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Increased insula coactivation with salience networks in insomnia

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ABSTRACT

Insomnia is among the most prevalent and costly of all sleep-related disorders. To characterize the neural mechanisms underlying subjective dysfunction in insomnia, we examined brain activity in 17 female insomniacs and 17 female healthy controls using simultaneous functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) while they were resting and while they were trying to fall asleep. In examining the dynamic regional activity within intrinsic brain networks, we found that, compared with controls, insomniacs had greater involvement of the anterior insula with salience networks, as well as insula BOLD correlation with EEG gamma frequency power during rest in insomniacs. This increased involvement of the anterior insula was associated with negative affect in insomniacs. Aberrant activation of the insula, which integrates temporal and bodily states, in arousal networks may underlie the misperception of sleep quality and subjective distress in insomnia.

1. Introduction

Insomnia is a disorder of all-day impairment from sleep-related distress that involves a perceived difficulty falling asleep, staying asleep, or obtaining refreshing sleep. Afflicting up to 10% of the population (Ohayon, 2002), insomnia may persist for months or years and predicts the development of other disorders, such as Major Depressive Disorder (Ford & Kamerow, 1989). Researchers have proposed multiple psychological and biological explanations for the symptoms of insomnia (Harvey & Tang, 2012), including dysfunction in neural circuitry like the brainstem systems controlling sleep-wake (Lu, Sherman, Devor, & Saper, 2006), faulty sleep drive (Krystal & Edinger, 2010), psychological factors, or multiple causes (Riemann et al., 2009).

An important framework for understanding insomnia is 'hyperarousal,' or the posited heightened activity of neural, metabolic, electrophysiological, and neuroendocrine systems in insomniacs (Bonnet & Arand, 2010). Importantly, however, a key aspect of insomnia is the subjective reporting of more sleep dysfunction, such as increased sleep latency, than is recorded by 'objective' measures such as polysomnography. Thus, the diagnosis of insomnia is based on the subjective report of psychological distress, particularly during the sleep-to-wake transition. This suggests a limitation of polysomnography for capturing a neural phenotype of insomnia. Alternative imaging methods may elucidate the neural basis of hyperarousal, and one of the few studies to examine neural activity in individuals diagnosed with insomnia reported anomalies in both wakefulness-promoting regions and regions that underlie the neural response to stress (Nofzinger et al., 2004). Using positron emission tomography, these investigators found that insomniacs failed to reduce activation in limbic system structures, particularly in the medial temporal cortex, amygdala, insula, and anterior cingulate cortex. Notably, there were no differences between insomniacs and healthy controls in EEG measures of sleep, including sleep onset latency, sleep efficiency, and spectral characteristics of sleep.

Psychological states during the sleep-to-wake transition are challenging to assess, as are the brain systems underlying these states. Task-based functional magnetic resonance imaging (fMRI), in which participants respond to external cues or process information, is counterproductive to the quiescent process of sleep onset that is disrupted in insomnia. In contrast, intrinsic network imaging, which does not require a specific task or even participant engagement or alertness, is particularly well suited to provide novel insights concerning dynamic brain functions underlying psychological processes in insomnia. This method can provide a dynamic

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portrait of brain networks even in the absence of a guided task (Raichle et al., 2001). In intrinsic network imaging, the blood-oxygen level dependent (BOLD) signal in the brain is organized into networks of regions with coherent activity. Although the study of these networks and their relation to cognitive and affective states is nascent, these intrinsic network analyses are promising methods for determining regions with aberrant coactivation with canonical networks in neurological and psychiatric disorders (Sheline, Price, Yan, & Mintun, 2010). Regions with aberrant coactivation may elucidate the underlying neural basis for neurological and psychiatric disorders.

Intrinsic network imaging offers a powerful tool to investigate brain regions and networks involved in insomnia without disrupting an individual's current mental state with more intrusive or invasive methods. This method also enables targeting of specific networks putatively involved in arousal and insomnia. In the present study, we examined late-night, intrinsic network fMRI in 17 female adults diagnosed with insomnia and 17 female healthy-sleeping controls. To assess sleep-onset dysfunction in insomniacs, we imaged participants in two conditions: resting-state and 'fall asleep,' in which participants were asked to let themselves fall asleep. We focused specifically on the role of affective regions within resting-state networks that include arousal-promoting structures that have been implicated in insomnia (Nofzinger et al., 2004).

2. Methods

2.1. Participants

We recruited females, ages 18–40, who self-reported insomnia or healthy sleep. Participants were excluded for any past or present DSM-IV Axis I disorder, any past or present sleep disorder except insomnia, current use of prescription psychotropic or hypnotic medication, BMI greater than 30, and any exclusionary criteria for the MRI environment. We recruited only females because they have a higher prevalence of insomnia than do males (Ohayon, 2002), as well as to increase the homogeneity of the sample and the power of this study.

Eligible participants were administered the Structured Clinical Interview for Diagnosis of DSM-IV-TR Axis I disorders (First, Gibbon, Spitzer, & Williams, 1997) and the Duke Structured Interview for Sleep Disorders (Edinger, Wohlgemuth, Radtke, Marsh, & Quillian, 2001; Stepanski et al., 2004). No participant met any criteria for any DSM-IV-TR Axis I disorder or any sleep disorder, other than insomnia in insomniacs: DSM-IV-TR insomnia, ICSD-2 psychophysiological insomnia, or ICSD-2 idiopathic insomnia. Insomniacs had to retrospectively report at least 30 total minutes of sleep difficulty at least 3 times a week for at least 2 months, along with subjective distress. These criteria were selected to balance DSM-IV-TR and ICSD-2 criteria (Ohayon & Reynolds, 2009), while reflecting evolving nosologies of insomnia (Edinger et al., 2011). Participants then completed demographic information, the Beck Depression Inventory II (BDI-II) (Beck, Steer, & Brown, 1996), Beck Anxiety Inventory (BAI) (Beck, Epstein, Brown, & Steer, 1988), the Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989), the Dysfunctional Beliefs and Attitudes about Sleep scale (DBAS-16) (Morin, Vallières, & Ivers, 2007), the Insomnia Severity Index (ISI) (Bastien, Vallières, & Morin, 2001), the Ford Insomnia Response to Stress scale (FIRST) (Drake, Richardson, Roehrs, Scofield, & Roth, 2004), the Fatigue Severity Scale (FSS) (Krupp, LaRocca, Muir-Nash, & Steinberg, 1989), and specific information about current (within the last month) and past (past six months) sleep.

Several factors suggest that this is a viable clinical group. Differences between the two groups in scores on the insomnia severity index (ISI), Ford Insomnia Response to Stress scale (FIRST), and Pittsburgh Sleep Quality Index (PSQI) clearly indicate that the insomnia group experiences greater subjective sleep distress than does the control group. Indeed, all but one of the insomnia group participants had at least subthreshold insomnia based on the ISI (Bastien et al., 2001); interestingly, this is not the same individual who reported less than 30 min of sleep latency. More than half (8 of 17) of the insomnia participants reported at least clinically severe levels of insomnia, based on the ISI.

2.2. fMRI acquisition

Eligible participants were instructed to abstain from using over-the-counter medications that may affect sleep for a week prior to the scan and to limit the consumption of caffeinated beverages on the day of the scan. At midnight, participants completed a high-resolution SPGR anatomical scan and two 20-min spiral-in/out scans: a resting-state scan, with the instruction to "rest quietly with your eyes closed," and a 'fall asleep' scan, with the instruction to "rest quietly with your eyes closed and let yourself fall asleep." Following each scan, participants rated using a button box both their alertness during the previous scan and their post-scan alertness on a modified version of the Karolinska sleepiness scale (Kaida et al., 2006); ratings on this scale ranged from 1 to 9, with 1 corresponding to "wide awake," and 9 corresponding to "in deep sleep." High-resolution anatomical scans were obtained with an SPGR sequence with a resolution of 0.859 mm × 0.859 mm × 1 mm. Resting-state and 'fall asleep' scans were whole-brain spiral-in/out scans (Glover & Law, 2001), with 30 oblique axial slices with a thickness of 4 mm (1 mm skip) and an in-plane voxel size of 3.4375 mm × 3.4375 mm (TE = 30 ms, FOV = 22 cm, flip angle = 80°, and TR = 2.04 s) and 600 time frames for each scan for a total time per scan of 20 min, 24 s. Before and after the session, participants completed the PANAS (Watson, Clark, & Tellegen, 1988).

2.3. fMRI preprocessing

For the two spiral-in/out scans, we used modified NITRC (NITRC.org) and custom-designed scripts to preprocess data. RETROICOR (Glover, Li, & Ress, 2000) was used to remove time-locked cardiac and respiratory artifacts, and RVHRCOR (Chang, Cunningham, & Glover, 2009) was used to remove low-frequency heart rate and respiratory volume artifacts. We discarded the first 6 TRs because of T1 equilibrium effects. We then applied slice timing correction, motion correction, skull-stripping, and linear and quadratic detrending. Functional scans were registered to the MNI152 average brain template (Mazziotta, Toga, Evans, Fox, & Lancaster, 1995). Motion files were used to 'censor' (remove) TRs in which the derivative value of any of six motion parameters (*x*-shift, *y*-shift, *z*-shift, rotation, pitch, yaw) exceeded a Euclidean norm of 1.2. Insomniacs and healthy controls did not differ in the number of TRs removed during the rest scan, $t(32) = 0.397$, or the 'fall asleep' scan, $t(32) = 1.792$, both $p > 0.05$.

Nuisance signal timecourses in spiral-in/out volumes arising from white-matter, and CSF were calculated from segmented anatomical scans and were regressed from spiral-in/out volumes along with the 6 motion parameters. The demeaned residuals were then subjected to Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) using FSL. We initially used the Laplace approximation to the Bayesian evidence of the model order to determine the number of components, but the length and resolution of the scans produced hundreds of components that proved impractical for analysis, as noted previously (Yourganov et al., 2011). Consequently, we selected 25 components for resting and 'fall asleep' scans based on previous dual regression studies (Filippini et al., 2009).

Visually identified components corresponding to known noise and artifacts resulting from scanner noise, movement, residual white matter or CSF signal, or residual physiological noise were filtered from the resulting volumes (Kelly et al., 2010). Given the size of the volumes and lengths of the scan, multiple noise components persisted after filtering; consequently, this procedure was repeated a total of three times on each scan session. The MELODIC component of dual-regression requires equivalent length data, thus excluding the use of motion-censored data blocks. Subsequent analyses that were later conducted on the original non-de-noised datasets indicated that the statistical contrasts did not differ from analyses conducted on de-noised datasets. Insomniacs and healthy controls did not differ in the number of noise components removed, $t(32) = 1.44$, $p > 0.05$.

2.4. fMRI analyses

All individual de-noised datasets from each scan were concatenated and decomposed into 25 spatiotemporal components for each of the two scan types. Components of interest were analyzed by dual regression (Filippini et al., 2009; Zuo et al., 2010). Briefly, the spatial maps derived from the temporal concatenation ICA were used to produce a timeseries for each component for each individual. Next, these timeseries were used to produce spatial maps of the corresponding component for each individual. A *z*-statistic of this resulting spatial map was subjected to non-parametric permutation testing, with 5000 permutations and a variance smoothing equal to the FWHM. The result of the permutation analysis is a test of between-group differences in each of the 25 component maps. Thresholding of group statistics was based on threshold-free cluster enhancement. Results are presented for clusters that reach a family-wise error corrected value of $p < 0.05$; uncorrected values of $p < 0.001$ are also shown for illustrative purposes.

2.5. EEG acquisition and preprocessing

EEG was acquired using a MRI-compatibile EGI HydroCel 256-electrode dense-array Geodesic Sensor Net at a sampling rate of 250 Hz. No signal quality decline was observed during the scan session. Using NetStation, the TR marker was used to filter out the MR artifact using a moving average of 5 TRs. Bad channels were visually identified and replaced with a spline interpolation. The resulting file was imported into the EEGlab toolbox in Matlab (R2011b). The first 6 and last 5 TRs, which remain contaminated with MR-related artifacts, were censored. The first three harmonics of the slice frequency (14.6 Hz, 29.3 Hz, 44.0 Hz) were removed using a finite impulse response (FIR) notch filter in Matlab. Using PPG markers, the ballistocardiographic artifact was removed using a principal components method (Niazy, Beckmann, Iannetti, Brady, & Smith, 2005), with an optimal basis set of 4 components. The resulting file was resampled to 125 Hz, re-referenced to average,

and segmented using the TR trigger marker; the result is a file with 589 epochs of 2.04 s each across 256 channels.

2.6. EEG/fMRI analysis

For each epoch, signals were averaged from (10–20 system) Fp1, Fp2, F7, F8, F3, F4, Fz, C3, C4, P3, P4, Pz T3, T4, T5, T6, O1, O2. Epochs in which extreme values occurred (signal > 300 μV) were censored. A fast-Fourier transform (FFT) was applied to the square root power of the averaged electrode signal and output in 0.5 Hz frequency bins to calculate power spectra. EEG/fMRI timecourses were constructed using a finite impulse response bandpass filter. The total power of a TR of each EEG frequency was calculated using a Teager Energy Operator. This timeseries was then log-transformed and fitted to the data from each fMRI voxel using ordinary least squares regression as implemented by 3dDeconvolve in AFNI. The ratio of the power in a particular band to the total EEG power of all bands at any given TR was calculated (De Munck, Gonçalves, Mammoliti, Heethaar, & Lopes da Silva, 2009), as this was more robust against noise-related broadband amplitude modulation. This timeseries was regressed against the fMRI data using 3dDeconvolve to produce *z*-statistic maps. TRs with excessive motion or extreme EEG values were censored from analysis (Jansen et al., 2012). EEG/fMRI results for the frequency band correlations were determined at the voxel-wise $p < 0.001$, cluster-wise $p < 0.05$, while the exploratory analyses of insomniacs and healthy controls are presented at a voxel-wise $p < 0.005$ level.

3. Results

3.1. fMRI

The 17 insomniacs and 17 healthy controls were equivalent in age and education level (Table 1). As expected, insomniacs reported more sleep dysfunction than did controls as measured by the PSQI, ISI, and FIRST, and also reported greater sleep onset latency, less overall sleep, and more impaired sleep function (Table 1). Sleep dysfunction was also present before the scan session (Supplementary Results). Insomniacs had higher BDI and BAI scores than did healthy controls, but scores for both groups were well below the clinical cut-offs for these questionnaires. Participants completed two 20-min, task-free fMRI scans beginning at 12:35 AM and 1:00 AM, which was close to their habitual bedtimes (Table 1). Insomniacs and healthy controls did not significantly differ in bedtimes or waketimes.

Using data from all participants, we conducted independent components analysis (ICA) to extract maps of brain networks for rest and 'fall asleep' scans that were specific to this study but that corresponded to previously described intrinsic networks. Among these networks, we were particularly interested in 'salience' networks (Deen, Pitskel, & Pelphrey, 2011), which include structures implicated in arousal and insomnia, such as the anterior cingulate cortex and insular cortex. Examining the spatially distinct salience networks maps generated from data combined across both groups, we found evidence in both the rest and the 'fall asleep' scans of three previously-reported salience networks (Deen et al., 2011): ventral anterior insula (vAI) salience network (Fig. 1A), dorsal anterior insula (dAI) salience network (Fig. 1B), and posterior insula salience network. We then used a dual regression approach (Filippini et al., 2009) to examine whether insomniacs and healthy control participants exhibited different patterns of brain regions in which BOLD activity was either more or less strongly correlated with these salience networks. Briefly, for each scan, we used salience network maps from the combined group-level ICA to derive network timecourses, from which individual subject maps were derived; these subject maps were then analyzed using permutation-based statistical inference to test for group differences in spatial regions with more or less coactivation with the salience networks.

For the rest scan, we found no differences between insomniacs and healthy control participants in brain regions that coactivated with any of the salience networks. For the 'fall asleep' scan, we found increased coactivation of the anterior insula in insomniacs compared to healthy control participants with the vAI salience network (Fig. 1C) and the dAI network (Fig. 1D), but not with the posterior insula salience network. Thus, while attempting to fall asleep, insomniacs had greater coactivation of the anterior insula with the dAI and vAI salience networks than did healthy controls.

Because the fMRI analyses described above are dependent on the networks derived from the resting or 'fall asleep' scans, they do not allow direct comparisons of the overall effect of scan type (resting vs. 'fall asleep'), the overall effect of group (insomniacs vs. healthy controls, across both scan types), or the interaction of group and scan type. To test for these effects, we concatenated data from both groups in both resting and 'fall asleep' scans and conducted an ICA network extraction and dual-regression analysis as described above (Fig. S1A; insomniacs had greater insula coactivation with vAI salience network than did healthy controls (Fig. S1B). There were not, however, overall significant group effects in the dAI salience network, nor was there a significant effect of scan or a significant interaction of group and scan in any salience network. Thus, while insomniacs show heightened insula coactivation with the vAI salience network, this coactivation does not appear to be exclusive to the 'fall asleep' scan.

Insomniacs did not differ from healthy controls in their subjective ratings of their alertness following the anatomic, rest, or 'fall asleep' scans, $F(1,32) = 2.91$, $p > 0.05$; moreover, self-rated post-scan alertness after these scans did not decrease throughout the session, $F(2,31) = 2.28$, $p > 0.05$ (Fig. 2A). Insomniacs also did not differ from healthy controls in their retrospective ratings of alertness during any scan, $F(1,32) = 2.821$, $p > 0.05$, although in both groups there was a significant decrease of alertness across scans, $F(2,31) = 10.18$,

Table 1
Demographic, sleep, and affective characteristics.

	CTL mean	INSM mean	Cohen's d	*p*-value
Age in years	27.56 (6.83)	27.16 (6.67)	0.06	0.865
Education level	6.18 (1.63)	6.24 (1.71)	0.04	0.919
Sleep onset latency (last month)	11.82 (6.96)	46.71 (42.67)	1.14	0.002
Wake after sleep onset (last month)	2.41 (3.87)	43.23 (76.42)	0.75	0.035
Total sleep time (last month)	7.63 (0.60)	6.18 (1.06)	1.69	<0.001
Bedtime	11:42 PM (56.71)	11:54 PM (96.12)	0.16	0.645
Waketime	7:51 AM (41.80)	8:18 AM (108.25)	0.33	0.345
ISI	1.76 (2.77)	15.00 (3.77)	4.00	<0.001
FIRST	14.71 (3.82)	24.24 (5.07)	2.12	<0.001
PSQI	1.94 (1.48)	9.24 (3.38)	2.79	<0.001
BAI	0.82 (0.81)	3.88 (2.67)	1.55	<0.001
BDI-II	1.82 (3.13)	4.59 (4.51)	0.71	0.046
PANAS negative affect	16.18 (2.38)	15.88 (1.11)	0.16	0.674

Mean values, effect sizes, and *p*-values for group differences of demographic variables of insomniacs (INSM) and healthy controls (CTL). Standard deviations are presented in parentheses. Abbreviations: insomnia severity index (ISI), dysfunctional beliefs and attitudes about sleep scale (DBAS-16), Ford insomnia response to stress scale (FIRST), Pittsburgh sleep quality index (PSQI), beck anxiety inventory (BAI), Beck depression inventory II (BDI-II).

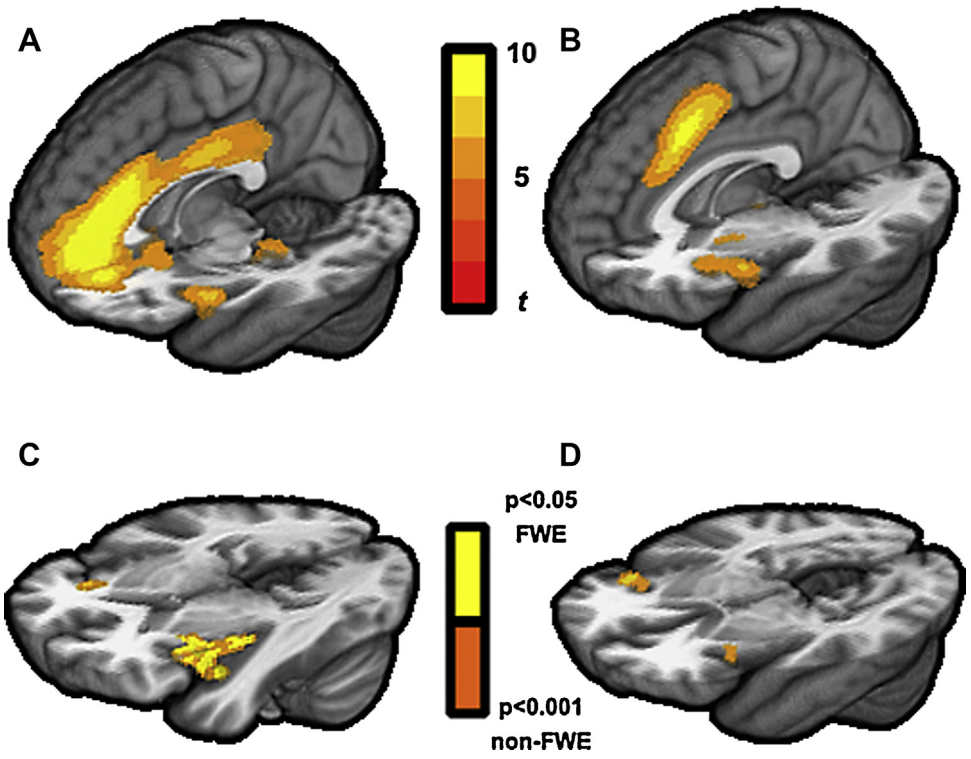

Fig. 1. fMRI BOLD networks derived from independent components analyses in the 'fall asleep' scan. These include the ventral anterior insula salience network (A) and dorsal anterior insula salience network (B) templates. Dual-regression analyses of regions that show increased coactivation in insomniacs in the ventral anterior insula salience network (C), as well as regions that show increased coactivation with dorsal anterior insula salience network (D), with colors corresponding to $p < 0.05$ family-wise error corrected and $p < 0.001$ uncorrected using non-parametric permutation testing.

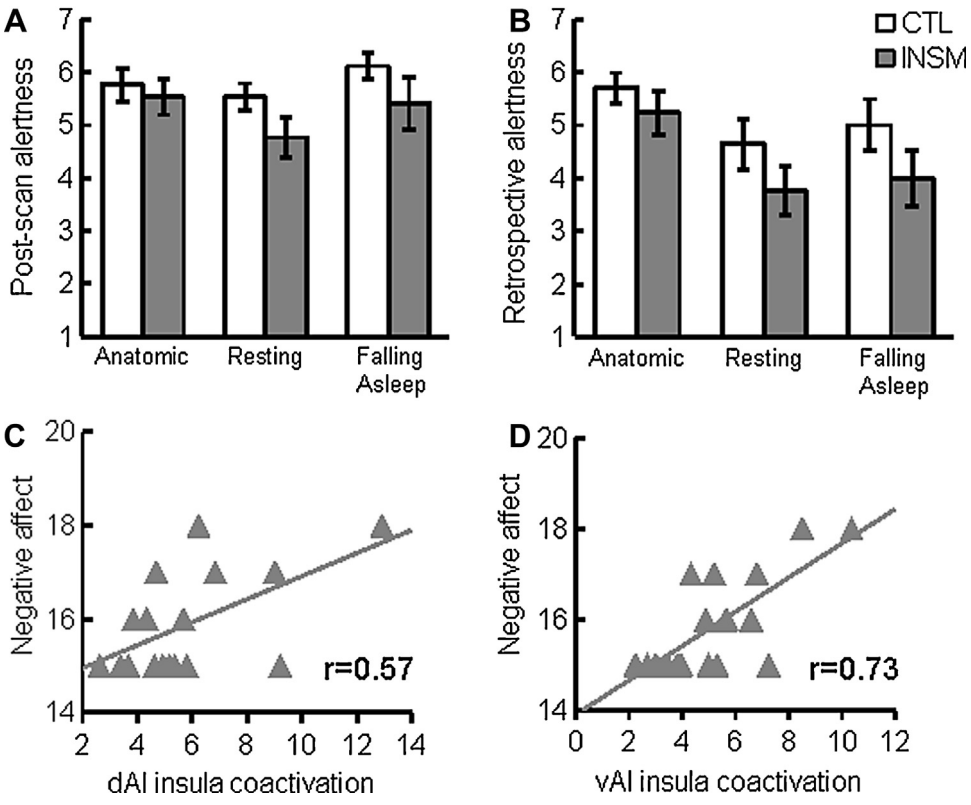

Fig. 2. Self-rated subjective alertness and fMRI BOLD network coactivation. Post-scan (A) and retrospective (B) alertness, as rated on a scale of 1 (deeply asleep) to 9 (wide awake) ± SEM. is moderately correlated with insula coactivation *z*-scores in dorsal anterior insula (C) and ventral anterior insula (D) salience networks. Retrospective alertness is not correlated with insula coactivation with dorsal anterior insula salience network (E) but is moderately correlated with insula coactivation with ventral anterior insula salience network (F). Insula coactivation in both dorsal (G) and ventral (H) anterior insula salience networks is significantly correlated with post-scan PANAS negative affect scores.

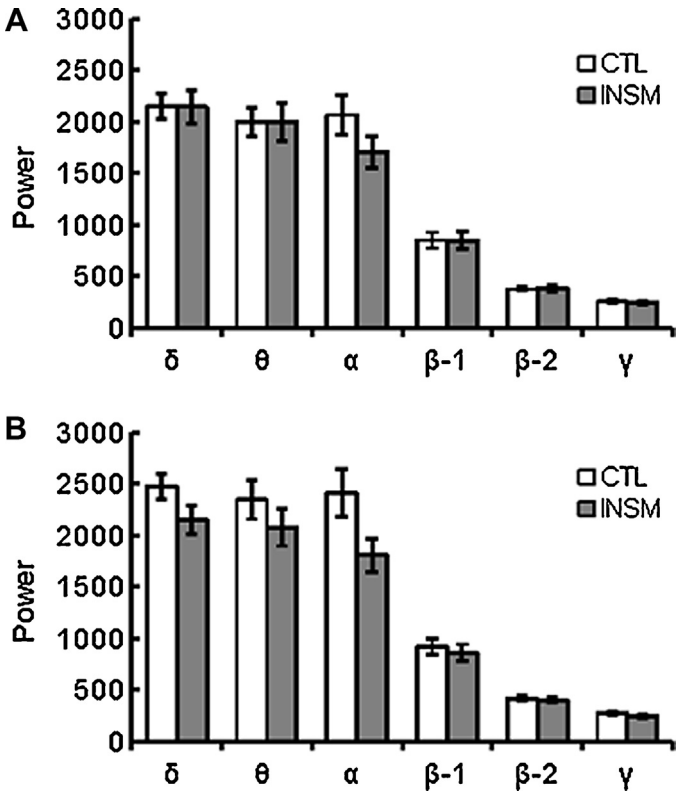

Fig. 3. EEG power of healthy controls (CTL) and insomniacs (INSM). EEG during rest (A) and 'fall asleep' (B), by power frequency ± SEM.

$p < 0.001$ (Fig. 2B). There were no interactions of group and time for either current ratings after scans, $F(2,31) = 0.76$, $p > 0.05$, or retrospective ratings of the scans, $F(2,31) = 0.42$, $p > 0.05$. Insomniacs and healthy controls also did not differ in negative affect, $t(32) = 0.462$, $p > 0.05$, or positive affect, $t(32) = 0.951$, $p > 0.05$. Exploratory analyses of the correlation between coactivation and affect within the insomnia group revealed that insula coactivation was significantly correlated with post-scan PANAS negative affect scores in both dAI, $p = 0.018$ (Fig. 2C), and vAI networks, $p < 0.001$ (Fig. 2D) but not PANAS positive affect scores, $p > 0.05$. Conducting these analyses including healthy controls or within the healthy control group alone did not yield significant correlations.

3.2. EEG

Next, we examined EEG measures in insomniacs and healthy control participants during the rest and 'fall asleep' scans. We did not observe obvious sleep episodes from visual inspection of the EEG. Overall power spectra were calculated for rest (Fig. 3A) and 'fall asleep' scans (Fig. 3B) in 0.5 Hz frequency bins. We examined overall frequency power in delta (δ: 0.5–4 Hz), theta (θ: 4–8 Hz), alpha (α: 8–14 Hz), beta-1 (β-1: 14–20 Hz), beta-2 (β-2: 20–35 Hz), or gamma (γ: 35–50 Hz) power bands using a repeated-measures multivariate analysis of variance (MANOVA; scan by frequency band by group). This analysis yielded a significant effect of scan, $F(1,32) = 10.270$, $p = 0.003$, a significant effect of frequency band, $F(1,32) = 111.151$, $p < 0.001$;the main effect of group was not significant, $F(1,32) = 1.352$, $p > 0.05$, nor was the interaction of group and frequency band, $F(5,28) = 1.546$, $p > 0.05$. There was a significant interaction of scan and frequency band, $F(5,28) = 3.415$, $p = 0.016$, and a significant interaction of group and scan, $F(1,32) = 4.800$, $p = 0.036$. Relative to healthy controls, insomniacs had reduced EEG power across multiple frequency bands during the 'fall asleep' scan compared to the resting scan. Post hoc analyses of individual frequencies reveal that α is the only individual band with significant differences between the two groups during the 'fall asleep' scan, $t(32) = 2.14$, $p = 0.040$, uncorrected. Notably, there were no significant differences between the two groups in the bands associated with non-REM sleep, δ and θ.

3.3. EEG/FMRI

We then constructed timeseries from each frequency band to examine whether insomniacs and healthy controls differed in BOLD correlates of EEG power. The full results for each power band are presented in Table S1. While there were few significant group differences in BOLD signal associated with lower frequency bands of EEG, we found for the resting state scan that healthy controls showed significantly greater BOLD signal associated with γ power ratio in the posterior cingulate (PCC) and medial prefrontal cortex (mPFC) than did insomniacs (Fig. 4A). Given the involvement of these structures in canonical resting state networks, specifically in default mode networks, we examined each group separately in an exploratory analysis to determine the BOLD signal associated with γ power ratio. In healthy controls, the pattern of BOLD signal includes PCC and mPFC (Fig. 4B); in contrast, in insomniacs, the pattern of BOLD signal includes bilateral insula, resembling the spatial pattern of the vAI network (Fig. 4C). We also examined the dorsal and ventral default mode networks in rest and 'fall asleep' using dual-regression, and found no differences between insomniacs and healthy controls in coactivations with these networks. There were no significant correlations in any participant between any EEG frequency band power timeseries and ICA-derived network timeseries.

4. Discussion

The present study is the first to characterize resting-state networks in insomniacs and healthy sleeping controls using combined EEG and fMRI. Using a dual regression approach, we found increased bilateral anterior insula BOLD coactivation with vAI and dAI salience networks, as well as insula-associated high-frequency γ power during rest in insomniacs. Notably, the insula is a key hub in the salience network itself, and the difference in coactivation between groups suggests a role for the salience network in insomnia. Indeed the aberrant insula contribution to salience networks is consistent with previous studies of insomnia in rodents (Cano, Mochizuki, & Saper, 2008) and humans (Nofzinger et al., 2004), suggesting that these networks, and in particular the anterior insula, contribute to the neural circuitry underlying insomnia. Anterior insula and nearby orbitofrontal cortex gray matter density have been previously implicated in insomnia (Stoffers et al., 2012). The insula has been posited to be a source of the slow waves that characterize deeper stages of sleep (Murphy et al., 2009), and the increased coactivation of the left insula with salience networks may interfere with the progressive generation of low-frequency EEG waves as part of the transition to sleep.

Whereas some studies have found few differences between insomniacs and controls in polysomnography-measured sleep (Rosa & Bonnet, 2000), others have documented persistent high-frequency activity during sleep in insomniacs (Krystal, Edinger, Wohlgemuth, & Marsh, 2002; Perlis, Smith, Andrews, Orff, & Giles, 2001). We did not observe increased high-frequency activity in insomniacs, although it is possible that the scanning environment impaired N1 sleep. Healthy controls had greater PCC and mPFC signal associated with γ power ratio than did insomniacs. Whereas the γ ratio power in healthy controls was associated with BOLD signal in PCC and mPFC, spatially similar to DMN networks, the γ ratio power in insomniacs was associated with BOLD signal in anterior

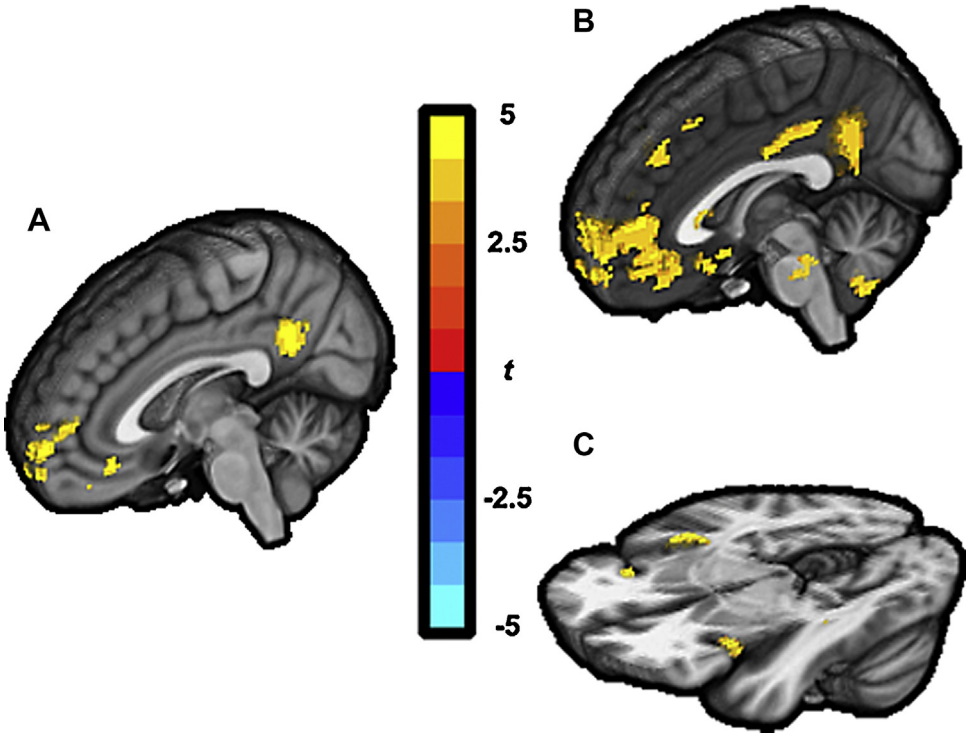

Fig. 4. EEG γ band power associated BOLD signal. Differences between healthy controls and insomniacs (A), $p < 0.001$, cluster corrected $p < 0.05$, and BOLD correlated with γ band in healthy controls (B) and insomniacs (C), both at uncorrected voxel-wise $p < 0.005$.

insula, similar to salience networks. While these associations were not evident in the rest scan, the γ ratio power BOLD associations with anterior insula might be indicative of impairments occurring outside attempted sleep onset in insomniacs. While there was a significant group by scan interaction in overall EEG spectra, there were only differences in α power between groups during the 'fall asleep' scan. Previous studies of EEG power in insomnia have found that insomniacs have reduced alpha power (Lamarche & Ogilvie, 1997), combined with a failure to reduce alpha power during the sleep onset period (Staner et al., 2003) and wakefulness (Freedman, 1986). It is not clear whether alpha power differences extend into the sleep period.

The insula is thought to play a role in affect, for example the anticipation of negative stimuli in anxious individuals (Simmons et al., 2011). Insomnia is associated with psychiatric conditions, including anxiety and major depression, and in this sample insomniacs have increased anxiety and depression scores compared to healthy controls, albeit below clinical thresholds. The increased insula coactivation observed could be indicative of this subthreshold anxiety, worry and rumination, or a signature of insufficient gating of the sensory stimuli of the fMRI environment (Hairston, Talbot, Eidelman, Gruber, & Harvey, 2010). However, another intriguing mechanism by which the insula may contribute to insomnia is through the subjective perception of sleep distress. DSM-IV, ICSD-9, and ICD-10 all define insomnia based on subjective distress, rather than on an objective measure of sleep disturbance, as insomniacs may misperceive the quantity or quality of their sleep relative to polysomnography (American Academy of Sleep Medicine, 2005; Association & DSM-IV, 2000; "WHO | International Classification of Diseases (ICD)," n.d.). We propose that the insula coactivation with salience networks underlies this subjective disturbance; indeed we found positive correlations in insomniacs between the degree of insula coactivation with salience networks and with self-reported alertness and negative affect. The insula has been proposed to integrate a variety of information, including interoceptive awareness, time perception, and emotional salience (Craig, 2009). Among other functions, the insula has been proposed to underlie facets of self-awareness, time dilation, and subjective salience, all of which have been proposed to play a role in insomnia (Harvey & Tang, 2012). Increased insula coactivation with salience networks may contribute specifically to the misperception of sleep and wakefulness. This misperception, whether a misestimate of time or some qualia of sleep satisfaction, may outweigh the fulfillment of homeostatic sleep need in insomnia.

Intrinsic network imaging has several advantages over task-based imaging for studying insomnia. Specifically, the lack of externally guided task—with accompanying visual or auditory stimuli—better simulates the mental state prior to sleep onset that is posited to be dysfunctional in insomnia. Furthermore, the focus on specific networks permits the identification of not only aberrant activity in a brain region but also the possible role of this brain region within a network of structures. Because intrinsic network imaging uses the same pulse sequences as task-based imaging and requires no additional setup, this is an important tool for the study of insomnia and other psychological disorders that may not be amenable to traditional task-based fMRI.

There are limitations of the current study that should be addressed in future investigations. For example, even though the insomnia group did report sleep dysfunction at the time of the scan (see Supplementary Results), a two-week sleep diary combined with actigraphy would be instructive in confirming the diagnosis of chronic insomnia. Currently, diagnoses of insomnia rely on subjectively reported distress, not on actigraphy; indeed, insomniacs who have objective short sleep may represent a different phenotype than do those whose sleep disturbance is primarily subjective (Krystal et al., 2002). Similarly, it is possible that the fMRI network findings we describe will vary as a function of individual differences in sleep variables as measured with traditional polysomnography. Certainly the fMRI environment is not an ideal sleep environment, especially combined with the 'first-night effect' reported in

polysomnography studies. Importantly, in present study there were no significant differences between the insomniac and control groups in δ or θ bands, suggesting that the group differences we observed in neural coactivation were not due to differences in sleep. Nevertheless, the present findings must be interpreted in the context of the study conditions. We did not use polysomnography to diagnose insomnia, and scoring sleep stage during scans may yield important information in future fMRI/EEG studies of this disorder.

In this study we have identified a role for the anterior insula in arousal-promoting BOLD signal networks in insomnia. This structure, as part of a network of structures involved in hyperarousal in insomnia, may be an important target for novel therapies for this disorder. In this context, it is noteworthy that administration of benzodiazepines has been found to reduce regional cerebral blood flow to multiple limbic system structures, including the anterior insula (Kajimura, 2004). Future studies could focus on directly altering BOLD signal in this structure using real-time fMRI or other interventions for the treatment of chronic insomnia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biopsycho.2013.12.016.

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