In Trauma-exposed Individuals, Self-reported Hyperarousal as Well as Sleep Architecture Predict Resting-state Functional Connectivity in Frontocortical and Paralimbic Regions

Supplementary Information

Supplementary Methods

Sleep Disorder Evaluation

Sleep disorders were evaluated using the Pittsburgh Structured Clinical Interview for Sleep Disorders (SCID-SLD), a widely used (1, 2), but unpublished scale consisting of a 14-page structured interview designed detect the major symptoms of sleep disorders as well as related impairments, medical issues and history based upon the International Classification of Sleep Disorders-2nd Edition (3) and the DSM-IV-TR (4).

Exclusion Criteria

Subjects were excluded if they had: a history of chronic childhood trauma or PTSD diagnosed prior to index trauma; history of neurological or major medical illness; DSM-IV psychotic, bipolar or autism spectrum disorders; reported sleep disorders other than Insomnia Disorder or detected obstructive sleep apnea (OSA) or periodic limb movement (PLMS) sufficient to warrant referral for treatment; current substance abuse, dependence or positive urine screen; use of anxiolytic or sleep medications; alcohol > 10 drinks/week or caffeine > 5 beverages/d; and MRI contraindications. Stable antidepressant use, mild Axis 1 anxiety disorders, dysthymia and remitted major depressive and substance use

disorders were allowed. A urine toxicology screen was conducted and any participants testing positive for 10 commonly abused substances were excluded.

Calculating the Composite Hyperarousal Index (CHI)

The CHI was calculated from the 6 Cluster E, hyperarousal items in the Clinician-Administered PTSD Scale for the DSM-5 (CAPS-5) and 6 hyperarousal items from the PTSD Checklist for DSM-5 (PCL-5), questions 15-20. The maximum potential points for each assessments' hyperarousal sections is 24 (0-4 range for each item x 6 items). Hyperarousal scores were converted to a 0-100 scale for both assessments, and then the two converted scores were averaged to get the final CHI. As an example, if a subject had a score of 14 out of 24 for the PCL-5 hyperarousal section, and an 11 out of 24 for the CAPS-5 cluster E section, then their score would be calculated as such: [(14/24)*100 + (11/24)*100]/2 = 52.083 CHI score.

Computation of Sleep Parameters from Evening-Morning Sleep Questionnaire Diary (EMSQ)

The evening portion of the EMSQ queried prior daytime activities and the time at which the participant began to attempt sleep. Morning portions queried time waking for the day, subjective SOL, subjective TST, and number and duration of nocturnal awakenings (summed as subjective wake time after sleep onset or WASO). Subjective sleep efficiency (SE) was computed from diaries as proportion of time in bed (TIB; duration between the time at which subject began

to attempt sleep and time of waking for the day) occupied by sleep [TIB – (SOL + WASO)].

Polysomnographic Montage

Electrodes were attached in the lab prior to each night of ambulatory PSG using the Somte-PSG ambulatory recorder (Compumedics USA, Inc., Charlotte, NC). The Somte-PSG recorder was contained in a customized cloth pack, worn on the chest, which encloses and protects all loose wires. It was set to begin recording before the subject's earliest anticipated bedtime. The montage included 6 EEG channels (F3, F4, C3, C4, O1, O2) referenced to contralateral mastoids (A1, A2), as well as 2 EOG channels (both outer canthi, above and below the eye), 2 channels of submental EMG, and 2 channels for ECG on the right clavicle and left 5th intercostal space. On the diagnostic night, additional channels for pulse-oximeter, respiration transducer belts, nasal cannula and tibialis channels were added to screen for obstructive sleep apnea (OSA) and period leg movement disorder (PLMD). Data were acquired at 256 Hz, using high and low pass filters of 0.16 and 102 Hz, respectively. All sleep records were scored by the same experienced, RSPGT research polysomnographer. Scoring followed the criteria of the American Academy of Sleep Medicine (5, 6) with 30-second epochs visually differentiated between wake, non-rapid eye movement sleep (N1, N2, N3) and REM sleep stages. The polysomnographer also assessed presence or absence of clinically significant OSA and PLMD.

Supplementary Results

Functional Connectivity of Each Seed to Regions of Anterior Cerebrum

Table S1 shows functional connectivity of each seed to clusters of contiguous voxels in regions of the anterior cerebrum irrespective of the relationship of such connectivity to symptom- or sleep-related regressors. As explained under the "Methods, rs-fMRI Data Analysis", to generate the functional connectivity maps, the averaged time series was obtained from each of the 5 fear-related seed regions (bilateral amygdala, bilateral anterior insular cortex, and dorsal anterior cingulate cortex) and 1 fear-regulatory (ventromedial prefrontal cortex) seed region. The correlation analysis was conducted between time series in the seed regions and voxels of the whole brain using the CONN functional connectivity toolbox v.17c (http://www.nitrc.org/projects/conn) (7). The correlation coefficient maps were then converted into z-maps by Fisher's r-to-z transformation to improve normality. In the within-group analyses using SPM for all 74 subjects, these correlation maps were restricted to an anterior cerebral mask and corrected for multiple comparisons within SPM8, using a family-wise error (FWE) correction threshold of $p<0.01$ at the whole-brain level.

Seed-to-Whole Brain Connectivity and Correlation Analyses

Table S2 shows results of second-level seed-to-whole brain connectivity analyses thresholded at cluster-defining threshold (CDT) $p<0.001$ and a liberal cluster-size threshold of ≥ 85 contiguous voxels. All of the results found within the anterior cerebral mask analyses were supported in the seed-to-whole brain

analysis. Additionally, five other posterior regions including the cerebellum, temporal gyrus, brain stem, lingual gyrus, and middle cingulate cortex were found (Table S2). Similarly to analyses of only an anterior cerebral mask, more rigorous analysis using SnPM was performed and the results were thresholded at a cluster-wise threshold of $p<0.1$ after family-wise error (FWE) correction with 5000 permutations. None of these new posterior clusters remained significant following the FWE correction using SnPM.

Table S1. The results of resting state functional connectivity of 5 fear-related and 1 emotion regulatory seed regions with anterior cerebral voxel clusters surviving family-wise error (FWE) correction across whole brain thresholded with $p < 0.01$.

Brain regions	Coordinates (mm)			Peak t value	Voxel size
	x	y	z		
dACC seed region					
SMA	2	0	62	8.04	1384
rACC	2	40	10	12.39	966
Left insula	-40	4	8	11.44	1565
Right insula	36	18	8	11.92	1538
Left thalamus	-8	-16	10	9.16	557
Right thalamus	10	-14	10	10.06	630
Left putamen	-22	6	10	7.34	708
Right putamen	24	14	0	7.97	821
Left amygdala	-22	-2	-14	6.63	105
Right amygdala	28	4	-18	6.87	57
Left amygdala seed region					
Left insula	-36	26	6	6.66	1325
Right insula	46	8	-8	7.86	461
rACC	2	42	4	6.08	259
Left inferior frontal cortex	-38	30	6	7.90	291
Right inferior frontal cortex	46	34	6	5.94	63
Left putamen	-22	10	-2	8.27	967
Right putamen	34	4	-2	7.49	497
Right thalamus	10	-12	-2	8.09	97
Right amygdala	28	0	-22	17.28	248
Left hippocampus	-28	-18	-14	14.27	652
Right hippocampus	28	-10	-14	16.75	516
Left temporal pole	-40	20	-28	10.05	1001
Right temporal pole	36	20	-28	11.59	799
Pons	-4	-22	-28	9.15	1603
Right amygdala seed region					
Right inferior frontal cortex	48	34	12	7.12	145
Left insula	-42	2	0	7.95	697
Right insula	46	2	-6	7.61	661
Left putamen	-28	-2	0	7.50	565
Right putamen	32	-4	0	7.55	499
vmPFC	2	30	-10	7.82	256
Left inferior orbito frontal cortex	-36	30	-10	7.68	258
Left amygdala	-22	-2	-18	21.52	220
Left hippocampus	-30	-14	-18	13.74	546
Right hippocampus	36	-10	-18	7.99	552
Pons	0	-18	-26	6.20	1114
Left temporal pole	-34	20	-26	9.57	945
Right temporal pole	40	20	-26	12.69	832

Brain regions	Coordinates (mm)			Peak t value	Voxel size
	x	y	z		
vmPFC seed region					
Left middle frontal cortex	-28	10	60	6.76	104
Left superior medial frontal cortex	-6	54	36	7.17	1028
Right superior medial frontal cortex	12	54	40	6.51	842
rACC	4	44	6	6.72	479
Left inferior orbito frontal cortex	-36	34	-16	11.07	759
Right inferior orbito frontal cortex	40	36	-16	10.53	534
Right temporal pole	40	14	-42	6.93	140
Middle cingulate cortex	2	-44	34	7.10	87
Left anterior insula seed region					
SMA	6	6	64	9.81	1001
Anterior cingulate cortex	2	34	22	15.68	2033
Right insula	38	18	-10	21.09	1075
Left putamen	-22	10	-6	11.82	864
Right putamen	28	8	-6	10.21	506
Left amygdala	-18	-2	-14	9.12	197
Right amygdala	26	2	-14	7.64	74
Pons	6	-16	-28	7.18	639
Left temporal pole	-34	18	-28	10.93	1046
Right temporal pole	36	14	-28	7.53	638
Right anterior insula seed region					
SMA	8	8	56	9.71	1050
Right middle frontal cortex	42	2	48	7.80	158
Anterior cingulate cortex	6	38	18	20.18	2477
Left insula	-40	16	0	19.20	1536
Left putamen	-24	8	0	8.21	771
Right putamen	24	12	0	9.6	1016
Left caudate	-6	14	0	7.58	464
Right caudate	12	12	0	10.77	452
Left thalamus	-6	-10	0	6.67	102
Right thalamus	10	-8	0	10.89	207
Left amygdala	-20	-2	-12	9.36	163
Right amygdala	26	-4	-12	7.24	207
Pons	-2	-20	-24	9.22	599
Left temporal pole	-34	18	-24	9.96	840
Right temporal pole	38	20	-24	12.70	947

Abbreviations: dACC – dorsal anterior cingulate cortex; SMA – supplementary motor areas; rACC – rostral anterior cingulate cortex; vmPFC – ventromedial prefrontal cortex.

Table S2. Correlation of posttraumatic symptom, sleep quality and sleep architecture variables with resting state connectivity of 5 fear-related and 1 emotion regulatory seed regions across whole brain thresholded at cluster-defining threshold p<0.001 and a cluster-size threshold of ≥ 85 contiguous voxels.

Predictor	Direction	Seed	Connected regions with seed	x, y, z (peak voxel)	peak t	k
Symptom						
PCL-5	Negative	right amygdala	right middle frontal cortex (BA9)	40,26,24	4.33	365
CHI	Negative	right amygdala	right middle frontal cortex (BA9)	40,26,26	3.73	170
Actigraphy						
Actigraph TST	Negative	left amygdala	dmPFC (SMA; BA8, 32, 6)	2,18,54	4.49	417
	Negative	vmPFC	right middle frontal cortex (BA9, 10)	30,34,36	4.03	288
	Positive	vmPFC	left hippocampus	-20,-12,-20	4.02	313
	Negative	left amygdala	right middle frontal cortex (BA10)	38,50,20	3.73	173
	Positive	vmPFC	right hippocampus	24,-12,-20	3.85	161
	Negative	left amygdala	left cerebellum	-36,-50,-42	4.01	90
	Positive	left amygdala	right middle temporal gyrus	36,-54,4	4.15	108
	Positive	vmPFC	left parahippocampus	-32,-24,-24	3.83	106
Actigraph SOL	Positive	dACC	right posterior insular cortex (BA13)	42,-10,0	4.4	292
	Positive	left amygdala	left insular cortex (BA13)	-40,6,-8	4.14	324
	Positive	vmPFC	right primary motor cortex (BA4)	28,-24,64	4.26	326
	Positive	left AIC	right middle frontal cortex (BA10)	30,52,14	3.76	233
	Positive	dACC	left posterior insular cortex (BA13)	-40,-16,2	3.67	153
	Positive	left amygdala	right insular cortex (BA13)	42,8,-14	4.13	169
	Positive	dACC	middle cingulate cortex (BA31)	-2,-36,48	4.09	97
	Negative	dACC	right middle temporal gyrus	46,-44,8	3.93	112
	Positive	left amygdala	left brainstem	-10,-20,-16	4.39	125
	Positive	left amygdala	left cerebellum	-34.-63.-20	3.59	85
	Negative	vmPFC	left cerebellum (posterior)	-12,-72,-16	3.87	142
	Negative	vmPFC	left cerebellum (anterior)	-28,-54,-28	4.03	141
	Negative	left AIC	left lingual gyrus	-2,-90,-4	4.28	102

Predictor	Direction	Seed	Connected regions with seed	x, y, z (peak voxel)	peak t	k
Actigraph SE	Negative	vmPFC	SMA (BA6)	2,-16,72	4.52	636
Diary						
Diary TST	Negative	left AIC	subgenual ACC (BA25)	6,12,-12	3.87	135
	Positive	dACC	rACC (BA24)	0,30,18	3.95	162
	Positive	left AIC	left inferior parietal cortex (BA40)	-52,-44,46	4.22	112
	Negative	left AIC	right middle temporal gyrus (BA22)	66,-52,8	3.72	96
	Positive	dACC	left superior temporal gyrus	-40,-16,-12	4.32	99
Diary SOL	Positive	right AIC	pre-SMA (BA8, 6, 9)	-2,42,52	4.48	492
	Positive	right AIC	left temporal pole (BA38, 21)	-42,18,-26	3.84	276
Nightmare and	Positive	dACC	rACC (BA24)	10,32,-8	4.89	309
Bad dream rate	Positive	left amygdala	left primary motor cortex (BA6, 4, 9)	-56,0,30	4.97	305
	Negative	left AIC	left orbitofrontal cortex (BA47)	-48,28,4	3.81	157
	Positive	vmPFC	SMA (BA6)	8,-8,70	3.87	133
	Positive	vmPFC	left postcentral gyrus	-26,-38,52	3.83	111
	Positive	vmPFC	right superior temporal gyrus	62,-24,-4	3.73	91
	Positive	vmPFC	right hippocampus	30,-28,-10	4.77	91
PSG						
SWS%	Positive	right amygdala	dmPFC (BA8, 9)	-6,28,50	5.21	643
	Positive	right amygdala	rACC (BA32)	8,44,8	4.14	218
	Positive	right amygdala	middle cingulate cortex (BA24)	2,-20,36	4.17	98

Abbreviations: PCL-5 – PTSD Checklist for DSM-5; CHI – composite hyperarousal index; BA – Brodmann area; TST – total sleep time; dmPFC – dorsomedial prefrontal cortex; SMA – supplementary motor areas; vmPFC – ventromedial prefrontal cortex; SOL – sleep onset latency; dACC – dorsal anterior cingulate cortex; AIC – anterior insular cortex; SE – sleep efficiency; rACC – rostral anterior cingulate cortex; PSG – polysomnography; SWS – slow wave sleep.

Table S3. Correlation between selected independent variables and functional connectivity scores using simple linear regression test.

Variables	R^2	β	SE	Partial R value	p	Bonferroni-corrected critical p-value
PCL-5						
R. amygdala seed – R. MFC	0.252	-0.003	0.001	-0.46	0.000054	<0.05
CHI						
R. amygdala seed – R. MFC	0.2	-0.003	0.001	-0.459	0.000039	<0.05
Actigraph TST						
L. amygdala seed – dmPFC	0.246	-0.001	0.0002	-0.507	0.000009	<0.01
vmPFC seed – R. MFC	0.179	-0.001	0.0003	-0.438	0.0001	<0.01
vmPFC seed – L. hippocampus	0.289	0.001	0.0001	0.547	0.000001	<0.01
L. amygdala seed – R. MFC	0.2	-0.001	0.0002	-0.46	0.000071	<0.01
vmPFC seed – R. hippocampus	0.237	0.001	0.0002	0.499	0.00001	<0.01
Actigraph SOL						
dACC seed – R. PIC	0.182	0.003	0.001	0.441	0.0001	<0.0083
L. amygdala seed – L. IC	0.25	0.003	0.001	0.511	0.00001	<0.0083
vmPFC seed – R. M1	0.239	0.003	0.001	0.501	0.000014	<0.0083
L. AIC seed – R. MFC	0.139	0.002	0.001	0.389	0.001	<0.0083
dACC seed – L. PIC	0.125	0.003	0.001	0.371	0.002	<0.0083
L. amygdala seed – R. IC				0.45	0.0001	<0.0083
Actigraph SE						
vmPFC seed – SMA	0.249	-0.013	0.003	-0.51	0.000008	<0.05
Diary TST						
L. AIC seed – subgenual ACC	0.195	-0.001	0.0002	-0.455	0.000085	<0.025
dACC seed – rACC	0.128	0.001	0.0003	0.375	0.002	<0.025
Diary SOL						
R. AIC seed – pre-SMA	0.257	0.004	0.001	0.514	0.000005	<0.025
R. AIC seed – L. temporal pole	0.182	0.004	0.001	0.44	0.000172	<0.025
Nightmare and Bad dream rate						
dACC seed – rACC	0.225	0.003	0.001	0.487	0.000026	<0.0125
L. amygdala seed – L. M1	0.282	0.003	0.001	0.541	0.000002	<0.0125
L. AIC seed – L. OFC	0.178	-0.005	0.001	-0.436	0.0001	<0.0125
vmPFC seed – SMA	0.198	0.004	0.001	0.458	0.000085	<0.0125
PSG SWS%						
R. amygdala seed – dmPFC	0.263	0.005	0.001	0.525	0.000017	<0.025
R. amygdala seed – rACC	0.149	0.005	0.002	0.404	0.001	<0.025

Abbreviations: R^2 – adjusted R square; β– unstandardized regression coefficient; SE – standard error; PCL-5 – PTSD Checklist for DSM-5; R. – right; MFC – middle frontal

gyrus cortex; CHI – composite hyperarousal index; TST – total sleep time; L. – left; dmPFC – dosal medial prefrontal cortex; vmPFC – ventromedial prefrontal cortex; SOL – sleep onset latency; dACC – dosal anterior cingulate cortex; PIC – posterior insular cortex; IC – insular cortex; M1 – primary motor cortex; AIC – anterior insular cortex; SMA – supplementary motor areas; ACC – anterior cingulate cortex; OFC – orbitofrontal cortex; rACC – rostral anterior cingulate cortex.

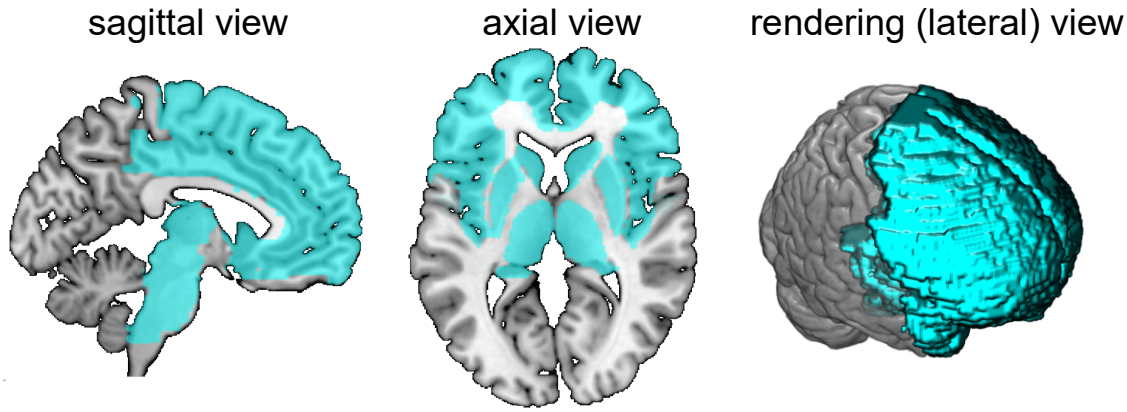

Figure S1. Anterior frontocortical/paralimbic mask. To focus on fear-related regions and to obtain higher statistical power, the second level analysis was restricted to this mask which contained: the hippocampus, amygdala, thalamus, basal ganglia, pons, brain stem, midbrain, putamen and caudate as well as Brodmann areas 3-6, 8-11, 13, 21-25, 28, 31-36, 38, 43-47.

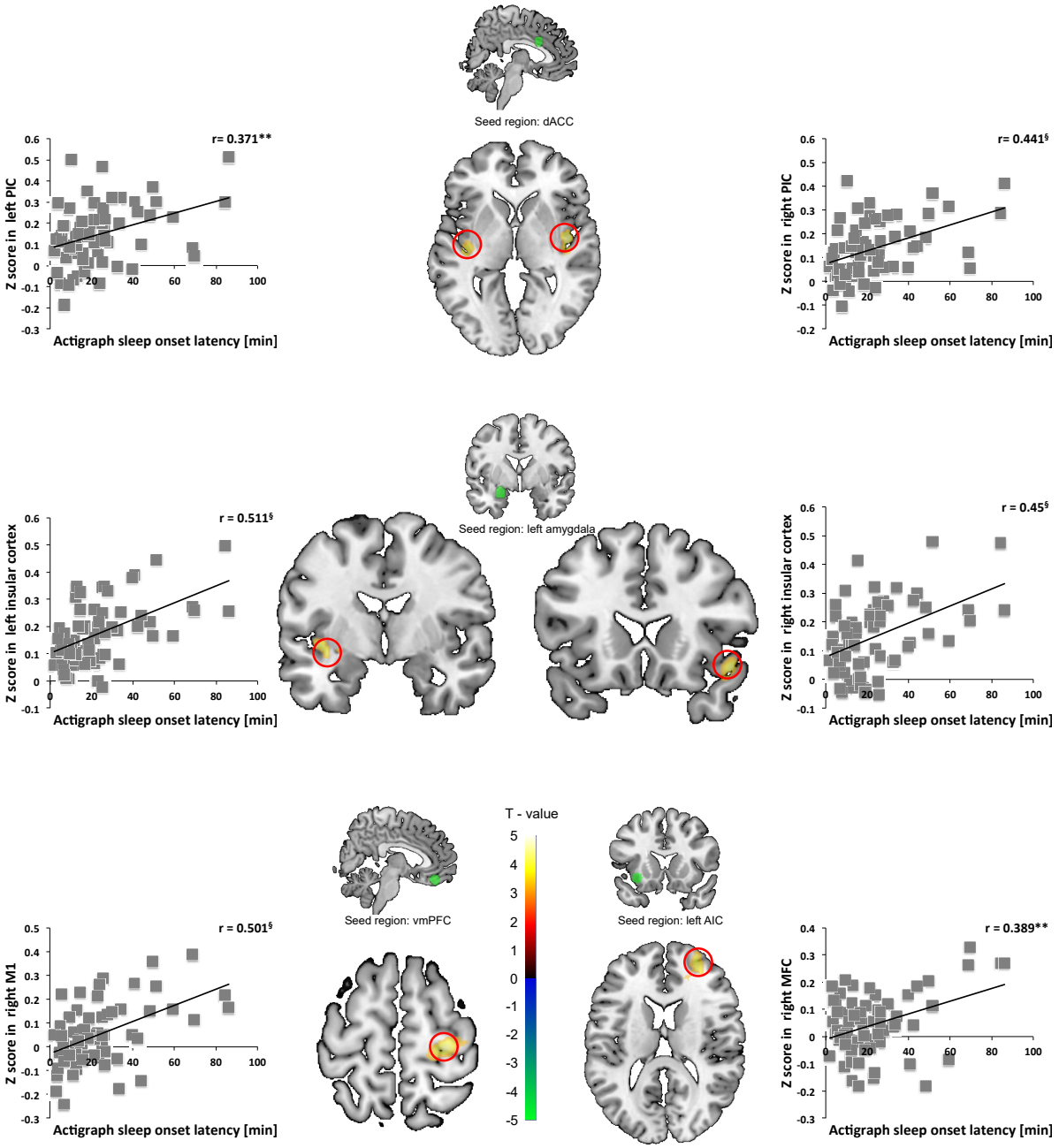

Figure S2. Actigraph sleep onset latency (SOL) predicts greater rsFC between the dACC seed and the bilateral posterior insular cortex (PIC) and correlates positively with extracted dACC-PIC mean Z-values *(upper panels)*. SOL predicts greater rsFC between the left amygdala seed and the bilateral insular cortex (IC) and correlates positively with extracted amygdala-IC mean Z-values (*middle panels*). SOL predicts greater rsFC between the vmPFC seed and the right primary motor cortex (M1) and correlates positively with extracted vmPFC-M1 mean Z-values *(bottom left panel)*. SOL predicts greater rsFC between left AIC seed and the right middle frontal cortex (MFC) and correlates positively with extracted AIC-MFC mean Z-values *(bottom right panel)*. ** p< 0.01; § p < 0.001.

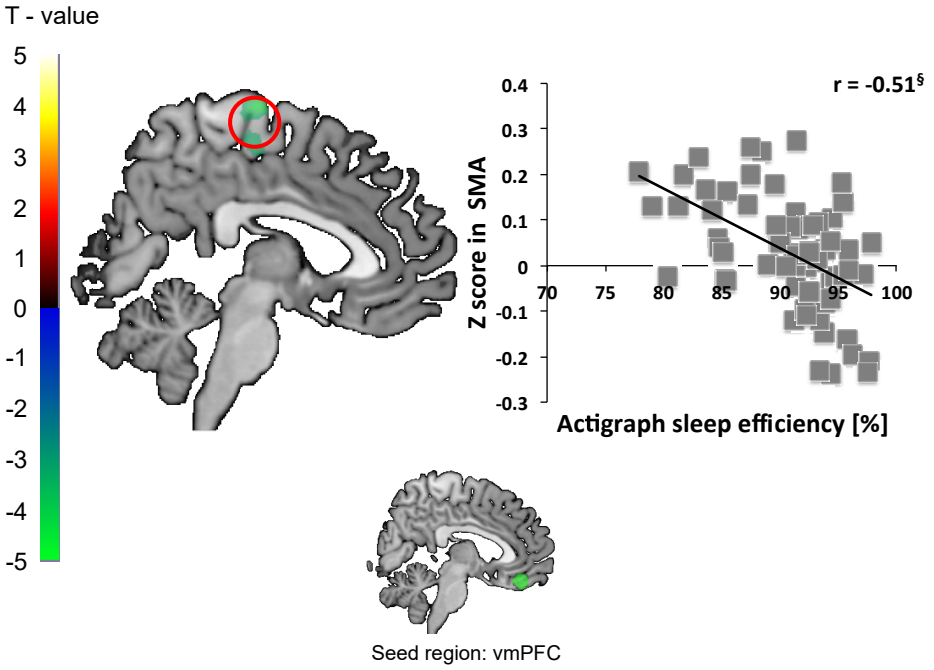

Figure S3. Actigraph sleep efficiency (SE) predicts lesser rsFC between the vmPFC seed and the supplementary motor area (SMA) and correlates negatively with extracted vmPFC-SMA mean Z-values. § $p < 0.001$.

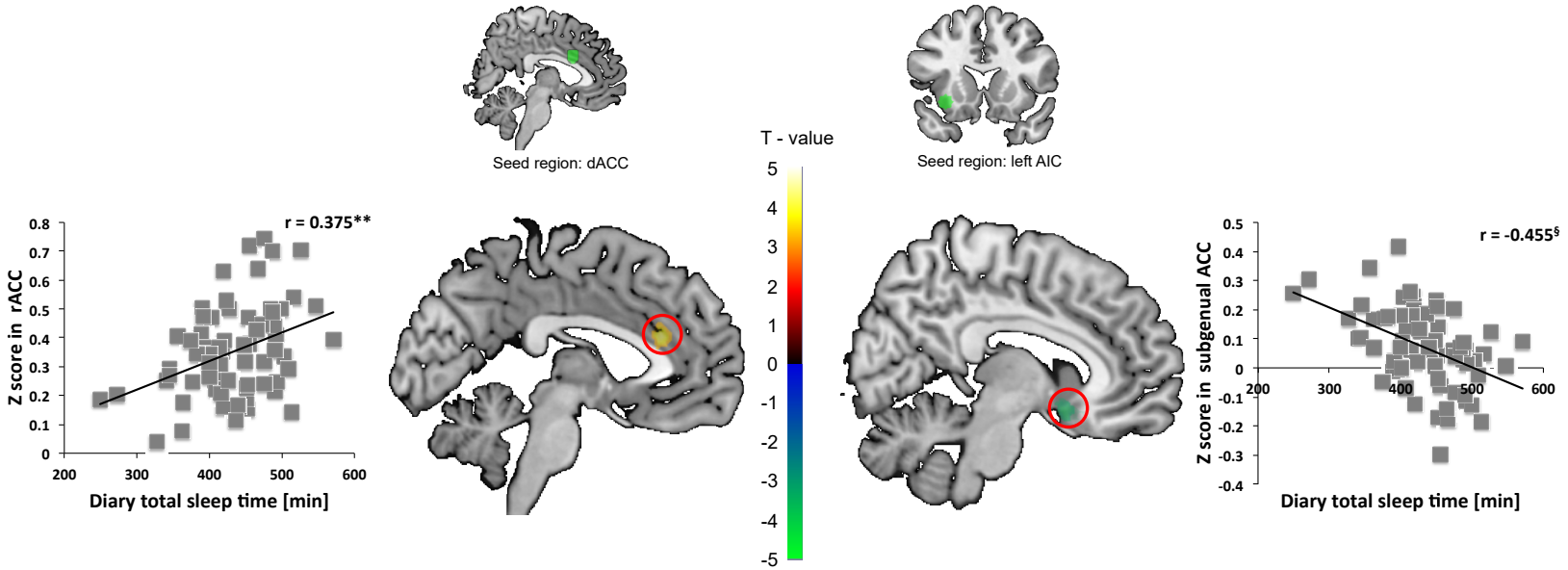

Figure S4. Diary total sleep time (TST) predicts greater rsFC between the dACC seed and rostral ACC (rACC) and correlates positively with extracted dACC-rACC mean Z-values *(left panel).* TST predicts lesser rsFC between the left AIC seed and the subgenual ACC (sgACC) and correlates negatively with extracted AIC-sgACC mean Z-values *(right panel).* ** p< 0.01; § p < 0.001.

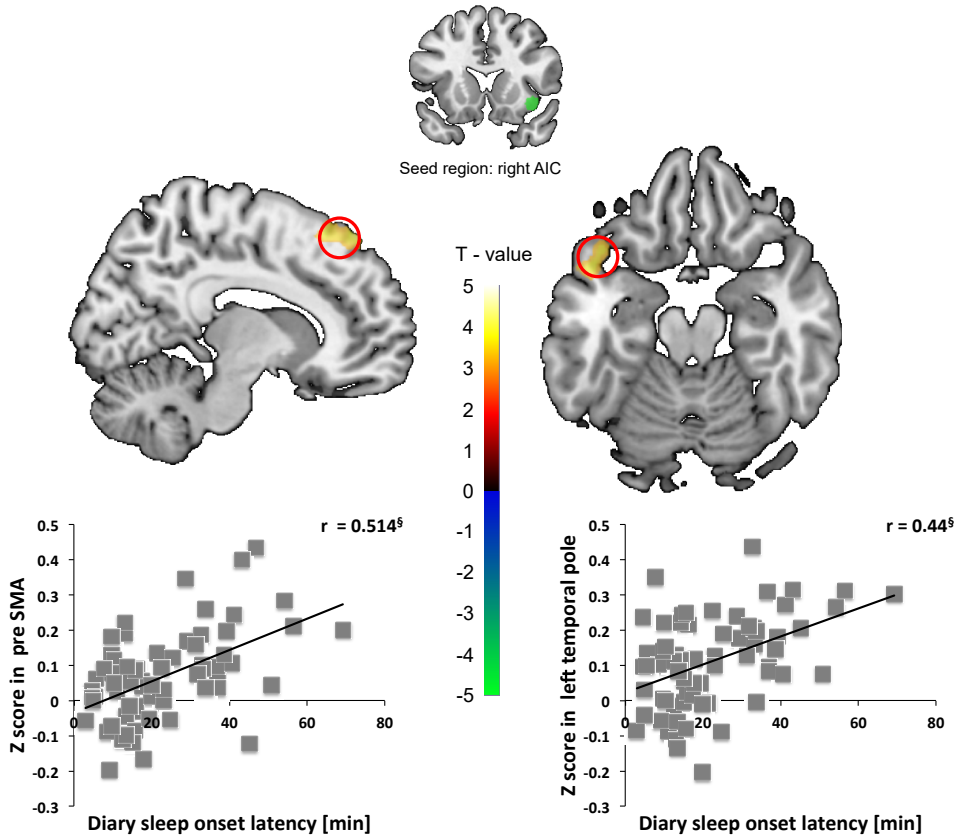

Figure S5. Diary sleep onset latency (SOL) predicts greater rsFC between the right AIC seed and the pre-supplementary motor cortex (pre-SMA) and correlates positively with extracted AIC-pre-SMA mean Z-values *(left panel).* SOL predicts more rsFC between the left AIC seed and the left temporal pole and correlates positively with extracted AIC-temporal pole mean Z-values *(right panel).* § $p < 0.001$.

Supplementary References

1. Insana SP, Hall M, Buysse DJ, Germain A (2013): Validation of the Pittsburgh Sleep Quality Index Addendum for posttraumatic stress disorder (PSQI-A) in U.S. male military veterans. *J Trauma Stress*. 26:192-200.

2. Stocker RPJ, Khan H, Henry L, Germain A (2017): Effects of Sleep Loss on Subjective Complaints and Objective Neurocognitive Performance as Measured by the Immediate Post-Concussion Assessment and Cognitive Testing. *Arch Clin Neuropsychol*. 32:349-368.

3. AASM (2005): *The International Classification of Sleep Disorders, Second Edition (ICSD-2).* Darien, IL: American Academy of Sleep Medicine.

4. APA (2000): *Diagnostic and Statistical Manual of Mental Disorders DSM-IV-TR Fourth Edition (Text Revision).* Washington, DC: American Psychiatric Association.

5. Iber C (2007): The AASM manual for the Scoring of Sleep and Associated Events: Rules Terminology and Technical Specifications. Westchester, IL: American Academy of Sleep Medicine.

6. Kales A, Rechtschaffen A. (1968): A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. California, LA: Brain Information Service/Brain Research Institute.

7. Whitfield-Gabrieli S, Nieto-Castanon A (2012): Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connet*. 2:125-141.