Thalamic altered spontaneous activity and connectivity in obstructive sleep apnea syndrome

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ABSTRACT

Obstructive Sleep Apnea (OSA) syndrome is a sleep disorder characterized by excessive snoring, repetitive apneas, and nocturnal arousals, which leads to fragmented sleep and intermittent nocturnal hypoxemia. Morphometric and functional brain alterations in cortical and subcortical structures have been documented in these patients via magnetic resonance imaging, even if correlational data between the brain's alterations, cognitive and clinical indexes are still not reported. Here, we examined the impact of OSA on brain spontaneous activity by measuring the fractional amplitude of low-frequency fluctuations (fALFF) in resting-state fMRI data of twenty drug-naïve OSA patients and twenty healthy controls matched for age, gender, and Body Mass Index. Patients showed a pattern of significantly abnormal subcortical functional activity as compared to controls, with increased activity selectively involving the thalami, specifically their intrinsic nuclei connected to somatosensory and motor-premotor cortical regions. Using these nuclei as seed regions, the subsequent functional connectivity analysis highlighted an increase in patients' thalamocortical connectivity at rest. Additionally, the correlation between fALFF and polysomnographic data revealed a possible link between OSA severity and fALFF of regions belonging to the Central Autonomic Network. Our results suggest a hyperactivation in thalamic diurnal activity in OSA patients, which we interpret as a possible consequence of increased thalamocortical circuitry activation during night-time due to repeated arousals.

INTRODUCTION

Obstructive sleep apnea (OSA) syndrome is the most common sleep-related breathing disorder in the world (Horner, 2008), typically causing a cascade of daytime symptoms including diurnal sleepiness and neurocognitive problems, such as deficits in memory, attention and executive functions (Bulcun, Ekici, & Ekici, 2012). Several functional neuroimaging techniques have been utilized to explore the biological mechanisms underlying OSA; however, the nature of alterations remains unclear[JD1]. A task-fMRI evaluation also suggested a recruitment of additional brain regions during learning and memory tasks in OSA patients as compared to control subjects (Ayalon, Ancoli-Israel, Klemfuss, Shalauta, & Drummond, 2006), possibly reflecting adaptive compensatory recruitment as the one observed after sleep deprivation in healthy subjects (Drummond et al., 2000). The vast majority of morphometric studies report gray matter concentration differences in cortical and subcortical regions such as the hippocampus, parahippocampal gyrus, cerebellum, temporal lobe, frontoparietal, and anterior cingulate cortices (Joo et al., 2010), findings partially confirmed recently in pediatric patients (Philby et al., 2017). Additionally, widespread white matter integrity alterations, including axons linking major structures within the limbic system, pons, frontal, temporal and parietal cortices, and projections to and from the cerebellum (Macey et al., 2008) were shown in OSA patients[SR2]. A positron emission tomography (PET) study suggested a right-lateralized decrease in brain metabolism with the involvement of precuneus, middle, and posterior cingulate gyrus, parietal–occipital and prefrontal cortices (Yaouhi et al., 2009), a result which has been recently replicated by using functional magnetic resonance imaging (fMRI) connectivity analysis (Santarnecchi et al., 2013). [JD3]Finally, a resting-state fMRI analysis by Li and colleagues (Li et al., 2016) revealed altered functional connectivity within regions of the Default Mode Network (DMN), with a negative correlation between delayed memory scores

and the strength of functional connectivity between posterior cingulate cortex (PCC) and right hippocampus. While these evidence point in the direction of widespread changes in brain functioning related to OSA, the link between the brain's alterations, cognitive and clinical indexes is still unclear.

Non-invasive measurements of spontaneous low-frequency fluctuations of BOLD (blood oxygenation level-dependent) signal as measured by resting-state fMRI has been successfully applied to the study of brain intrinsic activity, showing the interaction of anatomically and functionally defined resting-state networks even at rest (Fox et al., 2005). The amplitude of low-frequency BOLD fluctuations (ALFF, Zou et al., 2008) is reliably linked with functional states of the brain, to an extent that it has been suggested as a marker for pathological conditions like epilepsy (Zhang et al., 2010) and Alzheimer's disease (He et al., 2007). ALFF is thought to specifically measure the extent of Spontaneous Neuronal Activity (i.e. SNA; Zuo et al., 2010). In particular, higher ALFF seems to reflect higher spontaneous neuronal activity (Zou et al., 2008), even though higher values have been also reported in brain cisterns and large blood vessels, revealing its susceptibility to physiologically irrelevant noise. This caveat has been resolved by a modified version of ALFF, (i.e. fractional ALFF - fALFF), that uses the ratio of the power spectrum of low frequency (0.01–0.08 Hz) to the entire frequency range (e.g. 0–0.25 Hz for a TR = 2 s). fALFF successfully discriminated between the activity of cortical regions and liquor cisterns (Zou et al., 2008). To date, only one study exploring ALFF of untreated male OSA patients is available (Li et al., 2015), showing decreased activity in the right precuneus and bilateral PCC (both areas belonging to the DMN), that correlate with the lowest oxygen saturation and MoCA (Montreal Cognitive Assessment) score.

Here we expand on these preliminary findings by comparing the fALFF (instead of ALFF) at cortical and subcortical levels of treatment-naïve, both male and female patients with

moderate to severe OSA and healthy controls. By collecting polysomnographic data, we also aimed at detecting possible correlations with clinical and neuropsychological indexes, to evaluate if alterations in spontaneous activity are linked to OSA clinical manifestation and impaired cognitive performance.

Based on previous literature and the aforementioned considerations, we hypothesize: (i) that OSA patients will show alterations in sleep-related (i.e. induction, maintenance) brain structures; (ii) that clinical and cognitive symptoms severity will correlate with fALFF values of brain regions associated to the attention and working memory deficits observed in OSA.

MATERIALS AND METHODS

Participants

Forty-two participants were studied: 21 drug-naïve OSA patients and 21 age-and education matched healthy controls (patients: mean age 42.9 ± 7 years; 17 male; Body Mass Index: 29.5 ± 3; educations: 14.1 ± 2 years; controls: 41 ± 6 y.; 16 male; Body Mass Index: 25.7 ± 3; education: 12.4 ± 2 y.). All participants were right-handed (Oldfield Handedness scale) and monolingual native speakers. Inclusion criteria for patients were: (1) diagnosis of OSA (i.e. apnea/hypopnea index [AHI] >30), (2) age between 30 and 55 years, (3) no evidence of other medical disorders, with particular reference to current or past neurological and psychiatric ones, hypertension, diabetes, obesity (Body Mass Index [BMI] >30) or other sleep disorders and (4) no treatment received for the condition. Healthy controls had an AHI < 5, normal neurological physical examination, regular sleep-wake rate, absence of sleep disorders investigated through a sleep interview performed by a sleep medicine specialist (IS), and through a sleep diary/questionnaire.

Participants were excluded if they demonstrated (a) symptoms of cognitive deterioration (Mini-Mental State Examination < 27) (FN) or (b) brain structural abnormalities evaluated based on MRI images by an experienced neuroradiologist (CA). Because the higher prevalence of silent cerebrovascular lesions has been reported in patients with moderate-severe OSA (Nishibayashi, Miyamoto, Miyamoto, Suzuki, & Hirata, 2008), only subjects with a negative response to gray/white matter lesions detection on T1/T2-weighted images were included in the statistical analysis. One OSA patient and one control were therefore excluded, respectively for MRI lesions detected in T2-weighted images and electroencephalogram epileptiform alterations. All participants provided their written informed consent to the experimental procedure that was conformed to the Declaration of Helsinki. The study was approved by the University of Siena ethical committee.

Sleep quality assessment and in-patient evaluations

All participants including healthy controls reported regular sleep-wake schedules based on daily sleep diaries with an average total sleep time of 7.1 ± 1.2 h in the week before the study. Patients and control subjects underwent a habituation night at the center, aimed at reducing potential biases due to *first night effect* on both polysomnography (PSG) data and patterns of brain spontaneous activity (fMRI). Starting from day 2, participants underwent full nocturnal PSG (32 electroencephalogram -EEG- channels, video-recording, standard electro-oculogram, electromyogram, electrocardiogram, thoraco-abdominal movements, and snoring recording), MRI scanning, sleepiness (Epworth Sleepiness Scale -ESS; Johns, 1991), and mood (Beck Depression Inventory – BDI; Beck & Steer, 1984) evaluations, as well as a comprehensive neurocognitive assessment. The following electro-clinical indexes were derived from the PSG exam: Apnea-Hypopnea Index (AHI), minimal saturation values (Sa02min), time below 90% of saturation (T<90%), Apnea and Hypopnea mean duration in seconds (respectively mean apnea duration – MAD, mean hypopnea duration – MHD). Clinical scores related to pathology length and sleepiness levels were also collected (**Table 1**).

Cognitive assessment

Participants were tested using validated neuropsychological tests assessing the following cognitive domains/functions (FN): sustained and divided attention (Trail Making Test - TMT, form A and B, attentional matrices; Corrigan & Hinkeldey, 1987), verbal and visuospatial, working and long-term memory (Corsi visuospatial span; single-digit span; Rey Auditory Verbal Learning Test – RAVLT, Schoenberg et al., 2006) and executive function (Frontal Assessment Battery – FAB; Dubois, Slachevsky, Litvan, & Pillon, 2000).

Neuroradiological Acquisition

MRI examinations of all subjects were performed using a Philips INTERA scanner (Philips Medical Systems, the Netherlands). The scanning session included the acquisition of a whole-brain T1-weighted FFE 1-mm thick axial image (TR/TE=30/4.6 ms, flip angle=30 degrees, FOV=250mm, matrix 256x256), acquired in parallel to the anterior and posterior commissures; a T2-weighted Turbo Fluid Attenuated Inversion Recovery (FLAIR) 3-mm-thick axial image; a BOLD T2* weighted sequence acquired during rest condition (TR/TE=2500/40ms, 200 scans, 23 interleaved slices). Participants performed fMRI acquisition nearly 5-6 hours after their awakening. They were instructed to keep their eyes open during the video-monitored fMRI acquisition, try to relax and behave normally inside the scanner, easing mind-wandering and avoiding focusing on any particular topic. Additionally, to control for possible episodes of patients' sleepiness inside the scanner which might alter brain connectivity patterns, both patients and controls were allowed to sleep for two hours before the MRI acquisition (while monitored using PSG to quantify sleep efficacy), but not in the immediate 2 hours before the MRI session so to avoid sleep inertia upon awakening (Balkin & Badia, 1988). Patients' state inside the scanner was monitored via a remote camera for the entire duration of the MRI session.

Functional Imaging Data Preprocessing and Statistical Analysis

Functional image preprocessing and statistical analyses were carried out using SPM8 software (Wellcome Department of Cognitive Neurology, University College London; http://www.fil.ion.ucl.ac.uk/spm/) within the MATLAB environment (MathWorks, MA, USA). The first five volumes of functional images were discarded to allow for steady-state

magnetization. BOLD images were slice-time corrected following the interleaved descending acquisition criteria, realigned and resliced to mean volume to correct for head motion. Two recent studies suggested that head motion during MRI scanning might induce significant changes in functional connectivity (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012; Van Dijk, Sabuncu, & Buckner, 2012). To address this issue, we applied time series interpolation procedure based on displacement indexes proposed by Power and colleagues (2012), i.e. Frame-wise displacement (FD) and the RMS variance of the temporal derivative (DVARS). Functional time points showing FD > 0.5 mm and DVARS > 0.5 have been interpolated using a cubic spline function. Structural images were co-registered to the mean volume of functional images and subsequently segmented using a NewSegment routine in SPM8. Hidden Markov Random Field model was applied to remove isolated voxels. To obtain a more accurate spatial normalization we applied the SPM8 DARTEL module creating a customized gray matter template from all subjects' segmented images. Nonlinear normalization procedure with subsequent affine-only normalization to the Montreal Neurological Institute (MNI) template brain and voxel resampling to an isotropic 3x3x3 mm voxel size was then applied to functional MRI images. Linear trends were removed to reduce the influence of the rising temperature of the MRI scanner. All functional volumes were bandpass filtered at $0.01\ Hz < f < 0.08\ Hz$ for the ALFF analysis to reduce low-frequency drift and physiological high-frequency respiratory and cardiac noise.

Fractional ALFF computation

Filtered time-series were transformed into a frequency domain with a Fast Fourier Transform (FFT) using the Rest toolbox (http://www.restfmri.net/forum/REST) with subsequent calculation of the power spectra. Since the power of a given frequency is

proportional to the square of the amplitude of this frequency component of the original time series in the time domain, ALFF was defined as the square-root of the power spectrum, averaged across 0.01–0.08Hz at each voxel. As detailed in the introduction section of the manuscript, to improve the sensitivity of this measure, a ratio of the power of each frequency at the low-frequency range (0.01–0.08Hz) to that of the entire frequency range (0–0.25Hz) was calculated resulting in an index called fractional ALFF (fALFF; Zou et al., 2008). No spatial smoothing was applied on fALFF maps to better separate sources of alterations in smaller subcortical structures [JD4][ESan5](e.g. hypothalamus, thalamus nuclei).

Given the nature of fALFF representing local alterations in brain activity, the hypothesis that such alteration might also correspond to changes in the connectivity between the highlighted regions(s) and the rest of the brain seems legit. Therefore, in the case of significant differences between fALFF values of OSA respect to controls, a voxel-wise seed-based connectivity analysis using the clusters of significant between-group differences as seed regions was run.

Statistical analysis

Group differences in fALFF

Two-sample t-tests were used to assess group differences in age and years of education, while Pearson's Chi-square test was used to compare gender ratios (SPSS 21, SPSS Inc., Chicago). The comparison between fALFF levels of OSA patients and healthy controls was performed using a voxel-wise Analyses of Covariance (ANCOVA) model. Multiple comparisons correction was performed using Monte Carlo simulations with the following parameters: cluster [JD6][ESan7]connection radius 4mm, 1000 iterations, cluster threshold $p<.05$ corresponding

to 27 voxels on unsmoothed maps, voxel-level threshold p<.05. The same analysis was performed on functional connectivity maps of thalamic nuclei, by adopting a different cluster threshold (p<.05, 76 voxels on unsmoothed maps) due to the smoothing kernel applied (FWHM 4mm).

The link between fALFF, clinical and cognitive symptoms

To highlight possible links between clinical manifestation and brain spontaneous activity, voxel-wise correlation coefficients (Pearson's 'r' product-moment coefficient) were calculated between fALFF values and the following clinical scores: AHI, SA02min, T<90%, Apnea and Hypopnea mean duration in seconds (respectively mean apnea duration – MAD, mean hypopnea duration – MHD), pathology length and sleepiness. The resulting statistical maps were analyzed using a one-sample t-test (p<.05, Montecarlo correction for multiple comparisons) leading to the identification of brain regions whose fALFF level was significantly correlated with the corresponding clinical index. The same procedure was applied to neuropsychological scores, to identify regions whose alterations in fALFF values might correlate with the cognitive deficits observed in OSA patients. All analyses included age, BMI, normalized gray matter volume maps (obtained through voxel-based morphometry, VBM; Ashburner, 2007), and total brain volume (TBV) as covariates. Anatomical mapping of significant clusters was performed using the ANATOMY toolbox for SPM8. Seed-brain connectivity differences were calculated using the same procedure applied for fALFF maps, by comparing patients and controls' voxel-wise connectivity maps using the parameters specified for the aforementioned ANCOVA model.

Multivariate classification

Machine learning methods have been previously applied by our (Santarnecchi et al., 2014) and several other groups on an increasing number of neurological and psychiatric diseases (Wegrzyk et al., 2018; Zhong et al., 2017; Richiardi et al., 2012) to correctly identify patients respect to healthy controls. However, whether fALFF might represent a suitable marker in OSA has not been explored yet. To test such hypothesis, the mean fALFF value of 90 anatomically defined brain regions corresponding to the Anatomical Automatic Labeling (AAL; Tzourio-Mazoyer et al., 2002) atlas were included in a multivariate pattern analysis classification procedure (Cabral et al., 2016; Cabral et al., 2016). Values were analyzed using several generative and discriminative machine learning algorithms (n=14) based on a 40x90 matrix (subjects * brain regions), following a leave-one-out cross-validation approach implemented using the Weka software (Frank, Hall, Trigg, Holmes, & Witten, 2004). These procedures provide estimates of accuracy, sensitivity, specificity, and area under the Receiving Operating Curve (ROC) curve values in the context of patients' automated identification process based on their regional fALFF values respect to controls.

RESULTS

Demographic and neuropsychological data comparisons

Patients and control subjects did not differ in terms of age, gender, BMI, mood, and educational levels, while patients showed a significant increase in sleepiness scores (**Table 1**). Compared to healthy controls, patients reported a lower performance at the immediate recall of RAVLT ($p<.025$), TMT-B ($p<.027$), and attentional matrices ($p<.015$) (**Table 1**).

Please insert Table 1 around here

Group differences in fALFF

OSA patients showed a pattern of increased fALFF in lateral and anterior thalamic nuclei bilaterally. No other group-level differences in fALFF were found. Anatomical localization of thalamic clusters of increased activity is reported in **Figure 1** and **Table 2**. In light of the group comparison results, an attempt to identify thalamic-related differences in brain connectivity was made. To provide a more comprehensive analysis of possible thalamic alterations, an a-priori selection of regions of interests (ROIs) covering major thalamic nuclei (Ide, Kakeda, & Korogi, 2015) was done, using the FSL white matter tracts atlas (FMRIB Software Library, Oxford, UK). Bilateral thalamic nuclei connected to prefrontal, premotor, motor, somatosensory, parietal, temporal, and visual cortices were defined and then included in a voxel-wise seed-based functional, connectivity analysis. Zero-lag correlation values between the mean BOLD time series of each ROI and the rest of the brain were calculated for both patients and control subjects. Correlation maps were then smoothed using a 4mm FWHM kernel and converted to a normal distribution using Fisher's r-to-z transformation.

Please insert Figure 1 and Table 2 around here

Correlations between fALFF, clinical and neuropsychological data

Clinical scores. Significant negative correlations were observed between (i) AHI and fALFF of the left insular lobe and bilateral frontal, temporal, parietal and occipital cortices; (ii) MAD and fALFF of the anterior cingulate gyrus and caudate nuclei; (iii) MHD and fALFF related to bilateral prefrontal lobes, namely anterior cingulate cortex, middle frontal, middle orbital, and superior medial gyri (see **Table 3**).

Neuropsychological scores. Significant negative correlation between visuospatial memory scores and fALFF was observed in the right middle temporal and fusiform gyri, as well as cerebellum CRUS VI. Visuospatial memory scores were also positively correlated with fALFF values of left superior temporal gyrus.

No significant correlations were obtained between fALFF and $Sa0^2$min (minimal oxygen saturation), ESS, T<90%, as well as other neuropsychological scores. Details about statistical results, including correlation coefficients, and localization of fMRI results are reported in **Figure 2** and **Table 3**.

Please insert Figure 2 and Table 3 around here

Thalamic functional connectivity results

Increased thalamocortical connectivity was observed in OSA patients respect to controls (**Figure 3**). Specifically, differences were limited to the functional connectivity between seed regions placed in the somatosensory, premotor and motor thalamus nuclei and their links to bilateral primary and secondary somatosensory cortices (Brodmann Area - BA1, 2, 3 and 40),

superior parietal cortex (BA7), angular gyrus (BA 39), temporal lobe (BA20, 21 and 37), posterior cingulate cortex (BA23, 31), left superior and middle frontal gyri (BA10, 46).

Please insert Figure 3 around here

Multivariate classification results

Overall, classifiers based on fALFF values of 90 AAL regions of interest provided corrected accuracy values ranging between 45 and 71%. The best performance was obtained using a Simple Cart tree, a semi-supervised machine learning classification algorithm (Rovlias & Kotsou, 2004), that provided a correct classification of OSA patients for controls equal to 81,2% (CI=.6748-.9091; Sensitivity=.733; Specificity=.934; AUC .9561).

DISCUSSION

We investigated the presence of cortical and subcortical alterations in the amplitude of low-frequency BOLD signal fluctuations (fALFF) in drug-naïve patients with OSA, also exploring its discriminatory power and therefore its implementation as a possible biomarker for patients' detection. By comparing age, gender, and BMI-matched patients and healthy controls, we found a significant abnormal pattern of subcortical intrinsic activity in OSA patients, characterized by the greater amplitude of low-frequency oscillations in bilateral thalamic nuclei at rest, as well as significant differences in their functional connectivity with motor and somatosensory regions. Moreover, correlations with clinical and neuropsychological scores were also identified at the cortical level, suggesting a possible neural substrate for the cognitive impairment frequently accompanying OSA syndrome.

Thalamic alterations in OSA

Thalamus is part of the human arousal system, receives and projects several inputs/outputs to cortical and subcortical structures (Jones, 2003), and plays a fundamental role in sleep-waking transition (Paus, 2000; Spoormaker et al., 2010). Schwartz and colleagues (Schwartz & Roth, 2008) have suggested how pathological apneic episodes showed by OSA patients, in association with abnormal breathing effort and choking sensation might cause repeated nocturnal temporary brain activation, with an immediate restore of breathing muscles' tone to waking levels. More recently, Pattinson et al. (2009) have revealed activations of the anterior ventral, ventral postero-lateral, and ventrolateral thalamic nuclei in response to carbon dioxide stimulation (Pattinson et al., 2009). Accordingly, our study shows an increase in spontaneous activity (fALFF) in OSA patients, specifically related to anterior and lateral thalamic nuclei linked to somatosensory, motor, and premotor cortical regions and previously

associated to upper airway neuromuscular perception and control (Herrero, Barcia, & Navarro, 2002). We hypothesize that nocturnal chronic over-stimulation may lead to an up-regulation of spontaneous thalamic activity that, acting as a long-term potentiation (LTP) endogenous mechanism (Itami et al., 2016), result in the thalamic hyperactivation observed in our patients. Such plastic rearrangements are in line with the evidence of altered sleep-indexes (non-supine position's time and mobility index, **Table 1**) and with findings of enhanced ALFF in the motor cortices of patients affected by Essential Tremor (Yin, Lin, Li, Qian, & Mou, 2016).

Furthermore, testing for possible effects of increased thalamic fALFF on thalamocortical coupling, functional connectivity analysis suggests a pattern of increased thalamocortical connectivity in OSA patients, involving somatosensory, motor, and premotor thalamic nuclei. This finding supports our hypothesis of upregulated thalamocortical circuitry induced by a long-term exposition of obstructive sleep apnea syndrome, also emerged in a previous fMRI investigation based on different connectivity indexes (Santarnecchi et al., 2013). We cannot completely exclude that, considering the sleepiness score reported by our patients (see **Table 1**), the observed enhanced fALFF of thalamus nuclei could also reflect the effortful attempt that patients require to maintain wakefulness during the day, compared to healthy controls. However, in this regard it must be noticed that sleep-deprived subjects have been reported showing an opposite pattern of altered fALFF(Gao et al., 2015), making this hypothesis less likely. Moreover, considering (i) the thalamocortical connectivity alterations identified in our patients –suggesting an increased drive towards somatosensory and motor regions instead of a more plausible decrease induced by sleep (Spoormaker, Czisch, Maquet, &Jancke, 2011; Tagliazucchi & Laufs, 2014)- and (ii) the precautionary measures adopted before the MRI exam (see *Methods, Participants* section), we reasonably exclude the possibility of our patients being asleep during the MRI exam.

Both fALFF and functional connectivity of thalamic nuclei did not correlate with any clinical and neuropsychological scores. Such augmented thalamic activations could, therefore, represent an initial stage of illness or also a functional compensatory mechanism to microstructural alteration, accordingly to Algin and colleagues (Algin et al., 2012) that showed augmented choline to creatine ratio (Cho/Cr, an index of cellular membrane damage) in thalamic structures of OSA patients.

Disease severity and fALFF

OSA patients have been shown to report alterations in the regulation of autonomic functions (Harper et al., 2003; Hilton et al., 2001), however, the causal link between OSA pathophysiology and the autonomic system is still unclear. Multiple brain regions showed a correlation with clinical parameters, including (i) AHI and fALFF of left insular lobe, bilateral frontal, temporal, parietal and occipital cortices; (ii) MAD and fALFF of anterior cingulate gyrus; (iii) MHD and fALFF of bilateral prefrontal lobes, anterior cingulate cortex, and various frontal gyri. Interestingly, the vast majority of these regions are part of the network implied in controlling autonomic function, the Central Autonomic Network (CAN; Cechetto & Shoemaker, 2009). The insula, anterior cingulate cortex, ventromedial prefrontal cortex, amygdala, and cerebellum are the most important(Cechetto & Shoemaker, 2009; Gianaros & Sheu, 2009). As an example, in literature heart rate and blood pressure variations were associated with activity in medial prefrontal, anterior cingulate and insular cortices, caudal medial thalamus, hypothalamus, midbrain and ventral and dorsal pons (Rossi, Santarnecchi, Valenza, & Ulivelli, 2016; Harper, Bandler, Spriggs, & Alger, 2000). Additionally, maximal inspiration, Valsalva maneuver and isometric handgrip tasks caused activation of the insular cortex, medial prefrontal cortex, and thalamus (King, Menon, Hachinski, & Cechetto, 1999).

Here, we revealed a negative-correlation between fALFF values of almost all the key regions of the CAN (i.e. insula, anterior cingulate cortex, ventromedial prefrontal cortex, and cerebellum) with at least one clinical parameter (AHI, MAD, MHD) in OSA patients. Accordingly, alterations in autonomic parameters were previously demonstrated in OSA patients, as elevated heart rate, diurnal (Hedner, Ejnell, Sellgren, Hedner, & Wallin, 1988) and nocturnal (Somers, Mark, Zavala, & Abboud, 1989) hypertension, coronary vasoconstriction (Verrier & Dickerson, 1991), as well as increased susceptibility to arrhythmia (Rossi, Stradling, & Kohler, 2013). Our finding of negative correlations between fALFF values of the left insula and OSA severity (i.e. AHI) is consistent with previous studies showing how this sleep and autonomic related brain structure is highly responsive to hypoxic conditions (Macey et al., 2005; Williamson et al., 1997).

Anterior cingulate cortex (ACC) alterations in OSA patients have been already reported using PET (Pietrini et al., 1998). Joo and colleagues showed a reduction in grey matter concentration (Joo et al., 2010), as well as a minor white matter integrity (Macey et al., 2008; Macey et al., 2002) and non-atrophic cingulate gyrus, reveals a reduction in glucose metabolic activity in OSA patients (Yaouhi et al., 2009). Also, cingulate cortex seems very reactive to dyspnea (Peiffer, Poline, Thivard, Aubier, & Samson, 2001), breathlessness (Banzett et al., 2000) and sensation of “air hunger” and, as we anticipated, is involved in autonomic functions (i.e. CAN; Evans et al., 2002). In this view, chronic exposure to OSAS physiopathology could lead to an impairment of ACC activity, supporting the negative association between cingulate fALFF and MAD-MHD index. Finally, we found a negative correlation between putamen activity and OSA symptomatology. Putamen involvement in OSA neurobiology has been already stressed both in a morphometric (Yaouhi et al., 2009) and in a task-fMRI study (Ayalon, Ancoli-

Israel, & Drummond, 2009), respectively suggesting a decreased gray matter concentration and a reduced activation during a go-no-go task in this region.

Correlational analyses also showed negative coefficients between the spontaneous activity of cortical regions and OSA symptomatology. Firstly, we note a link between activity in frontal regions and AHI and MHD values, according to the first evidence of an altered frontal non-fractional ALFF (Li et al., 2015). Frontal regions might be very sensitive to OSA-induced hypoxia and hypercapnia, as indicated in animal studies (Piantadosi, Zhang, Levin, Folz, & Schmechel, 1997). Decreased NAA/cr ratio (N-acetyl aspartate to creatine, a biomarker of axonal integrity) in frontal regions has been previously reported (Algin et al., 2012), as well as prefrontal gray-matter loss and decrease in metabolism in OSA patients (Yaouhi et al., 2009).

Other cortical regions showed a negative correlation between their fALFF and clinical indexes, mostly located in the temporal cortices, left parietal and occipital lobes. Interestingly, a PET study by Corfield et al. (1995) revealed activations in the frontal lobe, temporal–occipital cortices, as well as the in cingulate gyrus and insula, caused by CO^2 stimulation (Corfield et al., 1995). Also, the same regions together with the thalamus, insular cortex, and basal ganglia, were found activated using fMRI during the inhibitory components of breath-holding (McKay, Adams, Frackowiak, & Corfield, 2008). Considering the sensitivity of these regions to apnea and hypopnea, long-term exposure to apneic events could result in their detrimental activity that correlates with OSAS scores. Finally, bilateral caudate nuclei negative correlations with mean apnea duration is supported by the gray matter concentration/volume decrease previously highlighted in this region (Torelli et al., 2011; Joo et al., 2010).

Considering all these evidences, fALFF of CAN regions need to be extensively investigated in animal models to confirm it as a possible marker of sleep apnea induced-

alterations. Additionally, we could longitudinally monitor it in OSA patients to reveal its potential role as useful parameter of clinical severity and, maybe, of treatment efficacy.

Neuropsychological-fALFF correlations in OSA patients

The correlation between fALFF and neuropsychological data showed significant results for the activity of the cerebellum and temporal lobes. Alterations of cerebellar functioning in OSA patients have been investigated in previous studies with PET, showing evidences of a positive correlation between spontaneous glucose metabolism in cerebellar regions and both motor and cognitive functioning (Yaouhi et al., 2009). In particular, cerebellum activity increases in response to the Valsalva manoeuvre(Harper et al., 2000), CO^2 stimulation and during inspiratory and expiratory loads (Gozal et al., 1996). Conversely, notwithstanding we do not refer to cognitive or motor data correlations, our results seem to show a reverse pattern, with an increase of cerebellar activity related to worst clinical outcome. As suggested for the thalamic hyperactivity, we hypothesize that increased cerebellar activity in response to hypopnea worsening might be due to motor function alterations related to augmented breathing effort during night.

Interestingly, temporal regions are the unique to show a transversal involvement in all our analysis (increased thalamo-cortical functional connectivity, correlation with clinical index and neuropsychological data). It must be noticed how the cognitive performance of our patients at visuo-spatial memory test negatively correlated withfALFF values of the right temporal lobe, akey region for memory processing. This lateralization is in line with the evidences of major right hemispheric brain structural alterations in OSA patients, as previously reported in (Yaouhi et al., 2009) and confirmed in a recent morphovolumetric analysis by our team (manuscript under preparation). In this context, the positive correlation between left

temporal lobe fALFF and performance in visuo-spatial memory could represent a compensatory mechanism for patients.

Automated patients' classification

Machine learning methods are widely tested today in medicine both for "classification" (i. e. differentiate patients from controls or divided patients into specific subgroups) as well as for "prediction" (i. e. anticipate the development/progression of pathology, (Alanazi, Abdullah, & Qureshi, 2017). In particular, a major focus of these specific types of artificial intelligence is represented by neurological and psychiatric disorders (Wegrzyk et al., 2018; Richiardi et al., 2012; Zhong et al., 2017). The advantages of insert these methods in the clinical routine are massive, for the clinicians as for the patients, in the light of a timing reduction for the correct diagnosis and for an eventual timely appropriate treatment (Santarnecchi et al., 2014).

Supervised machine learning results promote fALFF as a potential adjunctive index for neurofunctional classification of OSA patients. Even if results have not been cross-validated on an independent population, results put forward the identification of a meaningful biological basis for OSA, and could support the development of new clinical indexes (Woo, Chang, Lindquist, & Wager, 2017). Follow up studies, comparing the classification accuracy of fALFF with that of other potential marker highlighted in previous investigations (e.g. brain volumes, density, regional functional connectivity values) as well as EEG indexes, are needed.

Limitations of the study

The study focused on spontaneous brain activity data in patients often reported having significantly increased sleepiness during the day, as a consequence of fragmented nocturnal

sleep (Stepanski, 2002). Studies about the modulation of BOLD fluctuations amplitude during sleep have suggested a pattern of alterations circumscribed to sensory regions like visual, somatosensory and auditory cortices (Horovitz et al., 2008), but also to deep brain nuclei (Fukunaga et al., 2006). Consequently, we cannot completely exclude that the observed between-groups fALFF differences might be due to an unbalanced occurrence of sleep episodes inside the scanner in OSA patients more than healthy controls. Future studies should address this issue by simultaneously recording EEG-fMRI activity.Moreover, our results reflect alterations of fALFF within the canonical frequency window utilized for functional connectivity analysis (i.e. 0.01-0.08 Hz). Considering evidences of fALFF alterations within specific frequency bands documented in psychiatric (Yu et al., 2014) and neurological conditions (Han et al., 2011), as well as the possible influence of breathing-related artifacts over BOLD amplitude in higher frequency windows (i.e. >0.1 Hz), future investigation should deepen our results by computing fALFF for a wide range of frequency windows.

CONCLUSION

In line with several anatomical and functional evidences, our study indicates a persistent impact of sleep apnea condition onto brain resting-state dynamics in the daytime. The relationship between altered spontaneous activity in frontal, temporal and parietal lobes and pathology severity markers could be the substrate for overt neurocognitive deficits characterizing OSA patients. Even if the peripheral mechanistic cause is possible, the central control of upper airway musculature and of ventilation seems to be more than relevant in OSA patients. Recently, in fact, cortical electrical stimulation of amygdala and hippocampus in epileptic patients caused an immediate central apnea, and precedent studies on animals revealed the involvement of a large cortical network (Lacuey, Zonjy, Londono, & Lhatoo, 2017). Overall, OSA patients seem to reveal an important involvement of sensory-motor and autonomic brain networks, supporting the idea of a multilevel contribution to OSA syndrome involving both central nervous system and peripheral anatomo-functional pathology.

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Conflict of Interest Statement

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Author contributions

ES, RR, JD, SR and IS designed research; GV, ES, GS and AC acquired MRI data; IS and FN performed the clinical and cognitive evaluations; ES performed fMRI data preprocessing and statistical analyses; ES, JD, SR, GS and AR wrote the paper.

Data accessibility

Data will be available upon request from the corresponding author.

Abbreviations

AAL, anatomical automatic labeling; ACC, anterior cingulate cortex; AHI, apnea-hypopnea index; ALFF, amplitude of low-frequency oscillations; ANCOVA, analyses of covariance; BDI, Beck depression inventory; BOLD, blood oxygenation level dependent; BMI, body mass index;

CAN, central autonomic network; Cho/Cr, choline to creatine ratio; DMN, default mode network; DVARS, derivative of root mean square variance over voxels; EEG, electroencephalogram; ESS, Epworth sleepiness scale; FAB, frontal assessment battery; fALFF, fractional amplitude of low frequency fluctuations; FD, frame-wise displacement; FFT,fast Fourier transform; fMRI, functional magnetic resonance imaging; FLAIR, T2-weighted Turbo Fluid Attenuated Inversion Recovery; LTP, long-term potentiation;MAD, mean apnea duration; MHD, mean hypopnea duration; MNI, Montreal Neurological Institute; MoCA, Montreal Cognitive Assessment; NAA/cr, N-acetylaspartate to creatine ratio; OSA, obstructive sleep apnea; PCC, posterior cingulate cortex; PET, positron emission tomography; PSG, polysomnography; RAVLT, Rey auditory verbal learning test; ROC, receiving operating curve; ROI, regions of interest; Sa02min, minimal saturation values; SNA, spontaneous neuronal activity; TBV, total brain volume; TMT, trail making test; T<90%, time below 90% of saturation; VBM, voxel-based morphometry.

Reference List

Alanazi, H. O., Abdullah, A. H., & Qureshi, K. N. (2017). A Critical Review for Developing Accurate and Dynamic Predictive Models Using Machine Learning Methods in Medicine and Health Care. *J.Med.Syst., 41,* 69.

Algin, O., Gokalp, G., Ocakoglu, G., Ursavas, A., Taskapilioglu, O., & Hakyemez, B. (2012). Neurochemical-structural changes evaluation of brain in patients with obstructive sleep apnea syndrome. *Eur.J.Radiol., 81,* 491-495.

Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *Neuroimage., 38,* 95-113.

Ayalon, L., Ancoli-Israel, S., & Drummond, S. P. (2009). Altered brain activation during response inhibition in obstructive sleep apnea. *J.Sleep Res., 18,* 204-208.

Ayalon, L., Ancoli-Israel, S., Klemfuss, Z., Shalauta, M. D., & Drummond, S. P. (2006). Increased brain activation during verbal learning in obstructive sleep apnea. *Neuroimage., 31,* 1817-1825.

Balkin, T. J. & Badia, P. (1988). Relationship between sleep inertia and sleepiness: cumulative effects of four nights of sleep disruption/restriction on performance following abrupt nocturnal awakenings. *Biol.Psychol., 27,* 245-258.

Banzett, R. B., Mulnier, H. E., Murphy, K., Rosen, S. D., Wise, R. J., & Adams, L. (2000). Breathlessness in humans activates insular cortex. *Neuroreport, 11,* 2117-2120.

Beck, A. T. & Steer, R. A. (1984). Internal consistencies of the original and revised Beck Depression Inventory. *J.Clin.Psychol., 40,* 1365-1367.

Bulcun, E., Ekici, A., & Ekici, M. (2012). Quality of life and metabolic disorders in patients with obstructive sleep apnea. *Clin.Invest Med., 35,* E105.

Cabral, C., Kambeitz-Ilankovic, L., Kambeitz, J., Calhoun, V. D., Dwyer, D. B., von, S. S. et al. (2016). Classifying Schizophrenia Using Multimodal Multivariate Pattern Recognition Analysis: Evaluating the Impact of Individual Clinical Profiles on the Neurodiagnostic Performance. *Schizophr.Bull., 42 Suppl 1,* S110-S117.

Cechetto, D. F. & Shoemaker, J. K. (2009). Functional neuroanatomy of autonomic regulation. *Neuroimage., 47,* 795-803.

Corfield, D. R., Fink, G. R., Ramsay, S. C., Murphy, K., Harty, H. R., Watson, J. D. et al. (1995). Evidence for limbic system activation during CO2-stimulated breathing in man. *J.Physiol, 488 (Pt 1),* 77-84.

Corrigan, J. D. & Hinkeldey, N. S. (1987). Relationships between parts A and B of the Trail Making Test. *J.Clin.Psychol., 43,* 402-409.

Drummond, S. P., Brown, G. G., Gillin, J. C., Stricker, J. L., Wong, E. C., & Buxton, R. B. (2000). Altered brain response to verbal learning following sleep deprivation. *Nature, 403,* 655-657.

Dubois, B., Slachevsky, A., Litvan, I., & Pillon, B. (2000). The FAB: a Frontal Assessment Battery at bedside. *Neurology, 55,* 1621-1626.

Evans, K. C., Banzett, R. B., Adams, L., McKay, L., Frackowiak, R. S., & Corfield, D. R. (2002). BOLD fMRI identifies limbic, paralimbic, and cerebellar activation during air hunger. *J.Neurophysiol., 88,* 1500-1511.

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc.Natl.Acad.Sci.U.S.A, 102,* 9673-9678.

Frank, E., Hall, M., Trigg, L., Holmes, G., & Witten, I. H. (2004). Data mining in bioinformatics using Weka. *Bioinformatics., 20,* 2479-2481.

Fukunaga, M., Horovitz, S. G., van, G. P., de Zwart, J. A., Jansma, J. M., Ikonomidou, V. N. et al. (2006). Large-amplitude, spatially correlated fluctuations in BOLD fMRI signals during extended rest and early sleep stages. *Magn Reson.Imaging, 24,* 979-992.

Gao, L., Bai, L., Zhang, Y., Dai, X. J., Netra, R., Min, Y. et al. (2015). Frequency-dependent changes of local resting oscillations in sleep-deprived brain. *PLoS.One., 10,* e0120323.

Gianaros, P. J. & Sheu, L. K. (2009). A review of neuroimaging studies of stressor-evoked blood pressure reactivity: emerging evidence for a brain-body pathway to coronary heart disease risk. *Neuroimage., 47,* 922-936.

Gozal, D., Omidvar, O., Kirlew, K. A., Hathout, G. M., Lufkin, R. B., & Harper, R. M. (1996). Functional magnetic resonance imaging reveals brain regions mediating the response to resistive expiratory loads in humans. *J.Clin.Invest, 97,* 47-53.

Han, Y., Wang, J., Zhao, Z., Min, B., Lu, J., Li, K. et al. (2011). Frequency-dependent changes in the amplitude of low-frequency fluctuations in amnestic mild cognitive impairment: a resting-state fMRI study. *Neuroimage., 55,* 287-295.

Harper, R. M., Bandler, R., Spriggs, D., & Alger, J. R. (2000). Lateralized and widespread brain activation during transient blood pressure elevation revealed by magnetic resonance imaging. *J.Comp Neurol., 417,* 195-204.

Harper, R. M., Macey, P. M., Henderson, L. A., Woo, M. A., Macey, K. E., Frysinger, R. C. et al. (2003). fMRI responses to cold pressor challenges in control and obstructive sleep apnea subjects. *J.Appl.Physiol (1985.), 94,* 1583-1595.

He, Y., Wang, L., Zang, Y., Tian, L., Zhang, X., Li, K. et al. (2007). Regional coherence changes in the early stages of Alzheimer's disease: a combined structural and resting-state functional MRI study. *Neuroimage., 35,* 488-500.

Hedner, J., Ejnell, H., Sellgren, J., Hedner, T., & Wallin, G. (1988). Is high and fluctuating muscle nerve sympathetic activity in the sleep apnoea syndrome of pathogenetic importance for the development of hypertension? *J.Hypertens.Suppl, 6,* S529-S531.

Herrero, M. T., Barcia, C., & Navarro, J. M. (2002). Functional anatomy of thalamus and basal ganglia. *Childs Nerv.Syst., 18,* 386-404.

Hilton, M. F., Chappell, M. J., Bartlett, W. A., Malhotra, A., Beattie, J. M., & Cayton, R. M. (2001). The sleep apnoea/hypopnoea syndrome depresses waking vagal tone independent of sympathetic activation. *Eur.Respir.J., 17,* 1258-1266.

Horner, R. L. (2008). Pathophysiology of obstructive sleep apnea. *J.Cardiopulm.Rehabil.Prev., 28,* 289-298.

Horovitz, S. G., Fukunaga, M., de Zwart, J. A., van, G. P., Fulton, S. C., Balkin, T. J. et al. (2008). Low frequency BOLD fluctuations during resting wakefulness and light sleep: a simultaneous EEG-fMRI study. *Hum.Brain Mapp., 29,* 671-682.

Ide, S., Kakeda, S., & Korogi, Y. (2015). [Anatomy of the Thalamus]. *Brain Nerve, 67,* 1459-1469.

Itami, C., Huang, J. Y., Yamasaki, M., Watanabe, M., Lu, H. C., & Kimura, F. (2016). Developmental Switch in Spike Timing-Dependent Plasticity and Cannabinoid-Dependent Reorganization of the Thalamocortical Projection in the Barrel Cortex. *J.Neurosci., 36,* 7039-7054.

Johns, M. W. (1991). A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep, 14,* 540-545.

Jones, B. E. (2003). Arousal systems. *Front Biosci., 8,* s438-s451.

Joo, E. Y., Tae, W. S., Lee, M. J., Kang, J. W., Park, H. S., Lee, J. Y. et al. (2010). Reduced brain gray matter concentration in patients with obstructive sleep apnea syndrome. *Sleep, 33,* 235-241.

King, A. B., Menon, R. S., Hachinski, V., & Cechetto, D. F. (1999). Human forebrain activation by visceral stimuli. *J.Comp Neurol., 413,* 572-582.

Lacuey, N., Zonjy, B., Londono, L., & Lhatoo, S. D. (2017). Amygdala and hippocampus are symptomatogenic zones for central apneic seizures. *Neurology, 88,* 701-705.

Li, H. J., Dai, X. J., Gong, H. H., Nie, X., Zhang, W., & Peng, D. C. (2015). Aberrant spontaneous low-frequency brain activity in male patients with severe obstructive sleep apnea revealed by resting-state functional MRI. *Neuropsychiatr.Dis.Treat., 11,* 207-214.

Li, H. J., Nie, X., Gong, H. H., Zhang, W., Nie, S., & Peng, D. C. (2016). Abnormal resting-state functional connectivity within the default mode network subregions in male patients with obstructive sleep apnea. *Neuropsychiatr.Dis.Treat., 12,* 203-212.

Macey, P. M., Henderson, L. A., Macey, K. E., Alger, J. R., Frysinger, R. C., Woo, M. A. et al. (2002). Brain morphology associated with obstructive sleep apnea. *Am.J.Respir.Crit Care Med., 166,* 1382-1387.

Macey, P. M., Kumar, R., Woo, M. A., Valladares, E. M., Yan-Go, F. L., & Harper, R. M. (2008). Brain structural changes in obstructive sleep apnea. *Sleep, 31,* 967-977.

Macey, P. M., Woo, M. A., Macey, K. E., Keens, T. G., Saeed, M. M., Alger, J. R. et al. (2005). Hypoxia reveals posterior thalamic, cerebellar, midbrain, and limbic deficits in congenital central hypoventilation syndrome. *J.Appl.Physiol, 98,* 958-969.

McKay, L. C., Adams, L., Frackowiak, R. S., & Corfield, D. R. (2008). A bilateral cortico-bulbar network associated with breath holding in humans, determined by functional magnetic resonance imaging. *Neuroimage., 40,* 1824-1832.

Nishibayashi, M., Miyamoto, M., Miyamoto, T., Suzuki, K., & Hirata, K. (2008). Correlation between severity of obstructive sleep apnea and prevalence of silent cerebrovascular lesions. *J.Clin.Sleep Med., 4,* 242-247.

Pattinson, K. T., Mitsis, G. D., Harvey, A. K., Jbabdi, S., Dirckx, S., Mayhew, S. D. et al. (2009). Determination of the human brainstem respiratory control network and its cortical connections in vivo using functional and structural imaging. *Neuroimage., 44,* 295-305.

Paus, T. (2000). Functional anatomy of arousal and attention systems in the human brain. *Prog.Brain Res., 126,* 65-77.

Peiffer, C., Poline, J. B., Thivard, L., Aubier, M., & Samson, Y. (2001). Neural substrates for the perception of acutely induced dyspnea. *Am.J.Respir.Crit Care Med., 163,* 951-957.

Pelled, G. & Goelman, G. (2004). Different physiological MRI noise between cortical layers. *Magn Reson.Med., 52,* 913-916.

Philby, M. F., Macey, P. M., Ma, R. A., Kumar, R., Gozal, D., & Kheirandish-Gozal, L. (2017). Reduced Regional Grey Matter Volumes in Pediatric Obstructive Sleep Apnea. *Sci.Rep., 7,* 44566.

Piantadosi, C. A., Zhang, J., Levin, E. D., Folz, R. J., & Schmechel, D. E. (1997). Apoptosis and delayed neuronal damage after carbon monoxide poisoning in the rat. *Exp.Neurol., 147,* 103-114.

Pietrini, P., Teipel, S. J., Bartenstein, P., Rapoport, S. I., Moller, H. J., & Hampel, H. (1998). PET and the effects of aging and neurodegeneration on brain function: basic principles. *Drug News Perspect., 11,* 161-165.

Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage., 59,* 2142-2154.

Richiardi, J., Gschwind, M., Simioni, S., Annoni, J. M., Greco, B., Hagmann, P. et al. (2012). Classifying minimally disabled multiple sclerosis patients from resting state functional connectivity. *Neuroimage., 62,* 2021-2033.

Rossi, S., Santarnecchi, E., Valenza, G., & Ulivelli, M. (2016). The heart side of brain neuromodulation. *Philos.Trans.A Math.Phys.Eng Sci., 374*.

Rossi, V. A., Stradling, J. R., & Kohler, M. (2013). Effects of obstructive sleep apnoea on heart rhythm. *Eur.Respir.J., 41,* 1439-1451.

Rovlias, A. & Kotsou, S. (2004). Classification and regression tree for prediction of outcome after severe head injury using simple clinical and laboratory variables. *J.Neurotrauma, 21,* 886-893.

Santarnecchi, E., D'Arista, S., Egiziano, E., Gardi, C., Petrosino, R., Vatti, G. et al. (2014). Interaction between neuroanatomical and psychological changes after mindfulness-based training. *PLoS.One., 9,* e108359.

Santarnecchi, E., Sicilia, I., Richiardi, J., Vatti, G., Polizzotto, N. R., Marino, D. et al. (2013). Altered cortical and subcortical local coherence in obstructive sleep apnea: a functional magnetic resonance imaging study. *J.Sleep Res., 22,* 337-347.

Schoenberg, M. R., Dawson, K. A., Duff, K., Patton, D., Scott, J. G., & Adams, R. L. (2006). Test performance and classification statistics for the Rey Auditory Verbal Learning Test in selected clinical samples. *Arch.Clin.Neuropsychol., 21,* 693-703.

Schwartz, J. R. & Roth, T. (2008). Neurophysiology of sleep and wakefulness: basic science and clinical implications. *Curr.Neuropharmacol., 6,* 367-378.

Somers, V. K., Mark, A. L., Zavala, D. C., & Abboud, F. M. (1989). Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. *J.Appl.Physiol (1985.), 67,* 2101-2106.

Spoormaker, V. I., Czisch, M., Maquet, P., & Jancke, L. (2011). Large-scale functional brain networks in human non-rapid eye movement sleep: insights from combined

electroencephalographic/functional magnetic resonance imaging studies. *Philos.Trans.A Math.Phys.Eng Sci., 369,* 3708-3729.

Spoormaker, V. I., Schroter, M. S., Gleiser, P. M., Andrade, K. C., Dresler, M., Wehrle, R. et al. (2010). Development of a large-scale functional brain network during human non-rapid eye movement sleep. *J.Neurosci., 30,* 11379-11387.

Stepanski, E. J. (2002). The effect of sleep fragmentation on daytime function. *Sleep, 25,* 268-276.

Tagliazucchi, E. & Laufs, H. (2014). Decoding wakefulness levels from typical fMRI resting-state data reveals reliable drifts between wakefulness and sleep. *Neuron, 82,* 695-708.

Torelli, F., Moscufo, N., Garreffa, G., Placidi, F., Romigi, A., Zannino, S. et al. (2011). Cognitive profile and brain morphological changes in obstructive sleep apnea. *Neuroimage., 54,* 787-793.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N. et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage., 15,* 273-289.

Van Dijk, K. R., Sabuncu, M. R., & Buckner, R. L. (2012). The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage., 59,* 431-438.

Verrier, R. L. & Dickerson, L. W. (1991). Autonomic nervous system and coronary blood flow changes related to emotional activation and sleep. *Circulation, 83,* II81-II89.

Wegrzyk, J., Kebets, V., Richiardi, J., Galli, S., de Ville, D. V., & Aybek, S. (2018). Identifying motor functional neurological disorder using resting-state functional connectivity. *Neuroimage.Clin., 17,* 163-168.

Williamson, J. W., Nobrega, A. C., McColl, R., Mathews, D., Winchester, P., Friberg, L. et al. (1997). Activation of the insular cortex during dynamic exercise in humans. *J.Physiol, 503 (Pt 2),* 277-283.

Woo, C. W., Chang, L. J., Lindquist, M. A., & Wager, T. D. (2017). Building better biomarkers: brain models in translational neuroimaging. *Nat.Neurosci., 20,* 365-377.

Yaouhi, K., Bertran, F., Clochon, P., Mezenge, F., Denise, P., Foret, J. et al. (2009). A combined neuropsychological and brain imaging study of obstructive sleep apnea. *J.Sleep Res., 18,* 36-48.

Yin, W., Lin, W., Li, W., Qian, S., & Mou, X. (2016). Resting State fMRI Demonstrates a Disturbance of the Cerebello-Cortical Circuit in Essential Tremor. *Brain Topogr., 29,* 412-418.

Yu, R., Chien, Y. L., Wang, H. L., Liu, C. M., Liu, C. C., Hwang, T. J. et al. (2014). Frequency-specific alternations in the amplitude of low-frequency fluctuations in schizophrenia. *Hum.Brain Mapp., 35,* 627-637.

Zhang, Z., Lu, G., Zhong, Y., Tan, Q., Chen, H., Liao, W. et al. (2010). fMRI study of mesial temporal lobe epilepsy using amplitude of low-frequency fluctuation analysis. *Hum.Brain Mapp., 31,* 1851-1861.

Zhong, X., Shi, H., Ming, Q., Dong, D., Zhang, X., Zeng, L. L. et al. (2017). Whole-brain resting-state functional connectivity identified major depressive disorder: A multivariate pattern analysis in two independent samples. *J.Affect.Disord., 218,* 346-352.

Zou, Q. H., Zhu, C. Z., Yang, Y., Zuo, X. N., Long, X. Y., Cao, Q. J. et al. (2008). An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. *J.Neurosci.Methods, 172,* 137-141.

TABLES

	patients (*n*=20)	**controls (*n*=20)**	***p*-value**
Age (years)	42.9 ± 7	41 ± 6	0.78
Gender	17 M; 3 F	16 M; 4 F	-
Body Mass Index (BMI)	29.5 ± 3	25.7 ± 3	0.17
Education (years)	14.1 ± 2	12.4 ± 2	0.34
Epworth sleepiness scale	13.8 ± 4	5.2 ± 2	**<0.01**
Beck Depression Inventory	6±2	4±3	0.41
Pathology length (years)	6.9 ± 3	-	-
OSA severity (L=light; M=medium; S=severe)	1 L; 2 M; 17 S	-	-
PSG data			
Apnea-Hypopnea Index (AHI)	38.3 ± 15	-	-
Oxygen desaturation index (ODI)	35.7 ± 11	-	-
Oxygen saturation (minimal)	75.2 ± 7	-	-
Desaturation time (<90%)	13.4 ± 5	-	-
Apnea duration (mean)	23.9 ± 3	-	-
Ipopnea duration (mean)	35.7 ± 9	-	-
Apnea duration (max)	84.2 ± 33	-	-
Ipopnea duration (max)	105.4 ± 37	-	-
Non-supine position's time (mean%)	49 ± 24	-	-
Mobility Index (mean)	2.2 ± 1	-	-
Neuropsychological data			
RAVLT (immediate recall)	42 ± 7	48 ± 10	***0.025***
RAVLT (delayed recall)	10 ± 3	11 ± 3	0.402
Corsi visuo-spatial span	4 ± 0.7	4 ± 0.8	0.34
Single digit span	5 ± 0.6	5 ± 0.6	0.274
Trail making test - A	30 ± 16	25 ± 10	0.225
Trail making test - B	56 ± 15	46 ± 14	***0.027***
Attentional matrices	48 ± 5	44 ± 5	***0.015***
Frontal Assessment Battery	15 ± 1	15 ± 1	0.272

Table 1. Demographic, clinical and neuropsychological data of study participants. P-values refer to two-tailed independent samples t-tests. Means ± standard deviations are reported.

Increased fALFF	***Voxels***	**MNI coordinates**			**Peak t-value**
cluster 1	98				
Right thalamus - Somatosensory		15	-15	6	3.72
Right thalamus - Motor		21	-24	6	3.06
Right thalamus - Prefrontal		6	-24	6	2.81
cluster 2	87				
Left thalamus - Prefrontal		-12	-18	9	3.55
Left thalamus - Somatosensory		-18	-21	0	2.81
Left thalamus - Temporal		-24	-33	3	2.32
Left thalamus - Parietal		-21	-27	3	2.34

Table 2. OSA and Control subjects fALFF comparisons results. Peak t-values and MNI coordinates refer to significant clusters obtained in fALFF maps using two-tailed independent samples t-test ($p<.05$, Monte Carlo correction).

Clusters location	*voxels*	MNI coordinates			Pearson *r*
Apnea-Hypopnea Index					
cluster 1	1074	x	y	z	
Right Middle Occipital Gyrus		36	-72	33	-0.71
Right Middle Temporal Gyrus		51	-60	15	-0.65
Right Inferior Parietal Lobule		36	-45	42	-0.63
Right Angular Gyrus		57	-48	30	-0.62
Right Supra Marginal Gyrus		54	-42	30	-0.58
Right Superior Temporal Gyrus		57	-36	18	-0.54
Right Middle Temporal Gyrus		45	-51	18	-0.53
Right Superior Occipital Gyrus		30	-81	45	-0.52
cluster 2	1009	x	y	z	
Left Middle Cingulate Cortex		-12	-24	51	-0.72
Left Superior Frontal Gyrus		-21	27	39	-0.67
Left Insula Lobe		-36	6	3	-0.66
Left Superior Orbita lGyrus		-15	27	-15	-0.66
Left Rolandic Operculum		-48	-3	12	-0.66
Left Inferior Frontal Gyrus (p. Opercularis)		-45	9	6	-0.66
Left Superior Medial Gyrus		-9	30	30	-0.65
Left Middle Frontal Gyrus		-27	18	39	-0.64
cluster 3	961	x	y	z	
Right Precentral Gyrus		42	6	42	-0.73
Right Inferior Frontal Gyrus (p. Opercularis)		57	15	21	-0.71
Right Middle Temporal Gyrus		51	-42	-3	-0.69
Right Putamen		30	3	-9	-0.63
cluster 4	912	x	y	z	
Left Cuneus		-12	-60	21	-0.76
Left Middle Occipital Gyrus		-33	-69	21	-0.71
Left Angular Gyrus		-48	-57	30	-0.68

Left Superior Occipital Gyrus		-15	-87	18	-0.63
Left Fusiform Gyrus		-30	-60	-3	-0.61
Mean Apnea Duration	526	x	y	z	
Left Caudate Nucleus		-6	6	-9	-0.74
Right Mid Orbital Gyrus		6	24	-9	-0.71
Right Caudate Nucleus		9	15	-9	-0.71
Left Thalamus - Temporal		6	3	0	-0.7
Right Anterior Cingulate Cortex		3	30	-3	-0.61
MeanHypopneaDuration	3074	x	y	z	
Left Middle Orbital Gyrus		-21	45	-15	-0.71
Right Inferior Frontal Gyrus (p. Triangularis)		45	36	24	-0.66
Right Anterior Cingulate Cortex		6	27	18	-0.65
Left Anterior Cingulate Cortex		-3	27	18	-0.65
Right Middle Orbital Gyrus		36	57	-9	-0.62
Left Middle Frontal Gyrus		-27	9	48	-0.62
Right Middle Frontal Gyrus		39	24	42	-0.61
Right Superior Medial Gyrus		12	66	15	-0.6
Left Cerebellum – Lobule VIIa Crus I		-21	-87	-27	0.59
Corsi Span	415	x	y	z	
Right Middle Temporal Gyrus		60	-33	-12	0.69
Right Fusiform Gyrus		42	-60	-18	-0.68
Right Cerebellum		24	-60	-18	-0.67
Left Superior Temporal Gyrus		-57	-21	9	-0.65

Table 3. Results of correlation analyses between fALFF, clinical and neuropsychological indices. Pearson product-moment coefficient "*r*" values and MNI coordinates refer to brain regions showing significant correlation with clinical and neuropsychological scores in OSA patients ($p<.05$, Monte Carlo correction).

FIGURES LEGENDS

Fig 1. Regions showing differences in fALFF values between OSA patients and healthy controls. Panel A shows axial, sagittal, and coronal views of fALFF maps resulting from the comparison between OSA and control subjects ($p<.05$, Monte Carlo correction), highlighting increased bilateral thalamus spontaneous low-frequency fluctuation in OSA. Panel B shows the cyto-architectonic mapping of significant clusters on to different thalamus regions, divided using thalamo-cortical projection criteria. Rows displayed areas that overlap with significant clusters, while columns represent different probability classes. Dark-blue/dark-red color-code representation indicates over/underrepresentation of different regions into clusters. Quotient (Q) stands for how much more likely this area was observed in the functionally defined volume. Both left and right thalamic clusters show an overlap with thalamic nuclei linked to somatosensory, motor, and premotor cortices. Images are displayed in radiological convention.

Fig 2. Correlation with clinical and cognitive symptoms. Different panels show clusters of significant correlation (Pearson Product-Moment Coefficient, "r", values) between fALFF values and, respectively, (A) apnea-hypopnea index (AHI), mean (B) Apnea (MAD) and (C) Hypopnea (MHD) duration in seconds, (D) visuo-spatial short-term memory. OSA worsening seems to correspond to an overall decrease of brain spontaneous activity mainly involving frontal regions and left parietal areas. Images are displayed in radiological convention.

Figure 3. Differences in thalamic functional connectivity. Panel A, B and C respectively show group-related differences between the seed-based functional connectivity of thalamus nuclei linked to somatosensory, premotor and motor cortex. Clusters represent increased

functional connectivity in OSA patients respect to healthy controls ($p<.05$, Monte Carlo correction). Surface images (column one and two) show significant fALFF clusters plotted on medial and lateral sagittal views of a Population-Average, Landmark and Surface-based cortical map representing left and right hemisphere. Plots on the right represent clusters overlay onto a flat right and left hemisphere tissues with colour-coded Broadmann areas labels (column three and four). No patterns of decreased connectivity were identified. Note: BA= Broadmann area; primary and secondary somatosensory cortex (BA 1, 2, 3 and 40); superior parietal cortex (BA 7); angular gyrus (BA 39); temporal lobe (BA 20, 21 and 37); posterior cingulate cortex (BA 23, 31); superior and middle frontal gyri (BA 10, 46).

FIGURES

Figure 1

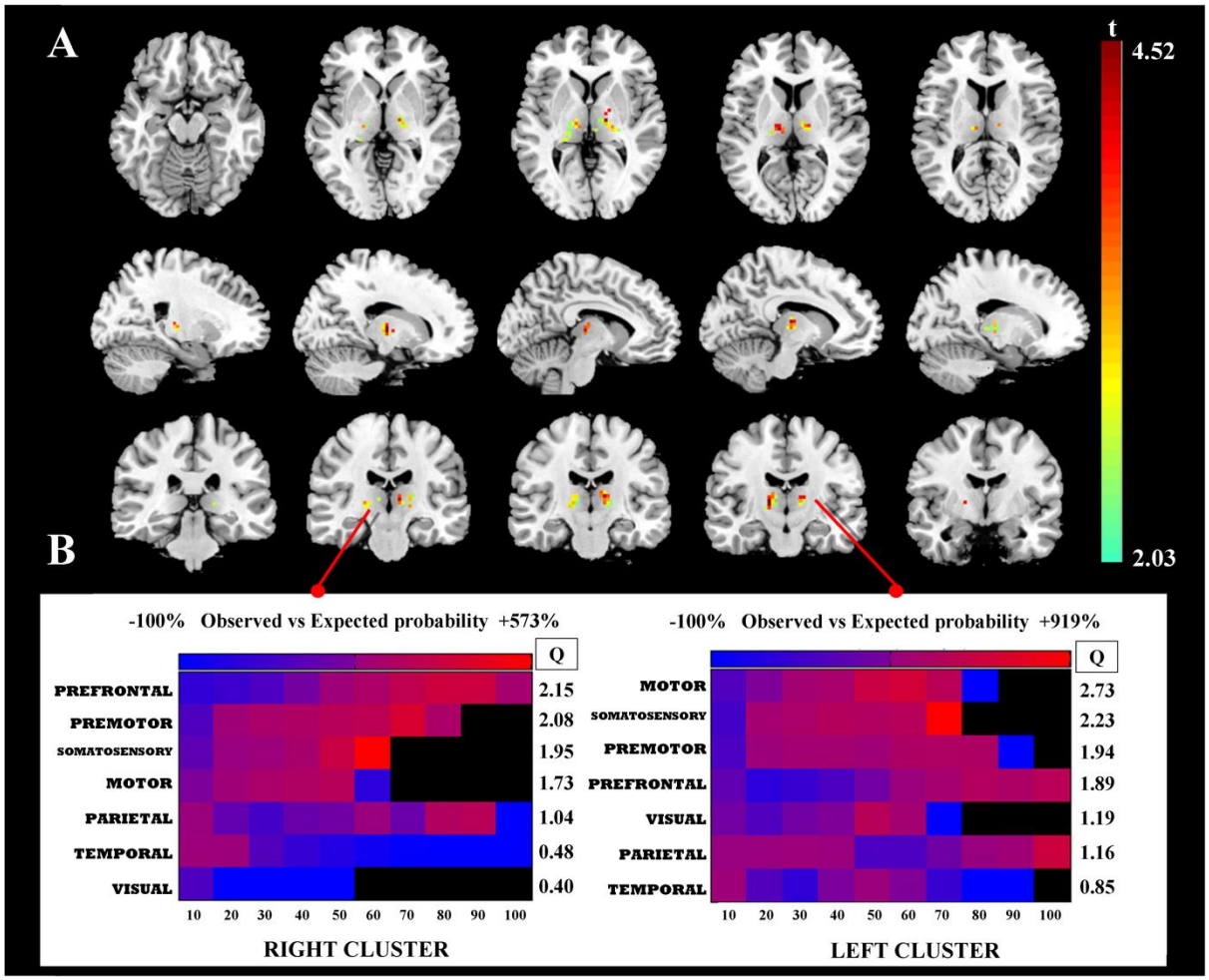

A
t
4.52
2.03
B
-100% Observed vs Expected probability +573%
Q
PREFRONTAL 2.15
PREMOTOR 2.08
SOMATOSENSORY 1.95
MOTOR 1.73
PARIETAL 1.04
TEMPORAL 0.48
VISUAL 0.40
10 20 30 40 50 60 70 80 90 100
RIGHT CLUSTER
-100% Observed vs Expected probability +919%
Q
MOTOR 2.73
SOMATOSENSORY 2.23
PREMOTOR 1.94
PREFRONTAL 1.89
VISUAL 1.19
PARIETAL 1.16
TEMPORAL 0.85
10 20 30 40 50 60 70 80 90 100
LEFT CLUSTER

Figure 2

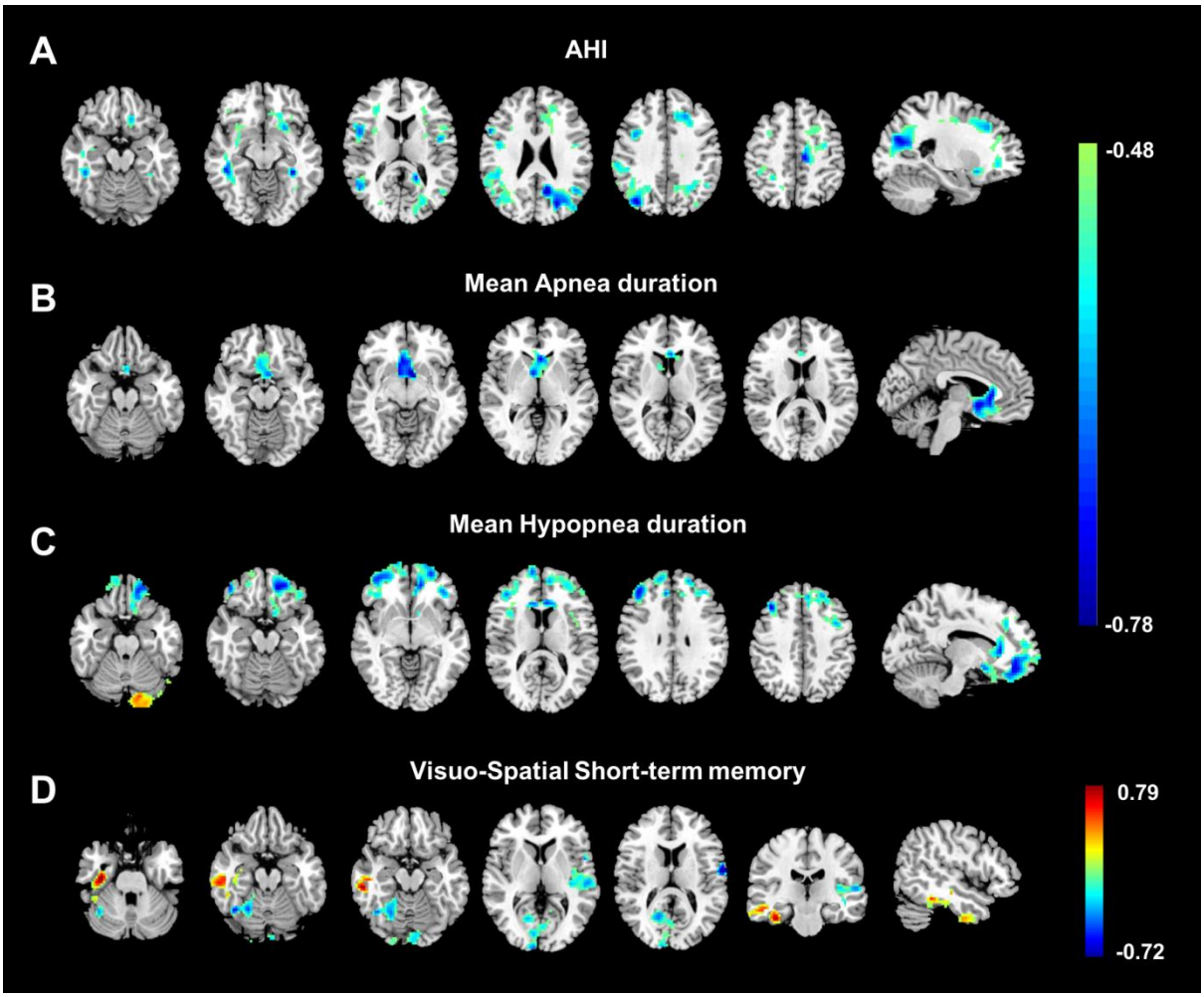
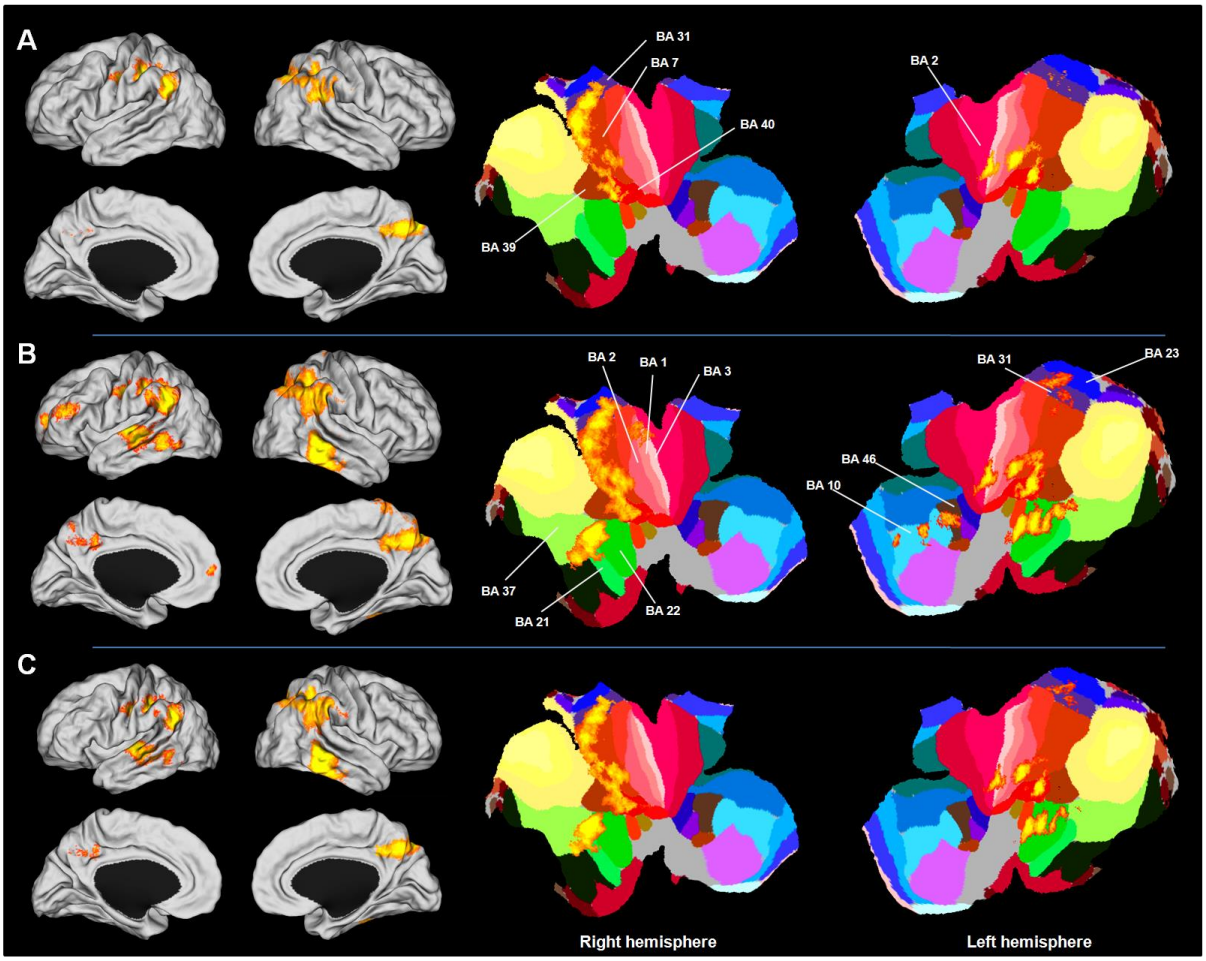

A
AHI
-0.48
B
Mean Apnea duration
C
Mean Hypopnea duration
-0.78
D
Visuo-Spatial Short-term memory
0.79
-0.72

Figure 3


A
BA 31
BA 7
BA 40
BA 39
BA 2
B
BA 2
BA 1
BA 3
BA 37
BA 21
BA 22
BA 31
BA 23
BA 46
BA 10
C
Right hemisphere
Left hemisphere