (8 males, mean age 25.8 ± 3.6) were enrolled. All participants underwent a protocol including sleep and psychological evaluation scales and multimodal brain MRI with resting-state functional MRI and 1H-MR spectroscopy.

Results: The independent component analysis disclosed an altered resting-state functional connectivity (FC) in the patients' sensory motor network, with a higher connectivity strength in opercular cortex, precuneus, occipital pole, and lingual gyrus. The seed-based analysis revealed a decreased FC between posterior cingulate cortex (PCC) and several cerebral areas. Finally, spectroscopic imaging revealed a reduced content of glutamine in the PCC ($p < 0.001$).

Interpretation: The increased connectivity in the sensory-motor network of DoA patients could constitute a "facilitatory medium" enhancing motor circuit activation, while the connectivity and metabolic alterations of PCC might represent a trait functional feature, contributing to a dysfunctional arousal process and the difficulty to reach a complete awareness during DoA episodes. In addition, these alterations at rest might be related to daytime impairment reported by patients, requiring new strategies for DoA management.

KEYWORDS
brain functional connectivity, brain spectroscopy, NREM parasomnias, sleep arousal, sleepwalking

INTRODUCTION

Disorders of arousal (DoA) consist of involuntary motor and emotional behaviors arising from deep NREM sleep, characterized by an impairment of awareness, amnesia for the episode, and a partial interaction with the environment [1]. Sleep is no longer seen only as a global process, but also as a complex set of local phenomena termed "local sleep," where different neural characteristics differentiate the basic states of consciousness, that is, wakefulness, NREM, and REM sleep [2]. These physiological states tend to cycle in a stable manner but the transitions from one to the other may be indistinct, leading to the co-occurrence of sleep and wakefulness in different regions of the brain and resulting in mixed states, well exemplified in sleep parasomnias [2], a model to analyze the complex interplay among cognitive, motor, and emotional behaviors. The exact pathophysiological mechanisms and the neuronal networks underlying DoA are complex and neurophysiologic evidence includes studies capturing in vivo episodes as well as indirect evidence collected during normal sleep or wakefulness [3–15]. The first single-photon emission computed tomography (SPECT) study capturing an episode of sleepwalking showed a "dissociation" between more activated areas, such as the posterior cingulate cortex (PCC) and anterior cerebellum, and deactivated areas, such as the fronto-parietal associative cortices, suggesting the selective activation of thalamo-cingulate networks with persistent inhibition of other thalamo-cortical pathways [3]. Stereo-EEG studies disclosed the persistence of deep sleep rhythms over fronto-parietal associative cortices and hippocampi, accounting for the unawareness and frequent amnesia during DoA episodes, together with wakefulness EEG rhythms over the cingulate cortices, motor, and limbic areas, responsible for the motor and emotional features of the episodes [4–8]. Indirect neurophysiologic evidence suggests subtle alterations mainly consisting in increased excitability of the motor and cingulate cortex, both during sleep devoid of clinical episodes and also wakefulness [9, 10]. A functional MRI (fMRI) study revealed an alteration of the sleep–wake transition in DoA patients, involving the motor, cingulate cortices, and also the thalamus [15].

To date, no evaluation of resting-state networks was performed, thus potential differences in rest condition compared to normal subjects remain unknown. Potential differences at rest between patients and controls might show that the neurophysiologic alterations are not restricted to sleep but could also involve wakefulness and possibly play a role in daytime consequences. Indeed, DoA should not be considered a benign condition anymore and a thorough comprehension of their mechanisms might be useful to improve their clinical management. Interestingly, a work exploring the different semiologies of cingulate epilepsy, hinted at a striking similarity between seizures arising from the anterior cingulate cortex (ACC) and NREM parasomnias [16]. In addition, a previous MR spectroscopy (MRS) study in patients with sleep-related hypermotor epilepsy (SHE), whose seizures often involve the cingulate cortex, disclosed a reduction of the N-acetyl-aspartate/creatine ratio in patients in the ACC [17].

The aim of this study was threefold: firstly, to conduct a resting-state fMRI in order to investigate both the brain functional networks integrity and the presence of potential alterations in brain connectivity. Secondly, to conduct a proton magnetic resonance spectroscopy (1H-MRS) on the anterior and posterior cingulate cortex. The cingulate cortex is a complex, heterogeneous region, both in terms of anatomical and functional organization, subserving a key role in cognitive, motor and emotional responses [18] and no data exist in DoA. Finally, to investigate potential correlations between MR features and clinical, psychological, and sleep parameters.

MATERIALS AND METHODS

Subjects and study design

Patients were consecutively recruited among subjects afferent to the Bologna Sleep Center (IRCCS Istituto delle Scienze Neurologiche, Bellaria Hospital) from December 2021 to February 2023. The patients all had a clinical diagnosis of DoA, based on International Classification criteria [1]. Healthy controls (HCs), not affected by DoA or other sleep disorders and matching for age and sex with DoA patients, were identified among acquaintances or partners of patients and consecutively recruited from November 2022 to June 2023. Inclusion criteria for DoA patients were an age >18 years old, both sexes, a clinical diagnosis of DoA. Inclusion criteria for controls were an age >18 years old, both sexes and the absence of any sleep disorder. Exclusion criteria for both DoA and controls were patients with conditions causing potential discomfort during the MRI, intellectual disability or cognitive impairment, a neurological, psychiatric, or relevant systemic condition, psychotropic therapy intake, seasonal therapy with steroids or antihistaminic drugs, previous history of brain trauma or neurosurgery, voluptuary substances abuse, pregnancy, shift worker condition, contraindication to the MRI exam.

Patients with a clinical diagnosis of DoA were enrolled in the study after the clinical and neurological assessment comprising a complete sleep assessment by a neurologist expert in sleep disorders (GM). The patients were instructed to maintain their normal life habits and sleep–wake rhythms in the days preceding the fMRI, in the absence of sleep deprivation schedules. On the day of the fMRI exam, the patients were evaluated by a trained neurologist expert in sleep disorders (GM) by means of sleep scales assessing sleepiness (Epworth Sleepiness Scale, ESS) [19], sleep quality (Pittsburgh Sleep Quality Index, PSQI) [20], severity of DoA (Paris Arousal Disorders Severity Scale, PADSS) [21], and by expert psychologists (MR, MM) for the psychological assessment. This included the investigation of the presence and severity of depression signs (Beck Depression Inventory, BDI-II) [22], and of both temporary trait and stable state of anxiety (State and Trait Anxiety Inventory–Y, STAI-Y) [23]. Potential dimensional maladaptive personality traits were measured through The Personality Inventory for DSM-5–Brief Form (PID-5-BF) [24], while post-traumatic conditions and dissociative symptoms or dissociative identity disorders were assessed through the Childhood Trauma Questionnaire (CTQ) [25] and the Dissociative Experiences Scale (DES) [26] respectively. The Difficulties in Emotion Regulation Scale (DERS) [27] was used to evaluate the emotional arousal modulation, but also the awareness, understanding, and acceptance of emotions, and the ability to act in desired ways regardless of emotional state. Finally, the administration of the Perceived Stress Scale (PSS) [28] offered a measure of the participants' psychological stress, that is, the extent to which persons perceive that their demands exceed their ability to cope. After the fMRI acquisition, on the same day, patients were equipped to undergo a 48h VPSG, including two sleep nights. Video-polysomnographic recordings were performed using standard bipolar EEG (according to the International 10–20 system) and included 19 electrodes (Fp1, Fp2, F3, F4, F7, F8, Fz, C3, C4, Cz, T3, T4, T5, T6, P3, P4, Pz, O1, and O2), electrocardiogram, electro-oculogram, chin and both anterior tibialis electromyography, thoracoabdominal plethysmography bands, and synchronized audio-video recording.

This study was approved by the local ethical committee on the 20th May 2021 (n°21075). Informed consent of patients and controls was acquired according to the Declaration of Helsinki. A signed consent-to-disclose form was obtained by each individual.

Structural magnetic resonance imaging acquisition

The standardized brain MRI protocol was performed using a high-field Siemens MAGNETOM Skyra 3T MRI scanner equipped with a head–neck high-density (64 channels) array coil. The MRI protocol included T1-weighted 3D Magnetization-Prepared Rapid Gradient-Echo Imaging sequence [MPRAGE, 176 continuous sagittal slices, 1-mm isotropic voxel, no slice gap, echo time (TE) = 2.98 ms, repetition time (TR) = 2300 ms, Inversion Time (IT) = 900 ms, flip angle = 9°, acquisition matrix = 256 × 256, pixel bandwidth = 240 Hz, in-plane acceleration factor = 2, duration ~5 min] and T2-weighted 3D fluid-attenuated inversion recovery (FLAIR) sequence (SPACE, 176 sagittal acquisition slices, 1-mm isotropic voxel, no slice gap, TE = 428 ms, TR = 5000 ms, IT = 1800 ms, flip angle = 120°, acquisition matrix = 256 × 256, pixel bandwidth = 780 Hz, in-plane acceleration factor = 2, duration ~5 min).

Functional MRI

All participants underwent a standardized protocol that included 10 min of resting-state fMRI. Subjects were instructed to lie still with their eyes closed without falling asleep, trying not to think about anything specific. They were also instructed not to hold the signaling button with their hand.

Functional MRI was based on a T2*-weighted gradient-echo planar imaging (GE-EPI) sequence sensitive to blood oxygenation level-dependent contrast. Single-shot EPI sequence (56 continuous axial slices, 2.5 mm isotropic voxel, no slice gap, TE = 37 ms, TR = 735 ms, flip angle = 53°, acquisition matrix = 94 × 94, pixel bandwidth = 2130 Hz, no in-plane acceleration, multiband acceleration factor = 8, phase encoding AP, and 816 volumes).

MR spectroscopy

Single-voxel proton MR spectroscopy (suppressed-water Point RESolved Spectroscopy, PRESS) sequence was acquired within the ACC and PCC (volume = 8 mL, echo time/repetition time TE/TR = 30/2000 ms, number of averaged fids = 128, duration ~4 min). The voxel localization was performed using the three planes of high-resolution 3D T1 MPRAGE sequence (Figure 1a).

Neuroimaging analysis

T1-weighted images were skull-stripped using BET and registered to the Montreal Neurological Institute (MNI) standard brain with an affine followed by a non-linear approach, using the FLIRT and FNIRT tools from the FMRIB Software Library (FSL, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) [29, 30]. The freely available software FreeSurfer v6.0.0 (http://surfer. Nmr.mgh.harvard.edu/) was used to extract the volume, area and cortical thickness of the sub-parcellation of the cingulate cortex, according to the Destrieux atlas [31], basing on the 3D T1-weighted MPRAGE images (Figure 1b).

For functional MRI analysis, the data pre-processing was conducted using FSL [29, 30]. Motion correction was performed with the FSL-MCFLIRT tool. Using the output of FSL-MCFLIRT, an automatic pipeline has been developed for quality control of fMRI images: GE-EPI volumes that are displaced more than 1.5 mm in one of the three spatial directions and rotated more than 1.5° around the three spatial axes with respect to the central volume of the temporal fMRI series were removed. Images were then corrected for susceptibility distortions using the field-maps estimated from the diffusion sequences via FSL topup, skull-stripped, intensity-normalized, high-pass filtered, co-registered to the T1 volume and registered to the MNI standard brain. Spatial smoothing was performed using a full width at half maximum (FWHM) Gaussian kernel of 5 mm. Lastly, independent component analysis (ICA) was performed using a probabilistic approach as implemented in FSL-MELODIC [30]; the number of components was automatically estimated from the data. A single-session ICA was run and the so obtained components were manually classified between signal and noise, based on knowledge of resting-state networks (RSNs) patterns and of typical artifact characteristics, as described in the literature [32], using a conservative approach. Data denoising was then performed by regressing out the noise signals.

We investigated brain functional connectivity (FC) during resting-state, using two different approaches. The first was an independent component analysis (ICA), which is a data-driven approach

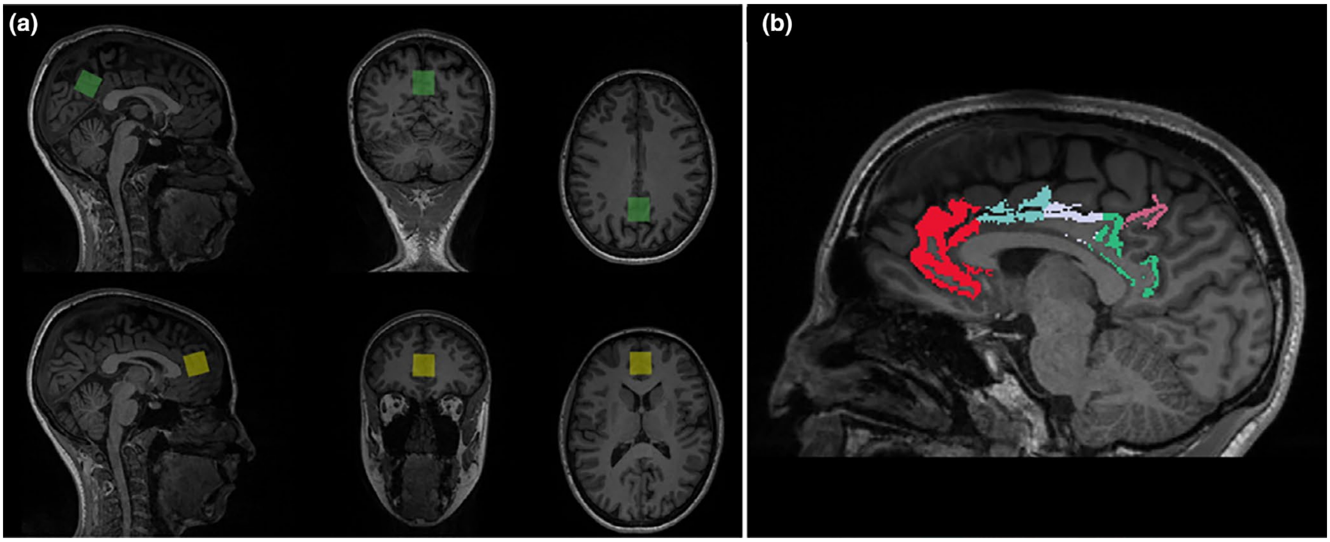

FIGURE 1 MRS localization and FreeSurfer segmentation of cingulate cortex. (a) 1H-MRS voxel ($20 \times 20 \times 20\,mm^3$) localization: In the PCC on the top, in the ACC on the bottom. (b) Five bilateral regions of the FreeSurfer parcellation of the cingulate cortex based on Destrieux atlas; from left to right: The anterior cingulate cortex (ACC), anterior mid-cingulate cortex (aMCC), posterior mid-cingulate cortex (pMCC), dorsal posterior cingulate cortex (dPCC), and ventral posterior cingulate cortex (vPCC). ACC, anterior cingulate cortex; MRS, magnetic resonance spectroscopy; PCC, posterior cingulate cortex.

that separates the resting-state fMRI data into a set of statistically independent spatial maps, grouping together temporally coherent brain regions (resting-state networks, RSNs) [32].

After pre-processing, group ICA with 50 components was performed on the temporally-concatenated functional images registered onto the MNI template. Components of interest were selected basing on their involvement of the cingulate cortex, via visual inspection, using the Yeo 17-Networks atlas as reference [33]. The selected networks corresponded to the default mode network (DMN), executive networks, salience network and sensory-motor network (SMN). To generate subject-specific RSNs maps a dual regression approach [34] was used, feeding into the regressions the group components of interest.

The second approach consisted in a seed-based analysis (SBA), which investigates the connectivity of specific apriori-chosen brain regions with the rest of the brain. The parcellation of different anatomic structures permits the evaluation of specific functional coupling, considering that some areas have different functions and connections depending on the anatomical area. SBA was performed by evaluating the pattern of FC of 5 seeds, selected as the five sub-segmentations of the cingulate gyrus identified by FreeSurfer according to the Destrieux atlas (Figure 1b). Analogously to ICA, SBA was performed via the dual-regression on the chosen seeds on the single-subject fMRI data.

For MR Spectroscopy analysis, the quality of the spectra was assessed through visual examination to determine the absence of artifacts. The assessment was conducted blindly with respect to the patients and controls diagnosis, according to the criteria for standard quality analysis [35]. Metabolite content was measured using the automatic software LCModel 6.3. In particular, N-acetyl-aspartate (NAA), choline (Cho), myo-Inositol (mI), glutamate (Glu), glutamine (Gln), and glutamate complex (Glx) related to creatine (Cr) or mI as internal reference were quantified. Starting from a prior known basis set of separate spectra for each the metabolites, LCModel optimized the line shape of all the peaks, their phase and frequency shift information [36].

Sleep analysis

Sleep stages were scored in 30-s epochs according to the AASM criteria [37]. The sleep data comprised the following: total sleep time, sleep efficiency, sleep latency, wake after sleep onset, REM sleep latency, sleep macrostructure, N3 stage fragmentation, microarousal index, and periodic limb movements index. In addition, for each patient, the video was carefully reviewed in order to identify DoA episodes and classify them into their three clinical entities, confusional arousal, sleep terror, or sleepwalking [1].

Statistical analysis

The normality of the distribution of all parameters was tested using the Shapiro–Wilk test.

Differences between DoA patients and HCs on clinical, sleep features and psychological tests were assessed using IBM SPSS vs.27 by means of the Student *t* test/Mann–Whitney test for normally distributed data or non-Gaussian distributed variables, respectively. For discrete variables a Pearson chi-squared test was adopted.

An analysis of covariance (ANCOVA) was used to compare ACC and PCC volumes between patients and controls, with total brain volume as covariate.

Group comparisons for fMRI connectivity maps were performed with a voxel-wise non-parametric bootstrap method based on the General Linear Model, using FSL's tool for permutation inference

(RANDOMISE) on neuroimaging data, with 5000 permutations. Results were corrected for multiple comparisons across voxels controlling for the family-wise error (FWE, $p<0.05$) and applying a threshold-free cluster enhancement method (TFCE). Age and sex were added as nuisance regressors for fMRI analysis. All the statistically significant results at a threshold of corrected-$p<0.05$ are reported.

The spectroscopic analysis was performed with ANCOVA using sex and age as covariates. A Pearson correlation explored any potential association between volumetric and metabolite data. Significance was set at p values <0.05, after Bonferroni correction for multiple comparisons.

Correlations between MR-derived features and clinical, sleep and psychological tests were performed with Pearson/Spearman tests (depending in whether the data were normally distributed) in the full sample, or patients and HCs separately, controlling for age, sex and education. Within the DoA patients' group, correlations with clinical variables (disease duration, disease severity, episode frequency, and sleep fragmentation) were performed. Results were corrected for multiple comparisons across all clinical tests and investigated regions for each MR feature using the false discovery rate. Statistical significance was set at corrected $p<0.05$.

RESULTS

In the time period of the study, out of 22 possible candidates, 15 patients with DoA (9 males, mean age 26.3 ± 7.7) were enrolled (4 patients had contraindication to MRI, 2 patients had scheduled surgery procedures, and 1 patient refused to participate). In the same period, our study sample was paired with 15 age- and sex-matched HCs (8 males, mean age 25.8 ± 3.6). In Tables 1 and 2, patients' clinical and sleep features are shown. All patients had a history of sleepwalking and/or sleep terror (12 patients had both manifestations, 2 patients only sleepwalking, and 1 only sleep terror). All patients except one had onset of DoA in childhood or adolescence. A family history of DoA was present in 9 subjects and the frequency of DoA manifestations at the moment of the observation was mostly weekly. The scale assessing DoA severity at the clinical interview, including a phenomenological (PADSS-A), a frequency (PADSS-B) and a consequence of the disorder (PADSS-C) section, showed overall scores ranging from a minimum of 14 to a maximum of 30 [21]. During VPSGs, all patients recorded at least one behavioral episode of DoA consisting mainly in confusional arousals, sleep terrors in a few cases and also sleepwalking in 3 patients. Overall, we recorded 56 episodes, 29 during the first night and 27 during the second night, all arising from N3 stage of sleep. In the first VPSG, patients had shorter sleep latency ($p=0.03$), longer total sleep time ($p=0.002$) and longer absolute time of each sleep stage (N1 $p=0.03$, N2 $p=0.02$, N3 $p=0.03$, REM $p=0.006$) as well as more sleep cycles ($p=0.02$). Table 3 shows a comparison of sleep and neuropsychological scales between patients and controls. Compared to HCs, patients scored higher at sleepiness scale ($p=0.02$) and showed worse subjective sleep quality ($p=0.0007$). In addition, patients' scores at state and trait anxiety were above the cutoff and significantly higher compared to HCs ($p=0.01$

TABLE 1 Clinical and episode features of DoA subjects.

DoA				PADSS				VPSG episodes		
ID	Positive family history	Disorder duration (yrs)	Frequency at recruitment	Total (0–50)	A (0–34)	B (0–6)	C (0–10)	CA	ST	SW
1	Yes	12	Weekly	16	4	4	8	2	0	0
2	Yes	18	Weekly	21	11	4	6	4	0	0
3	Yes	7	Weekly	18	9	4	5	2	0	0
4	No	17	~ Nightly	20.5	11	4.5	5	6	0	1
5	No	~10	~ Weekly	21	11	3.5	6.5	8	0	0
6	No	11	Weekly	19	10	4	5	1	0	0
7	Yes	7	Monthly	18	11	3	4	4	0	0
8	Yes	25	Nightly	30	17	5	8	11	1	2
9	No	22	Weekly	15	6	4	5	1	0	0
10	Yes	~10	Monthly	14	6	3	5	3	0	0
11	Yes	21	Nightly	18	9	5	4	4	1	0
12	No	11	Multiple/year	14.5	10	2.5	2	1	0	0
13	Yes	12	Weekly	17	8	4	5	1	0	0
14	Yes	27	~ Weekly	15.5	9	3.5	3	1	0	1
15	No	14	Weekly	14	6	4	4	1	0	0

Abbreviations: CA, confusional arousal; DoA, Disorders of Arousal; PADDS, Paris Arousal Disorders Severity Scale, the best cut-off to identify DoA for the total score was at 13/14; ST, sleep terror; SW, sleepwalking; VPSG, video-polysomnography; yrs., years.

TABLE 2 Sleep parameters in DoA subjects.

Sleep parameters	Night 1	Night 2	*p* value
TST	513.7 ± 112.8	370.8 ± 85.4	**0.002**
Sleep cycles	5.3 ± 1.4	3.5 ± 1.1	**0.02**
SL	6.2 ± 4.1	12.3 ± 10.8	**0.03**
SE	91.3 ± 2.8	89.7 ± 4.8	0.30
WASO	41.5 ± 14.4	28.7 ± 14.9	0.09
N1 (%)	6.1 ± 1.7	5.8 ± 2.8	0.66
N1 min	30.0 ± 6.0	21.3 ± 11.1	**0.03**
N2 (%)	46.4 ± 5.6	45.5 ± 6.8	0.81
N2 min	239.6 ± 71.2	168.9 ± 44.6	**0.02**
N3 (%)	22.1 ± 3.8	25.3 ± 5.7	0.08
N3 min	111.4 ± 23.3	91.9 ± 25.7	**0.03**
R (%)	25.4 ± 2.4	23.4 ± 6.1	0.18
R min	130.5 ± 34.0	88.6 ± 32.3	**0.006**
Arousal index N3	6.3 ± 3.8	7.1 ± 2.6	0.27

Note: Sleep stages are reported as % of TST. $p \le 0.05$ are highlighted in bold.

Abbreviations: N1, NREM stage 1; N2, NREM stage 2; N3, NREM stage 3; R, REM sleep; SE, sleep efficiency; SL, sleep latency; TST, total sleep time; WASO, wake after sleep onset.

and $p = 0.04$ respectively). Moreover, differently to HCs, patients scored above cutoff also for depression signs ($p = 0.0005$), perceived psychological stress ($p = 0.03$), and difficulties in emotion regulation ($p = 0.04$). Finally, at the CTQ-SF, childhood experience of emotional abuse and/or neglect were reported more frequently among DoA patients compared to HCs ($p = 0.05$).

Multimodal imaging

As regards morphometric analysis, no between groups differences in cingulate cortex sub-parcellations were disclosed in terms of volume, area or cortical thickness.

The resting-state ICA showed a significant difference in SMN (Figure 2a) with increased connectivity in patients with opercular cortex, precuneus, occipital pole, and lingual gyrus (Table S1).

The resting-state SBA revealed a significant difference in the connectivity of the dorsal PCC (Figure 2b), with reduced connectivity in patients with several areas, including the pre and post-central gyrus, supplementary motor area (SMA), putamen, ACC, parietal superior lobule, precuneus, opercular cortex, hippocampus, and thalamus (Table S2).

MR spectroscopy of the PCC showed, as the only abnormality, a significant decrease of the Gln content ($p < 0.001$) in the patients (Figure 3). No significant differences were detected between patients and healthy subjects in the ACC.

No statistically significant correlations between fMRI/MRS features and clinical/sleep/psychological data were found after correction for multiple comparisons, neither in the full sample, nor in the patient subgroup.

DISCUSSION

We investigated the neurofunctional and metabolic characteristics at rest of a population of DoA subjects. DoA are traditionally labeled as a self-limiting, mostly pediatric disorder, but increasing evidence is challenging this view. Our DoA population comprised young adults free from comorbidities and therapies, reflecting the features of similar patients commonly arriving at adult sleep centers who may show slight diurnal impairment in different domains. While not reaching frankly pathological scores, our sample of DoA scored above cut off at sleepiness and insomnia scales, and showed a clinical profile characterized by pervasive stress, anxiety, depressive mood and emotion regulation difficulties, in line with literature evidence [38]. Daytime impairment, potentially impacting learning, could be partly responsible for the slight difference in education between patients and controls. Neurophysiologic literature evidence is scarce in these patients and this is especially true for neuroimaging studies [11–15]. We show the presence of an increased connectivity in the SMN in DoA patients, a decreased connectivity of the dorsal PCC with several brain areas and a metabolic alteration (a decrease in glutamine) in the PCC.

Increased connectivity in sensory motor network in DoA: a facilitation of motor circuits

We detected in DoA subjects an increased connectivity within the SMN affecting the frontal operculum, precuneus, occipital pole, and lingual gyrus (Figure 2a, Table S1). To date, this is the first evidence of an alteration of a RSN under basic conditions in DoA, being in accordance with some direct and indirect neurophysiological evidence. Stereo-EEG studies documented the activation of motor areas during in vivo episodes [8]. Also indirect evidence shows an increased activation of motor areas [9, 10]. In particular, a study using transcranial magnetic stimulation found a hypoexcitability of some inhibitory circuits belonging to GABA-A neurotransmission and cholinergic inhibitory circuits within the motor cortex of sleepwalkers [10]. A high-density (HD) EEG study disclosed a significant decrease in slow wave activity (indicating an increased activation) during NREM sleep in a centro-parietal cluster including the motor, sensory-motor, cingulate cortices and precuneus [9]. These alterations were not correlated with clinical features of DoA, suggesting that local alterations of neuronal excitability, mainly in the motor and cingulate cortex, might be present independently from clinical episodes and predispose to their occurrence [9]. Similarly, an EEG power seed-based study found an increased connectivity in the beta frequency band between the motor cortex and the anterior and posterior cingulate cortices during arousals, unrelated to DoA events [15]. To a certain extent, a higher activation of the motor cortex is physiologically present also in normal subjects during sleep arousal [39]. However, DoA patients seem to show an extreme deregulation of a physiological arousal process, as they are prone to perform motor activities

TABLE 3 Sleep scales and psychological assessment in DoA subjects and healthy controls.

	DoA ($n = 15$)	HC ($n = 15$)	p value
Age (yrs)	26.3 ± 7.7	25.8 ± 3.6	0.62
Sex (M/F)	9/6	8/7	0.73
Education (yrs)	16.1 ± 2.4	18.6 ± 2.5	**0.01**
ESS (n.v. <11)	4.7 ± 2.8	2.6 ± 1.6	**0.02**
PSQI (n.v. <6)	5.6 ± 2.1	2.9 ± 1.6	**0.0007**
STAI-Y			
STAI-State (n.v. <40)	42.9 ± 12.0	31.9 ± 7.1	**0.01**
STAI-Trait (n.v. <40)	45.3 ± 13.5	35.8 ± 9.7	**0.04**
BDI-II (n.v. <14)	11.4 ± 6.9	3.7 ± 3.3	**0.0005**
PID-5-BF			
Negative affect	1.09 ± 0.57	1.04 ± 0.43	0.78
Detachment	0.73 ± 0.45	0.56 ± 0.35	0.25
Antagonism	0.72 ± 0.42	0.53 ± 0.40	0.23
Disinhibition	0.92 ± 0.56	0.61 ± 0.46	0.11
Psychoticism	0.93 ± 0.49	0.76 ± 0.45	0.31
PSS (n.v. <14)	18.2 ± 5.14	13.87 ± 5.45	**0.03**
CTQ-SF			
Emotional abuse (n.v. <11)	9.0 ± 3.70	6.87 ± 1.77	**0.05**
Physical abuse (n.v. <9)	5.4 ± 1.06	5.27 ± 1.03	0.57
Sexual abuse (n.v. <14)	6.07 ± 4.13	5.67 ± 2.58	1
Emotional neglect (n.v. <11)	10.73 ± 3.56	8.4 ± 2.64	**0.05**
Physical neglect (n.v. <16)	5.53 ± 0.83	5.33 ± 0.82	0.39
Minimization/denial (n.v. <2)	0.4 ± 0.63	0.27 ± 0.46	0.07
DES (n.v. <31)	9.64 ± 7.04	5.64 ± 3.74	0.06
Amnestic dissociation	44.67 ± 45.65	22.67 ± 26.58	0.12
Absorption and imaginative involvement	192.67 ± 137.14	119.33 ± 73.14	0.08
Depersonalization/derealization	32.67 ± 32.17	16.0 ± 21.31	0.11
DERS (n.v. <81)	86.33 ± 17.10	74.0 ± 14.49	**0.04**
Non acceptance of emotional responses	11.33 ± 2.50	11.0 ± 2.27	0.71
Difficulties engaging in goal-directed behavior	15.00 ± 3.42	12.4 ± 5.58	0.14
Limited access to emotion regulation strategies	19.4 ± 4.48	17.93 ± 2.69	0.29
Impulse control difficulties	11.0 ± 5.07	8.47 ± 1.85	0.08
Lack of emotional clarity	10.93 ± 3.79	9.47 ± 2.39	0.22
Lack of emotional awareness	7.2 ± 2.83	6.13 ± 2.47	0.28

Note: $p \leq 0.05$ are highlighted in bold.

Abbreviations: BDI-II, Beck Depression Inventory, second version; CTQ-SF, Childhood Trauma Questionnaire-Short Form; DERS, Difficulties in Emotion Regulation Scale; DES, Dissociative Experiences Scales; DoA, disorders of arousal; ESS, Epworth Sleepiness Scale; F, females; HC, healthy controls; M, males; n, number; n.v., normative values; PID-5, Personality Inventory for DSM-5; PSQI, Pittsburgh Sleep Quality Index; PSS, Perceived Stress Scale; STAI, State Trait Anxiety Inventory; yrs., years.

even with subthreshold triggers during NREM sleep. This increased and altered arousability has been considered as a dysfunctional survival reflex, where DoA subjects show a prompt motor reaction in response to a sudden threat, together with the persistence of sleep need over other brain areas [8]. In addition, DoA subjects show a higher ability to perform complex locomotor actions under cognitive load [40] as well as higher capacity to perform automatic movements [41]. All in all, DoA subjects seem

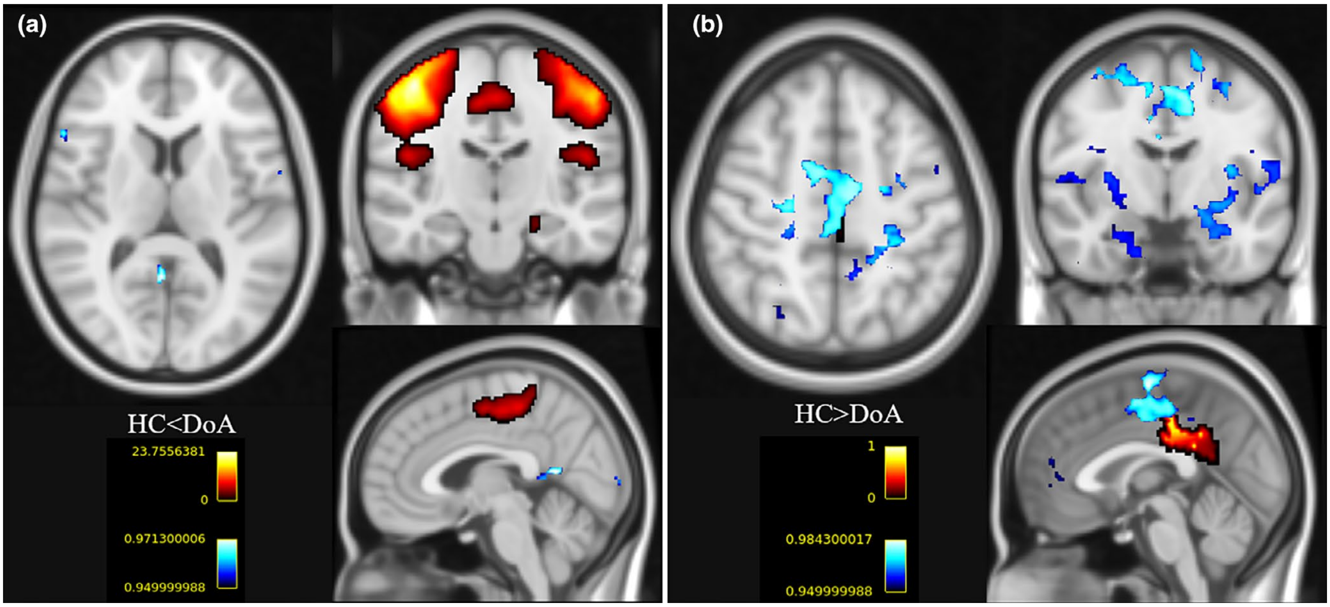

FIGURE 2 Resting-state fMRI alterations through ICA and SBA. (a) SMN (red) increased connectivity (blue) with occipital precuneus and right inferior frontal gyrus pars opercularis in DoA patients. (b) Left PCC (red) altered connectivity (blue) with several regions emerging from SBA: lower PCC connectivity in DoA patients with respect to HCs. DoA, disorders of arousal; HCs, healthy controls; ICA, independent component analysis; PCC, posterior cingulate cortex; SBA, seed-based analysis; SMN, sensory motor network.

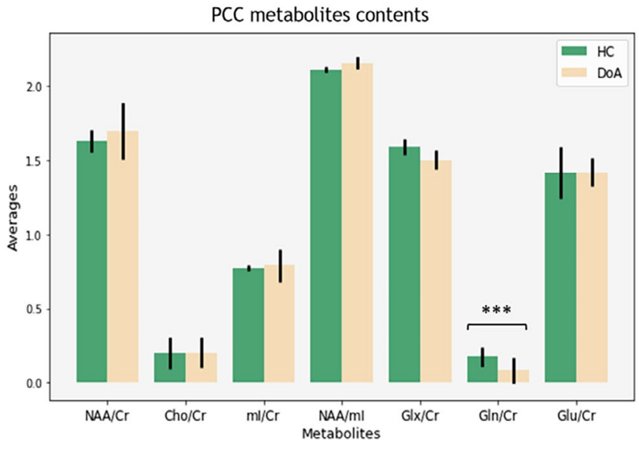

FIGURE 3 Single voxel proton MRS PCC analysis. Barplot representation of metabolites content in PCC between HCs and DoA patients. Gln/Cr content level is significantly different between the two groups. Cho, choline; mI, myo-inositol; Cr, creatine; DoA, disorders of arousal; Gln, glutamine; Glu, glutamate; Glx, glutamate + glutamine complex; HCs, healthy controls; MRS, magnetic resonance spectroscopy; NAA, N-acetyl-aspartate; PCC, posterior cingulate cortex.

to show a sort of "facilitation" of motor circuits, especially under unaware conditions.

In DoA subjects, the SMN had an increased connectivity with the frontal operculum, an area suggested to be involved in emotional facial expressions [42], with areas related to the visual system (occipital cortex), visual memory (lingual gyrus) [43], and integration of visuo-spatial imagery (precuneus) [44]. These activation could explain the facial expression changes in DoA [45] and the capacity of DoA to navigate into space.

Functional and metabolic alterations of posterior cingulate cortex in DoA

SBA of fMRI dataset showed a decreased connectivity of the left dorsal PCC with several cortical and subcortical areas in DoA subjects. This finding was associated with a significant decrease in glutamine content in the PCC assessed by MR spectroscopy. The PCC is one of the most important multi-sensorial integration hubs, involved in arousal and awareness processes, essential for visuospatial orientation and navigation in space, and in the integration between internal and external attention [46, 47]. Structural evidence has shown VBM alterations in this area, reinforcing the hypothesis that this hub might play a role in DoA pathophysiology [11, 12]. In addition, different studies documented an activation of PCC during DoA episodes. In particular, a SPECT study revealed an increased activation of the PCC and cerebellum during sleepwalking [3]. A similar higher activation of a centro-posterior zone mainly involving the precuneus and the superior parietal lobe was disclosed by a HD EEG study during confusional arousals, suggesting the implication of this area in a partial "regain of consciousness" along with the presence of some form of mental activity during DoA episodes [48]. In summary, the PCC appears to be a critical node in DoA patients, playing a major role in the development of these dissociated episodes, with blurred boundaries between sleep and wake. Consciousness is considered a combination of two main components: arousal (the level of consciousness), and awareness (related to the content of consciousness) [49]. Arousal involves the activity of subcortical structures including the brainstem reticular formation, hypothalamus, and basal forebrain, while awareness is related to the activity of a widespread set of frontoparietal associative areas [49]. In particular, self-awareness encompasses the posterior cingulate/precuneal cortices, medial frontal cortex, and bilateral temporoparietal junctions while the external awareness network involves the lateral frontal and parietal cortices [49]. Arousal and awareness are usually positively correlated but there are exceptions such as vegetative state (fully aroused but unaware), seizures or, as in our case, NREM parasomnias [49]. DoA episodes are, in fact, believed to be triggered by an arousing stimulus (either internal or external), in absence of (or maybe with a partial) awareness. It seems, then, that the arousal process promoted by subcortical structures and reaching the PCC induces a partial regain of consciousness [47, 48] (or more properly self-awareness) which, however, does not proceed to the ultimate level of complete awareness (and with that consciousness) typical of a wake state. In fact, higher order cognitive processing (including intentions to move/willed actions, the logic of mental activity, and the inductive reasoning) recognize mainly prefrontal areas, the ACC and the MCC as critical nodes [47]. To this extent, it may be proposed that, during DoA episodes, both the heterogeneous presence of local sleep/wake activities throughout different brain areas and also the breakdown of cortical connectivity typical of deep sleep [50], prevent the possibility to reach an integrated functional connectivity. Under this perspective, these disorders, currently collected under the label of disorders of arousal, more than an impairment of the arousal process per se, seem more specifically concerned by a difficulty to reach a complete awareness and, with that, consciousness.

In our study, we observed a decrease in glutamine content in the PCC. Glutamine is linked to the metabolic cycle of glutamate and GABA and this cycle is strictly linked to cortical glucose utilization, implying for glutamine a pivotal role in the energetic functioning of the cerebral cortex [51]. The PCC is one of the principal hubs of the DMN and has a metabolic rate almost 40% greater than average [46, 52]. The decrease in glutamine content in the PCC might hint to a need to re-establish a normal metabolic balance during the day, since an increased activity of this area is present during the night in DoA patients [3, 48]. Moreover, according to literature data showing that neurotransmitter concentrations within a node may modulate connectivity across a brain network [52], the significant decrease in glutamine found in the PCC may have influenced the connectivity of this central hub, which we found decreased with different cortical and subcortical areas (Figure 2b, Table S2).

Our results at the ICA in the SMN and the SBA on the PCC showed no correlation with the clinical, sleep and psychological parameters and scales. On the other hand, the analysis of MRS findings with clinical/sleep/psychological data, revealed some correlations between metabolite expression on the PCC and the scales mainly involving perceived stress, state and trait anxiety, and subitems of emotion dysregulation (Table S3). However, none of these survived the correction for multiple comparisons. Similarly to SMN hyperconnectivity and due to its role of multi-sensorial integration area, the alterations on the PCC may suggest a "functional impairment" during normal wakefulness without any activating condition. The lack of correlations may be influenced by the conservative statistic approach as well as the small sample, which is a limit of our study, hence one cannot exclude that a larger sample would allow some of these correlations to emerge. A tendency to negative stress coping strategies related to VBM alterations on the PCC, in fact, was already seen in DoA patients [12]. However, a major role for emotional control traditionally belongs to the anterior part of the cingulate cortex [18]. In addition, trait-like functional abnormalities which predispose to the occurrence of the episodes in DoA have similarly been suggested in other studies, where no correlations with clinical or sleep features were reported [9]. Although we found no alterations in the ACC, as we had postulated based on the idea that seizures arising from ACC may share overlapping motor features with parasomnia episodes [16], this may also depend on the different adopted parcellation of the cingulate cortex [18].

CONCLUSIONS

Our data add new findings in DoA mechanisms, suggesting a motor facilitation of DoA patients contributing to the occurrence of inappropriate behaviors during sleep. Interestingly, we also detected both metabolic and functional alterations on PCC, suggesting a substrate for a dysfunctional arousal process but also shedding light on the process to reach a complete awareness and, with that, consciousness. To address these complex issues, future studies should include standardized protocols with direct interview of patients during the episode or soon after. This approach would offer the possibility to collect information regarding the mental state, self-awareness, recall of the episodes, to compare episodes of different types and intensity and, with the possibility to use advanced methodic like high-density EEG, might add precious information on the neural mechanisms subtending DoA. Larger studies are warranted to confirm our findings. Furthermore, analyzing brain connectivity during a task or during sleep could be valuable to evaluate other structures involved in the pathophysiology of DoA.

AUTHOR CONTRIBUTIONS

Greta Mainieri: Conceptualization; writing – original draft; data curation; methodology; formal analysis. **Magali Jane Rochat:** Writing – review and editing; formal analysis; data curation; conceptualization. **Elena Cantoni:** Writing – review and editing; formal analysis; data curation. **Giovanni Sighinolfi:** Writing – review and editing; formal analysis; data curation. **Micaela Mitolo:** Conceptualization; data curation; writing – review and editing. **Giuseppe Loddo:** Conceptualization; writing – review and editing. **Francesco Mignani:** Data curation; writing – review and editing. **Susanna Mondini:** Writing – review and editing; data curation. **Raffaele Lodi:** Writing – review and editing; supervision; methodology. **Federica Provini:** Conceptualization; writing – review and editing; methodology; supervision; data

curation. **Caterina Tonon:** Conceptualization; methodology; writing – review and editing; data curation; supervision.

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CONFLICT OF INTEREST STATEMENT

Nothing to report.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Greta Mainieri https://orcid.org/0000-0002-6159-854X
Federica Provini https://orcid.org/0000-0001-9063-2658

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SUPPORTING INFORMATION

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