Sleep, regional grey matter volumes, and psychological functioning in adolescents

Adolescent sleep, brain structure, and function

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Summary

Changing sleep rhythms in adolescents often lead to sleep deficits and increased variability in sleep schedules. The adolescent brain, and in particular the rapidly developing structures involved in emotional control, are vulnerable to external and internal factors. In our previous study in adolescents at age 14, we observed a strong relationship between weekend sleep schedules and regional medial prefrontal cortex grey matter volumes. Here, we aimed to assess whether this relationship remained in this group of adolescents of the general population at the age of 16 (n=101; mean age 16.8 years; 55% girls). We further examined grey matter volumes in the hippocampi and the amygdalae, calculated with voxel-based morphometry. In addition, we investigated the relationship between regional grey matter volumes with psychological functioning. Sleep was assessed with self-reports and psychological functioning with the Strengths and Difficulties Questionnaire and tests on working memory and impulsivity. Later weekend wake-up times were associated with smaller grey matter volumes in the medial prefrontal cortex and the amygdalae, and greater weekend delays in wake-up time were associated with smaller grey matter volumes in the right hippocampus and amygdala. The medial prefrontal cortex region mediated the correlation between weekend wake up time and both externalising and internalising symptoms. Paying attention to regular sleep habits during adolescence could act as a protective factor against the emergence of psychopathology via enabling favourable brain development.

Keywords: Magnetic Resonance Imaging, Neurocognition, Prefrontal Cortex, Child and Adolescent Psychiatry

Abbreviations: Anatomical Automatic Labeling (AAL); Alcohol Use Disorders Identification Test (AUDIT); Cambridge Automated Neuropsychological Test Battery (CANTAB); Family Wise Error (FWE); Magnetisation Prepared Rapid Acquisition Gradient Echo (MPRAGE); Medial Prefrontal Cortex (mPFC); Montreal Neurologic Institute (MNI); functional Magnetic Resonance Imaging (fMRI); Region Of Interest (ROI); Statistical Parametric Mapping (SPM); Strengths and Difficulties Questionnaire (SDQ); Voxel-Based Morphometry (VBM); Weekday (WD); Weekend (WE);

Introduction

Sleep problems and psychiatric disorders increase sharply hand in hand during adolescence, but our understanding of the potential neurobiological links between them is only emerging (Suleiman et al., 2017). Late sleep, sleep deprivation, and social jet lag (the variability of sleep patterns between weekdays and weekends) have all been associated with a broad range of negative mental health consequences, including increased depressive and anxiety symptoms, increased risk-taking behaviours, as well as deteriorated executive functions (Merikanto et al., 2017; Owens, 2014; Smaldone et al., 2007). Furthermore, sleep disturbances seem to precede the onset of diverse psychiatric disorders (Tesler et al., 2013).

These studies support the theory that unhealthy sleep habits could affect the developing adolescent brain structure and thereby increase the vulnerability to various kinds of psychopathologies, but few studies on the relationship between adolescents' sleep habits and brain grey matter volumes have been published to date. In a sample of maltreated teenagers, reduced sleep efficiency was recently found to correlate with reduced GMV in hippocampus, inferior frontal gyrus and insula, suggesting that sleep might mediate the negative impact of adverse life events on brain morphology (Teicher et al., 2018). In a mixed sample of children and adolescents, weekday time in bed was found to correlate with regional grey matter volumes of the bilateral hippocampi and the dorsolateral prefrontal cortex (Taki et al., 2012). In our previous study of 14-year-old adolescents, we found late sleep during the weekend and short sleep during the week to be associated with smaller regional grey matter volumes, particularly in the medial prefrontal cortex (mPFC). In addition, there was a correlation between mPFC GMV and school performance (Urrila et al, 2017). Since sleep characteristics and brain morphology undergo

constant changes though adolescence (Hagenauer et al., 2009; Gied et al., 2009), it is important to study their interconnections repeatedly at different points of development.

The mPFC exerts an inhibitory top-down control of subcortical structures (S. S. Cho et al., 2013). Poor sleep and eveningness-prone or irregular sleep rhythms can negatively affect adolescents' emotion regulation, reward-related processing, and impulse inhibition by influencing the mPFC (Forbes et al., 2010; Galvan, 2010, Tashijan et al., 2017) as well as the amygdala and the hippocampus (Kreutzmann et al., 2015; Motomura et al., 2017; O'Byrne, et al., 2014; Yoo et al., 2007, Robinson et al., 2018). These structures have also been implicated in the etiology and maintenance of psychiatric disorders (Hoogman et al., 2017; Hulvershorn et al., 2011). Studying the effects of sleep especially on the mPFC, the amygdala, and the hippocampus would thus crucially contribute to understanding the development of psychopathology during adolescence. Our general hypothesis is that adolescents' sleep patterns affect brain regional grey matter volumes, which in turn lead to lower psychological functioning or even mild psychopathology. Understanding the trajectories that lead toward psychiatric disorders as early as possible in development would allow us to develop effective intervention and prevention strategies.

Aims of the study

In this follow-up study we aimed to assess whether our previous findings on the correlation between adolescents' sleep habits and brain grey matter volumes remained present at the age of 16, and to extend these findings by examining their relationships with psychological functioning.

Methods

Participants

Participants were recruited from schools near Paris, France, based on their age and absence of any major somatic condition. Written consent was obtained from all subjects in this study. The study was approved by the regional ethics committee (Comité de Protection des Personnes [CPP] Ile-de-France 7). The adolescents participated in a larger multi-centre study (http://www.imagen-europe.com/en/the-imagen-study.php) (Schumann et al., 2010) at age 14 (baseline), and were followed up at age 16. Only the French adolescents were assessed for their sleep habits and were thus eligible for this study. Details of the sample at baseline have been previously reported (Urrila et al, 2017). This study focuses on the sample at age 16, at which time point written informed assent and consent to study participation were obtained from a total of 138 adolescents and their parents, respectively. We excluded participants who did not complete the sleep questionnaires, those whose Magnetic Resonance brain images did not pass the quality control of the raw or the segmented images, participants with brain lesions, and those with marked alcohol consumption (alcohol use disorders identification test (AUDIT) total score >7 (Saunders et al., 1993)). In this study, we present data from the remaining 101 adolescents (mean age =16.83 years, SD=0.61; 56 girls; Table 1). At the time of the study, none of the participants were followed in the psychiatric care system and all participants were attending school regularly.

Table 1.Clinical, behavioral characteristics, and brain volumes in community adolescents at age 16

Variable		Mean or %	SD
Demographic variables	Age (years)	16.83	0.61
	Gender	55 % (n=56) female	
Sleep variables (N=101)	Wake up time WD	7:03	0:43
	Wake up time WE	10:01	1:15
	Bedtime WD	22:42	0:43
	Bedtime WE	00:05	1:10
	Difference wake up time WE-WD	2:59	1:17
	Difference bedtime WE-WD	1:23	1:04
	Time in bed WD	8:18	0:59
	Time in bed WE	9:56	1:00
	Difference time in bed WE-WD	1:40	0:59
Performance scores (N=82)	Delay discounting large amounts	-2.25	0.68
	Delay discounting medium amounts	-2.03	0.73
	Delay discounting geomean	-2.06	0.66
	Spatial working memory	7.59	7.80
Behavioural problems (N=74)	SDQ internalising	4.34	2.40
	SDQ externalising	7.36	2.68
Global brain measures (N=101)	Total grey matter volume	742.02	68.99
	Total white matter volume	477.22	49.28
	Total CSF	395.55	40.89
	Volume scaling factor	1.32	148.21

WD = weekday; WE = weekend; CSF = cerebrospinal fluid.

Sleep assessments

Sleep habits were assessed by asking the adolescents their usual bedtimes and wake up times during weekdays (WD) and weekends (WE). Time in bed was approximated by calculating the number of hours between bedtime and wake up time, separately for WD and WE. WE delay in sleep timing ("social jet lag") and weekly sleep debt was defined as the difference between weekday and weekend in sleep times and time in bed.

Psychological functioning

Symptom assessment was performed using the Strengths and Difficulties Questionnaire (SDQ) (R. Goodman, 1997), a child and adolescent self-report questionnaire used to identify internalising and externalising problems. It consists of 25 items, five items for each subscale: conduct problems, hyperactivity, emotional problems, peer problems, and prosocial behaviour. The items are scored 0 to 2, reflecting the answers "not true", "somewhat true" or "certainly true". The scores are then summed, generating five scale scores ranging from 0 to 10 with higher scores reflecting more problems in the first four scales or more prosocial behaviour in the last scale. In low-risk samples, conduct problems and hyperactivity are best combined into an 'externalising' subscale, and emotional problems and peer problems into an 'internalising' subscale (A. Goodman et al., 2010).

Other behavioural measures included the Kirby Delay-Discounting Questionnaire (Kirby et al., 1996), a monetary choice questionnaire assessing cognitive impulsivity through delay discounting by having participants choose between smaller immediately available rewards and

larger delayed rewards, and the spatial working memory task, a subtest of the computer-administrated Cambridge Automated Neuropsychological Test Battery (CANTAB) measuring executive functioning (Cognition, 2013). The spatial working memory test has been widely used in typically developing and clinical populations of children and adolescents (Faridi et al., 2015). The error score was used as the outcome variable.

MRI data acquisition and processing

MRI was performed on a 3T scanner (Siemens Trio). High-resolution anatomical MR images were obtained using a standardised 3D T1-weighted magnetisation prepared rapid acquisition gradient echo (MPRAGE) sequence based on the ADNI protocol (http://adni.loni.usc.edu/methods/mri-analysis/mri-acquisition/). The parameters were as follows: repetition time=2,300 ms, echo time=2.8 ms, flip angle=8°, 256x256x170 matrix, 1.1x1.1x1.1 mm voxel size.

The images were processed using Statistical Parametric Mapping 8 (SPM 8) using Voxel-Based Morphometry (VBM) (Ashburner et al., 2000). The "unified segmentation" algorithm was used to normalise and segment the T1-weighted images into grey matter, white matter and cerebrospinal fluid. Home-made tissue probability maps were used instead of the standard template of SPM based on fully grown and developed brains of adults, who have larger brain volumes. The modulated images were smoothed with a 10-mm full-width at half-maximum isotropic Gaussian kernel. Head size was measured by the volume scaling factor, which is based on the affine transformation performed during spatial normalisation (https://surfer.nmr.mgh.harvard.edu/fswiki/eTIV).

Statistical analyses

The outcome measures for the Kirby Delay-Discounting Questionnaire were calculated using an automated calculator (Kaplan et al., 2014). A logistic regression that allowed for continuous estimates of k (Wileyto et al., 2004), and a logarithmic transformation to normalise the distribution of k-values were applied. The geometric mean of the k-values is bounded by the lowest implied indifference k-value at which subjects chose the larger delayed reward and the highest indifference k-value at which the subject choses the immediate reward.

Group comparisons for socio-demographic and clinical data, and global brain volumes were performed within the framework of the general linear model (GLM) using R software (http://cran.r-project.org). Voxel-wise comparisons were carried out within the GLM framework of SPM8. Sleep variables were the main factors while age, gender and volume scaling factor were entered as confounding variables.

A priori masks were used to examine the hippocampus, amygdala, and medial prefrontal region with a region of interest (ROI) approach. The hippocampus and amygdala masks were created with the Anatomical Automatic Labeling (AAL) atlas in SPM. The medial prefrontal region was marked with a 10 mm radius from coordinates [-2, 34, -14] taken from our previous results on an analogous sample at age 14, which indicated a strong relationship between sleep habits and GMV in this region (Urrila et al, 2017). Subsequently, it was investigated whether psychometrics measures (spatial working memory, delay discounting, internalising and externalising problems) were associated with grey matter volumes in these regions of interest.

At the voxel-level, statistical significance was set to $p<0.05$ FWE (Family Wise Error) corrected for multiple comparisons. Brain locations were reported as x, y, and z coordinates in Montreal Neurologic Institute (MNI) space.

In addition, it was examined whether the sleep times that were significant in the ROI VBM analyses, were correlated with psychological functioning. Subsequently, causal mediation analyses were performed to determine whether the grey matter clusters could mediate the relation between sleep and psychological functioning variables. These analyses were performed if there was a significant relationship between sleep and psychological functioning variables and GMV of the same ROI.

The mediation analyses were performed with an algorithm using a set of general linear models to derive the mediation and direct effects from the total effect (Imai et al., 2010). The psychological functioning measures were entered as dependent factor, and sleep variables as independent factor within a linear regression model. For the mPFC ROI analysis, raw volume was extracted from the smoothed, normalized, and modulated images and entered as mediator variable. The hippocampus and amygdala volumes were extracted from the same images using the AAL masks instead of the significant clusters in order to better approximate the complete volumes. Volume scaling factor and age were entered as confounding variables. Gender was added for analyses without a gender interaction between sleep and psychological functioning scores. If there was a gender effect, we performed the mediation analysis separately for boys and girls. This mediation model was performed using 5000 Monte Carlo draws for nonparametric bootstrap. In causal

mediation analysis, a significant mediating effect is defined as a 95% confidence interval that does not include zero.

Results

Participant characteristics

On average, boys went to bed later during the week (boys = 22.53 ± 0.47, girls = 22.33 ± 0.33; $F(99)=2.41$, $p=0.02$) and woke up later during the weekend (mean wake up time: boys=10.23 ± 1:17, girls = 9:44 ± 1:10; $F(99)=2.63$, $p=0.01$) as compared to girls. There were no gender differences in any of the other sleep variables, nor in delay discounting, spatial working memory or internalising and externalising problems (Table 1).

Clinically relevant externalising symptoms (SDQ score ≥10), were present in 15 out of 81 adolescents (18.3%) and 10 out of 81 (12.2%) showed clinically relevant internalising symptoms (score ≥8).

Sleep and regional grey matter volumes

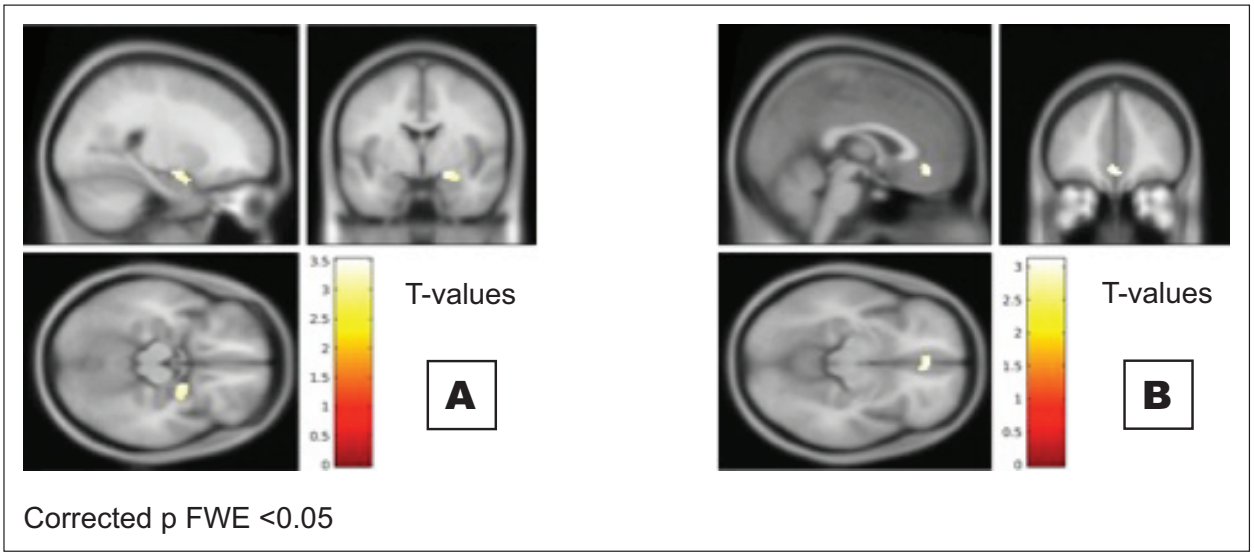
WE wake up time correlated negatively with GMV in the bilateral amygdalae and the mPFC (Table 2, Figure 1). WE delay in wake up time ('social jet lag') correlated negatively with GMV in the right hippocampal region and the right amygdala (Table 2). No other statistically significant correlations between sleep variables and regional grey matter volumes were found.

Table 2. Grey Matter Volume correlations with sleep measures in community adolescents at age 16 using regions-of-interest

Brain region	**Cluster**		**Peak**				
					coordinates		
	Size	p FWE	T-values	p FWE	x	y	z
Wake up weekend							
Amygdala							
Amygdala R	237	0.018	3.51	0.011	21	-6	-12
Amygdala L	3	0.047	3.04	0.041	-12	-3	-15
mPFC							
	203	0.029	3.11	0.02	2	42	-11
Variability wake up time							
Amygdala							
Amygdala R	51	0.0350	3.23	0.025	20	-3	-12
Hippocampus							
Hippocampus R	37	0.028	4.23	0.008	20	-25	-23

Size = number of voxels in cluster; MNI = Montreal neurological Institute coordinates in millimeters; R = right; L = left. MNI coordinates are given for the voxel of maximal statistical significance. Statistics significant at the p<0.05 FWE level; analyses are covaried for volume scale factor,age, and gender.

Figure 1. Later wake up time during the weekend was associated with reduced grey matter volumes in the A) left amygdala and B) mPFC



Sleep and psychological functioning

WE wake up time and WE delay in waking up correlated with measures of impulsivity as well as externalizing and internalizing problems (Table 3).

Table 3. Correlations between sleep variables and psychological functioning in community adolescents at age 16

	Wake up time WE	**Variability wake up time**
Delay discounting large	r=0.319**	r=0.292**
Delay discounting medium	r=0.217	r=0.194
Delay discounting geomean	r=0.265*	r=0.247*
SDQ externalising	r=0.511**	r=0.643**
SDQ internalising	r=-0.229	r=-0.327**
Spatial working memory errors	r=0.093	r=0.116

**=p<0.05, **=p<0.01, ***=p<0.001*

Grey matter volumes and psychological functioning

Smaller grey matter volumes in the mPFC region were associated with increased delay discounting (T=3.28, p<0.01) as well as internalising (T=4.99, p<0.001) and externalising (T=6.03, p<0.001) problems (Table 4). Additionally, smaller grey matter volumes in the amygdala (most significant cluster T=10.57, p<0.001), and hippocampal regions (T=11.01, p<0.001) were associated with internalising problems. There were no other significant relations between grey matter volumes and psychological functioning measures (Table 4).

Table 4. Grey matter volumes correlations with psychological functioning in 138 community adolescents at age 16

Psychological functioning	**Amygdala**		**Hippocampus**		**mPFC**	
	T-value	x,y,z	T-value	x,y,z	T-value	x,y,z
Delay discounting large	-	-	-	-	2.97*	-6,42,-15
Delay discounting medium	-	-	-	-	3.29*	-8,40,-17
Delay discounting geomean	-	-	-	-	3.28**	-8,40,-17
Spatial Working Memory	-	-	-	-	-	-
SDQ externalising	-	-	-	-	6.03***	-2,44,-14
SDQ internalising	10.57*** 4.40***	-30,-3,-29 36,0,-24	11.01*** 7.34*** 6.13***	-28,-7,-32 21,-45,-3 40,-15,-23	4.99***	4,30,-8

*- =no significant results, MNI coordinates and T-values are given for the voxel of maximal statistical significance, *=p<0.05, **=p<0.01, ***=p<0.001*

<u>*Mediation analyses*</u>

Causal mediation analyses showed that variability in mPFC volumes accounted for 30.5% of the total effect between wake up time during the WE and externalising problems, and for 65.2% of the total effect between wake up time during the WE and internalising problems (Table 5).

Table 5. Causal mediation analysis on the relationship between sleep variables and behavioural problems with medial prefrontal cluster volumes as mediator

		Wake up time WE – mPFC - SDQ externalising	**Wake up time WE – mPFC -SDQ internalising**
Mediation effect	Point estimate	0.393**	-0.354***
	95% CI	0.138-0.720	-0.55
Direct effect	Point estimate	0.9**	-0.176
	95% CI	0.396-1.393	-0.998
Total effect	Point estimate	1.293**	-0.53*
	95% CI	0.776-1.817	-1.004
Proportion total effect via mediation	Point estimate	0.305***	0.652*
	95% CI	0.113-0.579	0.149-3.419

Point estimate, estimate of the size of the effect; 95% CI, 95% confidence interval of the point estimate

**=p<0.05, **=p<0.01, ***=p<0.001*

Discussion

The present findings confirm and extend our previous findings that sleep patterns in adolescents from the general population correlate with regional brain grey matter volumes and psychological functioning. In particular, later WE wake up times were associated with smaller GMV in the mPFC and the amygdalae. Furthermore, a greater WE delay ('social jetlag') was associated with lower grey matter volume in hippocampus and amygdala regions. Regional grey matter volumes of the mPFC, amygdala, and hippocampus were associated with cognitive impulsivity and

behavioural problems. Causal mediation analyses showed that GMV of the mPFC mediated the relationship between weekend wake up time and externalising as well as internalising problems.

The results corroborate our previous findings in the sample at age 14, which showed an association between grey matter volumes in the mPFC region and weekend wake-up time (Urrila et al, 2017). This could indicate that weekend wake up times affect mPFC grey matter maturation throughout adolescence. The impact of sleep on the brain might be particularly significant and long-lasting during adolescence because the brain is still in development during this phase (Casey et al., 2008).

The relationship between WE delay ('social jetlag') and grey matter volumes could result from repeated challenges to the circadian regulatory mechanisms (Taylor et al., 2008). This can be compared with having regular *mini-jetlags*. A study on the short term effects of jetlag found reduced resting state activity in the mPFC and the left parahippocampal gyrus as well as other default mode network regions (Coutinho et al., 2015). In accordance with our finding of the association between GMV in the right hippocampal region and a difference in wake-up time, a study on chronic functional jetlag reported a relationship between sleep patterns and right temporal lobe atrophy, which included the parahippocampal gyrus (K. Cho, 2001). An alternative explanation is that later WE wake up times might reflect a late-prone biological rhythm rather than a social jetlag. Chronotype has recently been found to correlate with local GMVs and cortical thickness in a small sample of adult men (Rosenberg et al., 2018). However, late bedtimes, another characteristic of a late chronotype, were not related to the volumes of the regions of interest in our study.

The results of the current study further show that WE delay and mPFC grey matter volumes are both significantly associated with cognitive impulsivity as well as internalising and externalising problems. Since the mPFC is an important brain area for regulation of the limbic structures, it is plausible that impaired development of this structure due to unhealthy sleep habits can lead to cognitive and emotional control deficits (Alexander et al., 2011; Motomura et al., 2017; Yoo et al., 2007). This is the first study to show a mediating role of grey matter volumes in the mPFC in the relationship between sleep and internalising and externalising symptoms in healthy adolescents.

Internalising symptoms were also associated with smaller GMV in bilateral amygdala and parahippocampal regions. This finding is in accordance with previous research associating abnormalities in the amygdala, the parahippocampus, and the mPFC with emotional difficulties (Bootzin et al., 2011). Internalising symptoms are predictors of later educational underachievement, mental disorders, and impaired personal relationships (Colman et al., 2007). Externalising symptoms and impulsivity are both related to a host of maladaptive behaviours, including drug abuse, gambling, and poor academic and work success (Colman et al., 2009; Daugherty et al., 2010).

Bedtimes and time in bed were not significantly correlated with grey matter volumes at age 16. This is in contrast with our previous study in 14-year-old adolescents and a study by Taki and collaborators (Taki et al., 2012; Urrila et al, 2017), where weekend bedtimes were associated with smaller mPFC grey matter volumes, but the sample of these studies consisted of younger

children, who have not yet developed a delay in circadian rhythms and whose time in bed is still much more regulated by the parents. Therefore, time in bed during the week might be a more important factor in earlier brain developmental phases, while sleep timing and regularity in sleep timing might be more important later during adolescence. The current results also do not provide evidence of negative effects of sleep debt, as measured by time in bed difference between WD and WE, on the adolescent brain. It must be noted, however, that sleep debt could affect brain regions that were not examined in the current study.

The findings could be confounded by the developmental stage of the participants. However, we used a relatively large sample, drawn from the general population, with a very narrow age range, making the results less likely to be influenced by confounding factors associated with age and sampling bias. A limitation of this study is the use of self-report questionnaires to measure sleep and behavioural problems. Sleep diaries and polysomnographic or actigraphic recordings would have provided a more objective measure of sleep, and including parental reports could have completed the picture of behavioural problems in adolescents. As this is a cross-sectional study, it is difficult to see to which degree sleep habits are a cause or a consequence of reduced grey matter volumes. We theorise that sleep rhythms influence brain development, which in turn causes lower functioning. The results of the causal mediation analyses would also favour this interpretation. However, reduced grey matter volumes might be a pre-existing condition that contributes to cognitive impulsivity and behavioural problems as well as the development of specific sleep patterns. Lastly, the ROI approach does not allow exploration of effects of sleep brain on regions other than the chosen ROIs.

Conclusion

Overall, the present findings are consistent with and extend our previous report in 14 year-old adolescents, suggesting that the negative impact of irregularity of sleep schedules on the adolescent brain can result in reduced ability to regulate emotions and impulses. This highlights the importance of sleep habits in adolescents and supports the recommendation to keep variability in sleep times to a minimum in order to reduce the risk of psychiatric morbidity.

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