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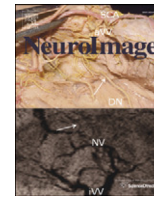

Sleep duration during weekdays affects hippocampal gray matter volume in healthy children

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ABSTRACT

Sleep is essential for living beings, and sleep loss has been shown to affect hippocampal structure and function in rats by inhibiting cell proliferation and neurogenesis in this region of the brain. We aimed to analyze the correlation between sleep duration and the hippocampal volume using brain magnetic resonance images of 290 healthy children aged 5–18 years. We examined the volume of gray matter, white matter, and the cerebrospinal fluid (CSF) space in the brain using a fully automated and established neuroimaging technique, voxel-based morphometry, which enabled global analysis of brain structure without bias towards any specific brain region while permitting the identification of potential differences or abnormalities in brain structures. We found that the regional gray matter volume of the bilateral hippocampal body was significantly positively correlated with sleep duration during weekdays after adjusting for age, sex, and intracranial volume. Our results indicated that sleep duration affects the hippocampal regional gray matter volume of healthy children. These findings advance our understanding of the importance of sleep habits in the daily lives of healthy children.

Introduction

Although the function of sleep remains debatable, sleep has been associated with the function and structure of the hippocampus. For example, one major theory about the function of sleep proposes that memory consolidation occurs predominantly during sleep, when the hippocampus sends information from memory to the neocortex for permanent storage (Axmacher et al., 2009). Additionally, sleep deprivation was shown to reduce the proliferation of cells and to suppress neurogenesis in the hippocampus of rats (Guzman-Marin et al., 2003, 2005). Even human patients with primary insomnia showed significant reductions in hippocampal volume (Riemann et al., 2007). Although the correlation between sleep and the hippocampus has been elucidated in studies on animals and on human patients and although the influence of chronic sleep loss on the cognition of healthy children has been examined (Jan et al., 2010), the correlation between sleep and the hippocampal volume of healthy children has not yet been clarified. Understanding the correlation between sleep and the hippocampus of children is especially important to identify the sleeping habits associated with the development of a healthy brain and sound cognition. Therefore, we aimed to analyze the correlation between sleep duration and the hippocampal gray matter volume using brain magnetic resonance images of 290 healthy children aged 5–18 years by applying voxel-based morphometry (VBM). This approach enabled global analysis of brain structure and without bias towards any specific brain region while permitting the identification of potential differences or abnormalities in brain structures (Ashburner and Friston, 2000). We hypothesized that there would be a significant positive correlation between sleep duration and the hippocampal gray matter volume in healthy children.

Materials and methods

Participants

All subjects were healthy Japanese children and the detail of the recruitment is written elsewhere (Taki et al., 2010). Briefly, we collected brain MR images from 290 subjects (145 boys, 145 girls; age range, 5.6–18.4 years) who did not have any history of malignant tumors, head traumas with a loss of consciousness lasting over 5 min, developmental disorders, epilepsy, psychiatric diseases, or claustrophobia. We announced that only right-handed children can participate in this study in an advertisement used in the subject recruitment, and also confirmed that all subjects were right-handedness using the self-writing questionnaire "Edinburgh Handedness Inventory" (Oldfield, 1971). We measured full-scale intelligence quotients (IQ) by having trained

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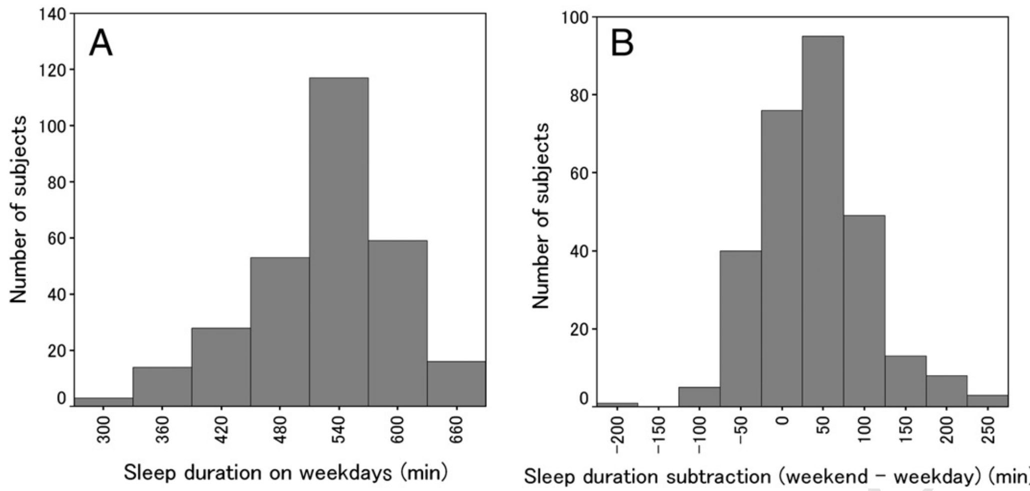

Fig. 1. Histograms showing duration of sleep on weekdays (A) and duration of sleep on weekends minus that on weekdays (B) for all subjects.

examiners administer the Japanese version of the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) (Fujita, et al., 2006) to subjects aged 16 years or older or the Wechsler Intelligence Scale for Children-Third Edition (WISC-III) to subjects younger than 16 years of age (Azuma, et al., 1998). The duration of sleep during weekdays and that during weekends was collected using self-answering questionnaire, and the duration of sleep during weekdays and the duration of sleep during weekdays subtracted from that during weekends for all subjects are shown in Fig. 1. We did not collect subjective sleep scales, such as sleepiness, from questionnaires or visual analog scales. Instead, we regarded duration of sleep during weekdays subtracted from that during weekends as the subjective scale of sleep. The characteristics of the subjects are shown in Table 1. As per the Declaration of Helsinki (1991), written informed consent was obtained from each subject and his/her parent prior to MR image scanning after a full explanation of the purpose and procedures of the study was provided. Approval for these experiments was obtained from the Institutional Review Board of Tohoku University.

Image acquisition

All images were collected using a 3-T Philips Intera Achieva scanner. Three-dimensional, high-resolution, T1-weighted structural images were collected using a magnetization-prepared rapid gradient-echo (MPRAGE) sequence. The parameters were as follows: 240×240 matrix, TR = 6.5 ms, TE = 3 ms, TI = 711 ms, FOV = 24 cm, 162 slices, 1.0-mm slice thickness, and scan duration of 8 min and 3 s.

Image analysis

Voxel-based morphometry (VBM) with Diffeomorphic Anatomical Registration using Exponentiated Lie Algebra (DARTEL) (Ashburner, 2007) was conducted. DARTEL has been shown to produce a more accurate registration than the standard VBM procedure (Klein et al., 2009) and enables increased sensitivity to findings such as the correlation between gray matter volume and several measures such as age. After image acquisition by MRI, all T1-weighted MR images were analyzed using Statistical Parametric Mapping 8 (SPM8) (Wellcome Department of Cognitive Neurology, London, UK) in Matlab (Math Works, Natick, MA, USA). First, the "New Segmentation" algorithm from SPM8 was applied to every T1-weighted MR image to extract tissue maps corresponding to gray matter, white matter, and cerebrospinal fluid (CSF). This algorithm, which is an improvement on the unified segmentation algorithm (Ashburner and Friston, 2005), uses a Bayesian framework to iteratively perform the probabilistic tissue classification and spatial non-linear deformation in terms of Montreal Neurological Institute (MNI) space. Although we were interested only in the probabilistic tissue segmentation at this point, this new Bayesian segmentation and warping algorithm, which included an improved set of tissue priors (Ashburner and Friston, 2009) for regularization, increased the robustness and accuracy of the segmentation over that of previous standard VBM algorithms. This step allowed us to obtain probability maps of the three aforementioned tissues for each subject and to have them all rigidly registered by ignoring the non-rigid part of the warping to a temporary common space (which happened to be as close to the MNI space as can be reached by a rigid transformation) because the subsequent DARTEL step focused on estimating the "pure non-linear" component of the transformation and used rigidly registered tissues as input. Next, these 290 segmented tissue maps were used to create a customized, more population-specific template using the DARTEL template-creation tool (Ashburner, 2007). DARTEL estimates the best set of smooth deformations working from every subject's tissues to their common average, applies the deformations to create a new average, and then reiterates the process until convergence is achieved. The

Table 1
Characteristics of subjects.

	Boys ($n = 144$)	Girls ($n = 146$)	P
Age [years], (mean ± SD, range)	11.0 ± 2.87, 5.6–17.1	11.6 ± 3.35, 5.8–18.4	0.100[a]
Full-scale IQ, (mean ± SD, range)	104.3 ± 13.24, 77–137	100.9 ± 11.13, 71–128	0.019[b]
Socioeconomic status[d], (mean, range)	4.04, 1–7	3.85, 1–7	0.252[c]
Sleep duration [min] (mean ± SD, range)	519.0 ± 69.4, 300–660	510.0 ± 75.1, 300–660	0.289[a]
Sleep subtraction[e] [min] (mean ± SD, range)	23.6 ± 61.8, −90–240	52.3 ± 63.6, −205270	<0.001[a]

[a] Student's *t*-test.
[b] Welch's *t*-test.
[c] Mann–Whitney *U*-test.
[d] Socioeconomic status was classified as follows; annual income below 2 million yen, 1; 2–4 million yen, 2; 4–6 million yen, 3; between 6 and 8 million yen, 4, 8–10 million yen, 5; 10–12 million yen, 6; more that 12 million yen, 7.
[e] Sleep subtraction was calculated by subtracting the duration of sleep during weekdays from that during weekends.

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smoothness and reversibility of the deformation are obtained from the diffeomorphic properties of DARTEL transformations. The template space was matched to the MNI space using an affine-only registration, which enabled us to match our images' custom coordinate space to the more standard MNI space (Bergouignan et al., 2009). We used a set of standard MNI tissues maps and a multivariate tissue-affinity-registration algorithm provided by SPM and DARTEL for that process. At the end of the process, each subject's gray matter map was warped using its corresponding smooth, reversible deformation parameters to transform it to the custom template space and then to the MNI standard space. We also computed the group means and variances of all these images to visually confirm that the process had operated correctly by searching for a low variance near major landmarks. The major advantage of creating a population-specific template on which to register the tissues is that this approach limits the amount of stretching of each image during the necessary step of spatial normalization. As described by Good et al. (2001), the warped gray matter images were then modulated by calculating the Jacobian determinants derived from the special normalization step and multiplying each voxel by the relative change in volume to obtain the gray matter volume. This modulation step was performed to correct for volume changes in nonlinear normalization. Finally, the warped modulated gray matter images were smoothed by convolving an 8-mm full-width at half-maximum isotropic Gaussian kernel. After completing these image analyses, we obtained smoothed modulated gray matter images to be used for the statistical analysis.

Statistical analysis

We used SPM8 for all statistical analyses. We performed multiple regression analysis, in which regional gray matter volume was used as a dependent variable, and age, sex, duration of sleep, and intracranial volume were used as independent variables to investigate the correlation between hippocampal regional gray matter volume and duration of sleep. Intracranial volume was calculated by summing the gray matter, white matter, and CSF volumes derived from the aforementioned imaging process. We performed region-of-interest (ROI) analysis by setting the ROI of the bilateral hippocampus using the "WFU_PickAtlas" (Lancaster et al., 2000; Maldjian et al., 2003) and performed small-volume correction within the ROI. We set the significance level at $p<0.05$ for the family-wise error rate.

Results

We found that the volume of the bilateral hippocampal body was significantly positively correlated with the duration of sleep during weekdays after adjusting for age, sex, and intracranial volume and after performing small-volume correction of the hippocampal ROI (left: $t=3.59$, $p=0.014$, family-wise error, corrected; right: $t=3.81$, $p=0.007$, family-wise error, corrected), as shown in Fig. 2. The whole-brain analysis showed that the duration of sleep during weekdays was substantially positively correlated with the regional gray matter volume of the bilateral hippocampal body (left: $t=3.59$, $p<0.001$, uncorrected; right: $t=3.81$, $p<0.001$, uncorrected) and the right dorsolateral prefrontal cortex ($t=3.95$, $p<0.001$, uncorrected) after adjusting for age, sex, and intracranial volume and using the liberal threshold ($p<0.001$, uncorrected; cluster size >100). Next, we subtracted the duration of sleep during weekdays from that during weekends. Although we found a significant negative correlation between the duration of sleep during weekdays and the difference between the duration of weekend and weekday sleep (partial correlation coefficient $[C]=-0.300$, $p<0.001$, adjusting for age

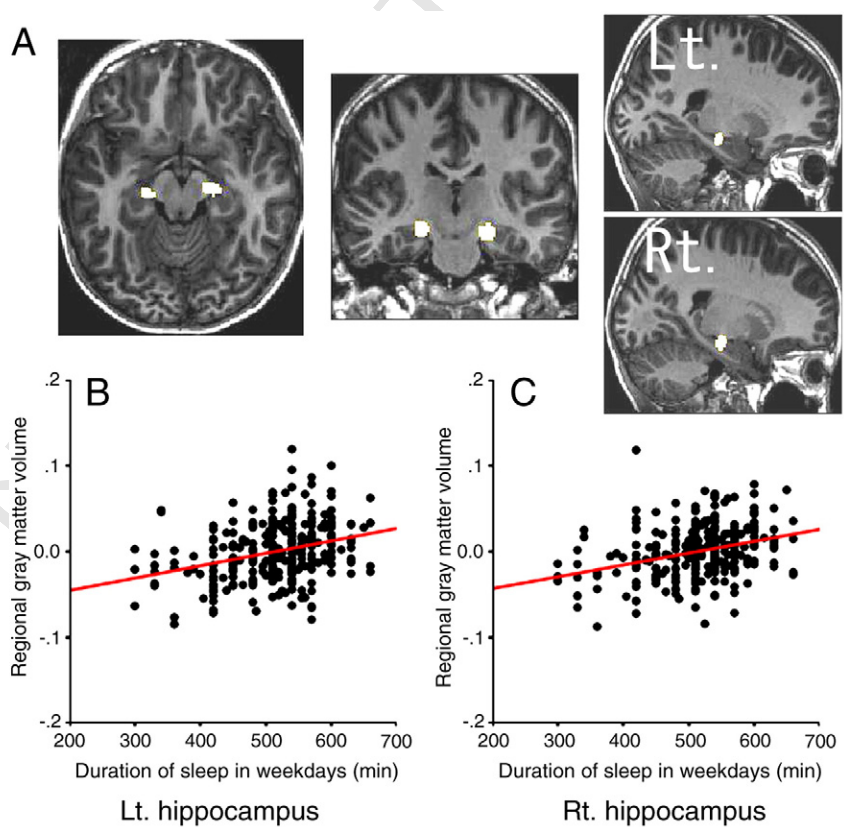

Fig. 2. Correlations between duration of sleep on weekdays and regional gray matter volume in the region-of-interest analysis of the bilateral hippocampus. (A) Gray matter regions showing significant positive correlations between duration of sleep on weekdays and regional gray matter volume according to axial, coronal, and sagittal views. (B) Correlations between duration of sleep on weekdays and regional gray matter volume in the left hippocampus. (C) Correlations between duration of sleep on weekdays and regional gray matter volume in the right hippocampus.

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and sex), no significant correlation between regional gray matter volume and this difference remained after adjusting for age, sex, and intracranial volume.

Discussion

We demonstrated that the regional gray matter volume of the bilateral hippocampus was significantly positively correlated with the duration of sleep during weekdays. Although the mechanisms underlying this significant positive correlation have not been clarified, the findings of several studies in rats and humans have supported these results. The generation of new neurons in dentate gyrus of the hippocampus has been confirmed in several mammals, including humans (Eriksson et al., 1998; Gould et al., 1999). However, sleep deprivation reduced the proliferation of cells in the dentate gyrus of the hippocampus (Guzman-Marin et al., 2003) and also suppressed neurogenesis in rats (Guzman-Marin et al., 2005). Even human patients with primary insomnia showed significantly smaller bilateral hippocampal volumes than did good sleepers after adjusting for age and sex (Riemann et al., 2007). Moreover, patients with obstructive sleep apnoea, a common sleep disorder, showed significant gray matter reduction in several regions such as the hippocampus (Canessa et al., 2011; Macey et al., 2002). In addition to these findings, the role of sleep may modulate synaptic contacts by downscaling synaptic strength to a baseline level that is energetically sustainable, which is beneficial for maintaining plasticity for a new environment and, as a result, learning and memory (Tononi and Cirelli, 2006). Specifically, sleep deprivation affects hippocampal activation and decreases memory performance (Van Der Werf et al., 2009; Yoo et al., 2007). Thus, sleep may be necessary, at the very least, for neurogenesis and for synaptic reorganization in the human hippocampus.

We did not find a significant correlation between regional gray matter volume and the difference between the duration of sleep during weekdays and that during weekends. A recent study proposed that the quality and the duration of sleep should be regarded as two separate domains – the subjective and objective, respectively – even though these domains do overlap to some extent (Dewald et al., 2010). As mentioned above, because the duration of sleep constitutes an objective aspect of sleep, we also analyzed the correlation between regional gray matter volume and the subjective (qualitative) aspect of sleep using the difference between weekday and weekend sleep duration. Our rationale was as follows. Given that school starts at about 8:30 AM on weekdays in Japan, weekday times of awakening are determined by school schedules; however, most Japanese children do not attend school during weekends. Thus, if the quality of sleep were low during the week, we would expect children to awaken later during weekends. For this reason, the subtraction process described above was employed to reflect the quality of sleep (a subjective aspect) during weekdays. The absence of a significant correlation between regional gray matter volume and the result of this calculation indicated that hippocampal volume is more crucial for the objective than for the subjective aspect of sleep. Thus, more sleep may be more beneficial for the hippocampus irrespective of the subjective aspects of sleep. However, we cannot conclude that excessive sleep would have a positive effect on hippocampal and cognitive functioning given that the number of the subjects demonstrating excessive amounts of sleep was rather small, as shown in Fig. 1. Indeed, the mean sleep duration of subjects in this study was 8.57 h, which approximates empirical evidence showing that children and adolescents require, on average, approximately 9 h of sleep per night (Mercer et al., 1998). Additionally, the mean difference between the duration of sleep during weekends and that during weekdays was 38 min in favor of weekends, which we evaluated as rather small. Thus, we believe that the duration of sleep obtained by the subjects in this study was generally appropriate, and we concluded that sufficient, but not excessive, sleep is beneficial for the hippocampus.

We found that the regional gray matter volume of the right dorsolateral prefrontal cortex had a substantial positive correlation with the duration of sleep during weekdays in the whole-brain analysis. Although we did not find the mechanism of the correlation, it is thought that maturational pattern of the dorsolateral prefrontal cortex is a plausible mechanism to account for the correlation. Postmortem studies of human brains showed that the time course of synaptogenesis was earlier in the visual cortex and auditory cortex than in the prefrontal cortex (Huttenlocher, 1979; Huttenlocher and Dabholkar, 1997; Huttenlocher et al., 1982). In addition, synapse elimination starts earlier in the visual cortex than in the auditory cortex, and that in the prefrontal cortex starts later than in both of the former regions (Huttenlocher and Dabholkar, 1997). These results suggest that brain maturation starts in the occipital lobe, and then moves to the temporal lobe, followed by the prefrontal cortex. In addition, recent neuroimaging studies have shown that brain gray matter maturation progresses with an increase in volume followed by a decrease in volume (Courchesne et al., 2000; Giedd et al., 1999; Gogtay et al., 2004; Shaw et al., 2008), which is thought to be related to synaptogenesis and synaptic elimination (Huttenlocher and Dabholkar, 1997) and with intracortical myelination (Paus, 2005), and the prefrontal cortex is known as one of the latest maturing regions (Gogtay et al., 2004). Because the period corresponding to the highest gray matter volume and brain perfusion of the prefrontal cortex is around adolescence (Gogtay et al., 2004; Taki et al., 2011), the dorsolateral prefrontal cortex may be especially affected by sleep pattern from the childhood to adolescence. Because the dorsolateral prefrontal gyrus is involved in higher cognitive functions, such as working memory (Baddeley, 2003; Klingberg, 2006) and executive function (Kramer et al., 2007; Zimmerman et al., 2006), sufficient sleep is thought to be important for several cognitive functions.

The present study had limitations. First, this is a cross-sectional study. Thus, although we have shown a relationship between sleep duration and hippocampal gray matter volume, we cannot clarify a causal relationship between sleep and hippocampal gray matter volume. Longitudinal studies are needed to clarify this issue. Second, as for the subjective sleepiness, we evaluated the subjective (qualitative) aspect of sleep using the difference between weekday and weekend sleep duration; however, we did not collect data on subjective sleepiness directly, such as asking whether the subjects felt sleepy during the daytime using questionnaires or visual analog scale. Therefore, further studies may help to clarify the correlation between subjective aspects of sleep, such as sleepiness, and brain structure.

In conclusion, our brain MRI study demonstrated that the duration of sleep during weekdays was significantly positively correlated with the regional gray matter volume of the bilateral hippocampus, suggesting that sufficient sleep has a beneficial effect on the hippocampus. These findings advance our understanding of the importance of sleep habits in the daily lives of children.

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