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CHRONIC SLEEP RESTRICTION CAUSES A DECREASE IN HIPPOCAMPAL VOLUME IN ADOLESCENT RATS, WHICH IS NOT EXPLAINED BY CHANGES IN GLUCOCORTICOID LEVELS OR NEUROGENESIS

A. NOVATI,^a H. J. HULSHOF,^b J. M. KOOLHAAS,^a
P. J. LUCASSEN^c AND P. MEERLO^{a*}

^aDepartment of Behavioral Physiology, Center for Behavior and Neurosciences, University of Groningen, Groningen, The Netherlands

^bDepartment of Molecular Neurobiology, Center for Behavior and Neurosciences, University of Groningen, Groningen, The Netherlands

^cSwammerdam Institute for Life Sciences, Centre for Neuroscience, University of Amsterdam, Amsterdam, The Netherlands

Abstract—Sleep loss strongly affects brain function and may even predispose susceptible individuals to psychiatric disorders. Since a recurrent lack of sleep frequently occurs during adolescence, it has been implicated in the rise in depression incidence during this particular period of life. One mechanism through which sleep loss may contribute to depressive symptomatology is by affecting hippocampal function. In this study, we examined the effects of sleep loss on hippocampal integrity at young age by subjecting adolescent male rats to chronic sleep restriction (SR) for 1 month from postnatal day 30 to 61. They were placed in slowly rotating drums for 20 h per day and were allowed 4 h of rest per day at the beginning of the light phase. Anxiety was measured using an open field and elevated plus maze test, while saccharine preference was used as an indication of anhedonia. All tests were performed after 1 and 4 weeks of SR. We further studied effects of SR on hypothalamic-pituitary-adrenal (HPA) axis activity, and at the end of the experiment, brains were collected to measure hippocampal volume and neurogenesis. Behavior of the SR animals was not affected, except for a transient suppression of saccharine preference after 1 week of SR. Hippocampal volume was significantly reduced in SR rats compared to home cage and forced activity controls. This volume reduction was not paralleled by reduced levels of hippocampal neurogenesis and could neither be explained by elevated levels of glucocorticoids. Thus, our results indicate that insufficient sleep may be a causal factor in the reductions of hippocampal volume that have been reported in human sleep disorders and mood disorders. Since changes in HPA activity or neurogenesis are not causally implicated, sleep disturbance may affect hippocampal volume by other, possibly more direct mechanisms. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

*Corresponding author. Tel: +31-(0)-50-363-2334; fax: +31-(0)-50-363-2331.

E-mail address: P.Meerlo@rug.nl (P. Meerlo).

Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BrdU, 5-Bromo-deoxyuridine; CORT, corticosterone; DCX, doublecortin; DG, dentate gyrus; FA, forced activity; GCL, granular cell layer; HC, home cage; HPA, hypothalamic-pituitary-adrenal axis; NeuN, neuronal nuclei; OD, optical density; PD, postnatal day; PFA, paraformaldehyde; SGZ, subgranular zone; SR, sleep restriction.

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Restricted sleep in our society is a problem that not only affects adults but is increasingly common among children and adolescents as well (Meijer et al., 2000; Van den Bulck, 2004; Bixler, 2009). One contributing factor is that adolescence is characterized by spontaneous changes in circadian organization resulting in a stronger tendency for evening activities and delayed sleep timing (Crowley et al., 2007; Roenneberg et al., 2007; Hagenauer et al., 2009). Subsequently, the combination of late evening activities and early morning school or work obligations prevents a large number of young people from getting sufficient sleep (Meijer et al., 2000; Van den Bulck, 2004). Lack of sleep at this age, as in adulthood, has various immediate effects, including tiredness, decreased attention, decreased motivation, reduced cognitive function, and decreased academic performance (Wolfson and Carskadon, 2003; Curcio et al., 2006). In addition, it might be that reduced sleep time at a younger age also affects ongoing brain development, perhaps leading to more persistent effects at later ages.

Many systems in the adolescent brain are still maturing and the morphology of several brain areas go through prominent changes in, for example, grey and white matter ratio (Sowell et al., 2002). The maturation of neurobehavioral systems in this early phase of life requires a high level of plasticity and is associated with strong emotional alterations that may increase the vulnerability to psychopathologies (Dahl and Gunnar, 2009). Indeed, the prevalence of mood disorders such as depression seems to increase from childhood to adolescence (Birmaher et al., 1996; Costello et al., 2002). This increase most likely is a complex interaction between endogenous developmental processes and external factors, one of which may be a recurrent lack of sleep. In agreement with this are various studies in young subjects that have linked the onset of anxiety and depression to short and disrupted sleep (Chang et al., 1997; Gregory et al., 2005; Buysse et al., 2008). In some studies, sleep problems preceded the onset of psychopathology with several years (Chang et al., 1997; Gregory et al., 2005).

One of the brain regions that appears to be particularly sensitive to sleep disruption is the hippocampus (Graves et al., 2003; McDermott et al., 2003; Ruskin et al., 2004; Van der Werf et al., 2009). The hippocampus plays an impor-

tant role in cognition and emotional regulation (Bannerman et al., 2004; Bast, 2007) and is one of the few brain regions that displays neurogenesis continuing from adolescence into adulthood (Abrous et al., 2005; Ming and Song, 2005). Lower levels of hippocampal neurogenesis and reduced hippocampal volume have been implicated in the etiology and symptomatology of emotional and depressive disorders (Sapolsky, 2000; Czéh and Lucassen, 2007; Perera et al., 2008; Boldrini et al., 2009; Lucassen et al., 2010). Moreover, experimental studies show that hippocampal integrity can be affected by prolonged restriction or disruption of sleep (McDermott et al., 2003; Roman et al., 2005a; Guzman-Marin et al., 2006, 2007) while clinical studies have reported a reduction in hippocampal volume in primary insomnia and sleep apnea (Morrell et al., 2003; Riemann et al., 2007). However, most of these data are based on studies in adult animals or humans. The impact of chronically disrupted sleep on hippocampal integrity at a young age, when the brain might be particularly sensitive, has so far received little attention.

In the present study, we applied an animal model of chronic sleep restriction that is aimed at mimicking chronically insufficient sleep as it often occurs in human society. Thus, rather than total sleep deprivation, rats were allowed to sleep part of the day but, presumably, not enough to fully recover (Roman et al., 2005b; Novati et al., 2008). We used the model in the adolescent period to study whether insufficient sleep (*i*) changes hypothalamic-pituitary-adrenal (HPA) axis activity, (*ii*) affects hippocampal volume and neurogenesis, and (*iii*) alters anxiety and anhedonic behavior.

EXPERIMENTAL PROCEDURES

Animals and housing

This study was performed with 48 male Wistar rats, 25 or 28 days old at the start of the experiments. Animals were housed in pairs in a room with a 12 h: 12 h light-dark cycle (lights on 9 AM–9 PM) and temperature of 21 ± 1 °C. Standard laboratory chow and water were provided ad libitum. Experiments were approved by the Ethical Committee of Animal Experiments of the University of Groningen.

Experimental design

Two experiments were performed in this study. In the first one, we examined effects of sleep restriction on anxiety and depression-like behavior measured in an open field test, an elevated plus maze and a saccharine preference test. Rats were sleep restricted throughout adolescence, from postnatal day (PD) 28 to 61. All behavioral tests were performed on consecutive days, after 1 and 4 weeks of sleep restriction. In the second experiment, rats were sleep restricted from PD 30 to 61. Blood samples were collected after 7 and 25 days of sleep restriction to assess plasma levels of stress hormones and brains were collected after 4 weeks of sleep restriction to measure hippocampal volume and examine hippocampal neurogenesis. To quantify survival of newly generated hippocampal cells, all rats received an intraperitoneal injection with the thymidine analogue 5-bromodeoxyuridine (BrdU) at PD 25, 5 days before the start of the experiment (100 mg/kg BrdU in saline, pH=7.0, Sigma, St Louis, MO, USA). As BrdU is incorporated into the DNA of cells in S phase of the cell cycle, it is used to label newborn cells (Kee et al., 2002). Within 1–4 days after

injection, BrdU labeled cells stop dividing and any change in the number of labeled cells thereafter indicates a change in survival (Dayer et al., 2003). Five days after the BrdU injection, at PD 30, the sleep restriction treatment started and continued until PD 61.

Sleep restriction and forced activity controls

For both experiments, 24 animals were assigned to one of the following groups ($n=8$ in each): chronic sleep restriction (SR), forced activity control (FA), and home cage control (HC). Details on our sleep restriction and control procedures have been reported before (Roman et al., 2005a; Novati et al., 2008). Briefly, SR was performed by placing rats in drums of 40 cm diameter, rotating at a speed of 0.4 m/min. Animals were kept awake 20 h per day (1 PM–9 AM) and were left undisturbed for the remaining 4 h at the beginning of the light phase (9 AM–1 PM). Electroencephalographic (EEG) recordings have shown that rats in the wheels have occasional brief sleep bouts. Since this adds up to no more than 10% of the time, the animals are severely sleep deprived on a daily basis, which is confirmed by a sleep rebound during the 4 h daily rest periods (Barf and Meerlo, unpublished results). To examine whether consequences of the treatment were caused by forced locomotion rather than sleep loss per se, a forced activity (FA) group was included as control. Animals of the FA group were housed in the same type of drums which were rotating at double speed for half the time (0.8 m/min for 10 h). As a result, the forced activity animals walked the same distance as sleep restricted animals, but had sufficient time to sleep. The 10 h forced activity took place during the last 10 h of the dark phase (11 PM–9 AM), that is, during the main activity phase of the rats. In addition to the FA controls, we also used undisturbed, naive controls that remained in their home cage throughout the experiment (HC).

Open field test

An open field test was performed to assess effects of SR on general explorative activity and anxiety (Meerlo et al., 1996). The animals were subjected to a 5-min test twice, on days 7 and 31 of the SR protocol, between the third and fourth hour of the daily rest period, before the SR animals were returned to the rotating drums. The open field consisted of a round arena (120 cm in diameter and 20 cm high walls) with a central zone and an outer zone (two imaginary concentric circles with diameters of 60 and 120 cm, respectively). Before the test of each new animal, the arena was thoroughly cleaned with water and soap to eliminate odor cues. Animals were transported in their home cage to the experimental room and placed in the outer zone of the arena. The behavior of the animals was recorded by a camera and analyzed with a computerized imaging analysis system (Ethovision, Noldus Information Technology, Wageningen, The Netherlands). The time spent in the central and outer zone and the total distance covered in each of the two zones were calculated.

Elevated plus maze

On days 8 and 32 of the SR period, during the last two hours of the daily rest phase, animals were subjected to an elevated plus maze test, a widely used nonconditioned anxiety test (Pellow and File, 1986). The plus maze consisted of a black wooden apparatus with two open and two closed arms, 55 cm above the floor. Each arm was 45-cm long and 10-cm wide and the closed arms had 50-cm high walls. Before the test of each new animal, the maze was thoroughly cleaned to eliminate odor cues. At the start of the test, the animals were placed in the centre of the plus facing a corner between a closed and an open arm. The test lasted 5 min and the behavior of the animals was recorded on video for later analysis. Time spent in open and closed arms as well as time in the centre between the arms was scored and expressed as percentage of the total time.

Saccharine preference

To examine the effects of SR on depression-like behavior, we performed a saccharine preference test, which is often used to measure anhedonic behavior (Moreau, 1997). On days 8 and 32 of the SR protocol, following the elevated plus maze test, rats were water deprived for 20 h (1 PM–9 AM) and then subjected to a two-bottle choice preference task during the 4 h of rest (9 AM–1 PM). One bottle contained the regular tap water and the other one a sweet 0.05% saccharin solution. The bottles were placed in central position on the top of the cage to avoid position preference. The liquid intake was measured by comparing the weight of the bottles before and immediately after the test. Water or saccharine solution intake was expressed as percentage of the total fluid intake.

Blood samples and hormones analysis

To measure effects of chronic SR on plasma levels of stress hormones, 0.5 ml blood samples were taken from the tail of the animals at the end of the daily sleep deprivation phase (9 AM) and at the end of the resting phase (1 PM), after 7 and 25 days of SR. Samples were collected within 1–2 min in cold Eppendorf tubes containing EDTA and centrifuged (4 °C, 2600 g, 15 min). The supernatant was stored at –80 °C for later analysis. Plasma concentrations of adrenocorticotrophic hormone (ACTH) and corticosterone (CORT) were determined by radioimmunoassay according to the manufacturer's instructions (MP Biomedicals, Orangeburg, NY, USA).

Brain collection

At PD, 61 brains of the rats from the second experiment were collected for immunocytochemical analysis. After injection with 2 ml/kg pentobarbital, the animals were transcardially perfused with 0.9% saline followed by a 4% solution of paraformaldehyde (PFA) in 0.1 M phosphate buffer. Brains were removed from the skull and post-fixed in 4% PFA for another 24 hours. Then they were kept in 0.01 M PBS overnight and subsequently cryoprotected by 30% sucrose for 48 h before freezing. With a cryostat, eight series of 30- μ m sections were cut and collected in 0.01 M PBS with 0.1% sodium azide until further processing.

Immunohistochemistry

Immunohistochemical staining for the neuronal marker NeuN was used to measure hippocampal volume. Differentiation of new hippocampal cells into neurons was examined by staining for doublecortin (DCX), a microtubule-associated protein that is found in immature neurons (Rao and Shetty, 2004; Couillard-Despres et al., 2005). Survival of newborn cells was assessed by staining for BrdU, which had been injected five days before the start of the SR protocol.

For BrdU immunostaining, first, DNA was denatured with 50% formamide in 2 \times saline sodium citrate (30 min, 65 °C), followed by repeated rinsing in saline sodium citrate. Sections were subsequently placed in a 2 M HCl solution (30 min, 37 °C) and then in 0.1 M borate buffer (pH=8.5). After treatment with 0.3% H₂O₂ (30 min, RT), the sections were first incubated in 3% normal serum and 0.1% Triton-X-100 in 0.01 M TBS and then in primary antibody (rat anti-BrdU, 1:800, Serotec, Oxford, UK) for 48 h at 4 °C. The sections were then incubated in 3% normal serum and 0.01% Triton-X100 before the secondary antibody (donkey anti-rat 1:400, Jackson ImmunoResearch, Suffolk, UK) was applied (2 h, RT). Reaction with avidine biotin complex (1:500, ABC Elite Vector Laboratories, Burlingame, CA, USA) was done for 2 h at room temperature before development in 0.2 mg/ml diaminobenzidine and 0.003% H₂O₂.

For DCX and NeuN immunostaining, sections were pre-treated for 30 min with 0.3% and 0.6% H₂O₂, respectively. After

blocking of aspecific staining with normal serum (3% for DCX and 0.3% for NeuN), sections were incubated with primary antibody (goat anti-DCX at 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA; or mouse anti-NeuN at 1:700; Chemicon, Temecula, CA, USA) for 48 h at 4 °C. Following 40-min exposure to a secondary antibody (Rabbit anti-Goat at 1:400 for DCX and goat anti-mouse at 1:400 for NeuN; Jackson ImmunoResearch, Suffolk, UK), sections were incubated with avidine-biotin complex (1:400 for DCX and 1:500 for NeuN; Elite Vector Laboratories, Burlingame, CA, USA) and the cell labeling was visualized through reaction with 0.2 mg/ml diaminobenzidine and 0.003% H₂O₂.

Quantification

All quantifications were conducted by an investigator blind to the experimental conditions. The number of BrdU positive cells was quantified in the granular cell layer (GCL) and subgranular zone (SGZ) of the dentate gyrus (DG) in every eighth section of the dorsal hippocampus (bregma –1.80 to –4.08; Paxinos and Watson, 1986) at a 400 \times magnification. Immunopositive cells less than one cell diameter away from the SGZ inner border were also included in the analysis, whereas other cells present in the hilus were excluded. In each section, the number of cells was divided by the length of the GCL in that section. The total number of cells per animal was expressed per mm GCL.

DCX immunoreactivity in the DG was quantified by measuring optical density (OD) with a computerized image analysis system (Leica Qwin, Rijswijk, The Netherlands) according to previously published methods (Dagyte et al., 2009). The OD of DCX expression was measured in the GCL and SGZ and corrected for non-specific background labeling measured in the corpus callosum. For each animal, DCX immunoreactivity was measured by delineating the entire GCL and SGZ in both hemispheres of three dorsal hippocampal sections (around bregma –2.50, –3.20, –4.00; Paxinos and Watson, 1986). OD values were expressed in arbitrary units corresponding to grey levels measured by the analysis system.

To estimate the total volume of the dorsal hippocampus and the volume of its cellular subregions, every eighth section was stained with NeuN antibody (11 sections total). We performed the volume measurements in the dorsal hippocampal area of 11 sections per animal (bregma –1.80 to –4.08; Paxinos and Watson, 1986) using the Leica Qwin image software (Rijswijk, The Netherlands). In each of these sections, the complete hippocampal area was outlined as indicated in Fig. 4A. The areas of the cellular subregions, particularly the GCL of the DG and the pyramidal cell layer of the CA1 and the CA2/3, were outlined as indicated in Fig. 4C. The volume estimate for the total hippocampus and the cellular subregions was based on the Cavalieri's method and was obtained by multiplying the sum of the section areas per animal, by section thickness and number of series (Walker et al., 2002; Czéh et al., 2010).

Statistics

Immunohistochemical and behavioral data were statistically tested with a one-way analysis of variance (ANOVA). Repeated measures ANOVA was used for the analysis of ACTH and corticosterone data. When treatment effects were detected with ANOVA, a post hoc Tukey test was used to assess differences between specific treatment groups. A paired *t*-test was applied to assess differences in preference between water and saccharin in each group of animals. The level of significance was set to *P*=0.05. Data in text, tables, and figures are expressed as average per group \pm SEM.

Table 1. Behavior in the open field test after 7 and 31 days of sleep restriction. Data show percentages of time spent and distance covered in the central zone of the arena

| | Day | Home cage | Forced activity | Sleep restricted |
|--------------|-----|-----------|-----------------|------------------|
| Time (%) | 7 | 3.9±1.0 | 2.3±0.4 | 2.9±0.8 |
| | 31 | 10.6±1.5 | 8.9±1.7 | 9.2±1.5 |
| Distance (%) | 7 | 11.3±4.9 | 3.8±0.9 | 5.9±0.8 |
| | 31 | 17.0±3.1 | 12.9±2.4 | 20.2±2.9 |

RESULTS

Behavior

The rats in the present study coped with the protocol of 1-month SR without visible signs of deterioration or illness. Growth was slightly suppressed in both the sleep-restricted rats and forced-activity controls as compared to the home cage controls, resulting in a significantly lower body weight by the end of the experimental period (SR: 234.6±8.1 g, FA: 222.6±7.1 g, HC: 260.1±6.4 g; treatment effect $F_{2,21}=7.01$, $P=0.005$; post hoc Tukey test $P<0.05$ for both SR and FA vs. HC).

No significant effect of SR was found on explorative behavior in the open field test (Table 1). In each of the two tests, after 1 and 4 weeks of treatment, all groups spent most of their time in the outer zone of the arena. Time spent in the central area and the distance traveled in the central area were small and did not differ between the groups (day 7: time $F_{2,21}=0.95$, $P=0.401$; distance $F_{2,21}=1.91$, $P=0.174$; and day 31: time $F_{2,21}=0.35$, $P=0.711$; distance $F_{2,21}=1.68$, $P=0.209$).

Also, SR did not affect anxiety-related behavior in the elevated plus maze test (Fig. 1). Rats in all three groups spent a large part of the time in the closed arms and no difference was found between groups in the percentage of time spent in open arms (day 8: $F_{2,21}=1.06$, $P=0.363$ and day 32: $F_{2,20}=0.083$, $P=0.921$).

A saccharine preference test was used to measure anhedonia (Fig. 2). On day 9 of the experiment, HC animals displayed a clear and significant preference for saccharine over water ($t_{1,7}=4.13$, $P=0.004$), which was less clear and not significant in the FA rats ($t_{1,7}=1.48$, $P=0.181$) and completely absent in the SR rats ($t_{1,7}=-0.34$, $P=0.742$). One-way ANOVA indicated a significant effect of treatment on saccharine intake ($F_{2,21}=6.07$, $P=0.008$), but post hoc Tukey test only showed a difference between the HC and SR rats ($P=0.007$). After 4 weeks of treatment, all three groups displayed a clear preference for the saccharine solution over water (HC: $t_{1,7}=20.16$, $P=0.001$; FA: $t_{1,7}=24.34$, $P=0.001$; SR: $t_{1,7}=2.89$, $P=0.023$). While the preference of the SR rats was on average still lower, ANOVA no longer indicated any significant treatment effect at this time point ($F_{2,21}=2.40$, $P=0.115$).

Stress hormones

To examine the level of HPA axis activation in response to SR, blood samples were collected after 7 and 25 days of

treatment (Table 2). Both immediately after the daily 20-h sleep deprivation and at the end of the daily 4-h resting phase, plasma ACTH and CORT levels in the SR animals were low and not different from FA or HC controls. Statistical analysis did not show significant differences in ACTH and CORT levels between experimental groups for any time point ($P>0.05$ in all cases).

Hippocampal neurogenesis

To investigate effects of chronic SR on survival of newly produced cells in the hippocampus, BrdU was injected in the rats 5 days before the beginning of the treatment. The number of BrdU-positive cells determined at the end of the experiment, that is, at 36 days after BrdU injection, was not different between the groups (Fig. 3B, $F_{2,21}=0.77$, $P=0.478$), suggesting that 4 weeks of SR or FA treatment had not affected survival of the newly formed cells in the dorsal hippocampus. Also the differentiation of new cells into neurons did not appear to be affected by the treatment as the optical density of DCX labeling in the GCL and SGZ of the DG did not differ between the groups (Fig. 3D, $F_{2,21}=1.32$, $P=0.288$).

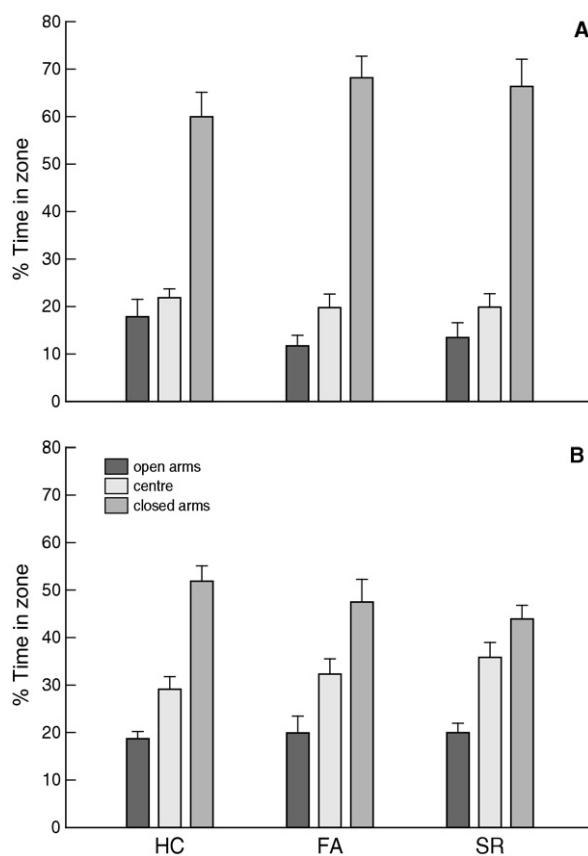


Fig. 1. Anxiety behavior in the elevated plus maze test on day 8 and 32 of the sleep restriction protocol (panel A and B, respectively). All rats had showed preference for the closed arms of the plus maze. The percentage of time spent in open arms was similar for all experimental groups on both test days.

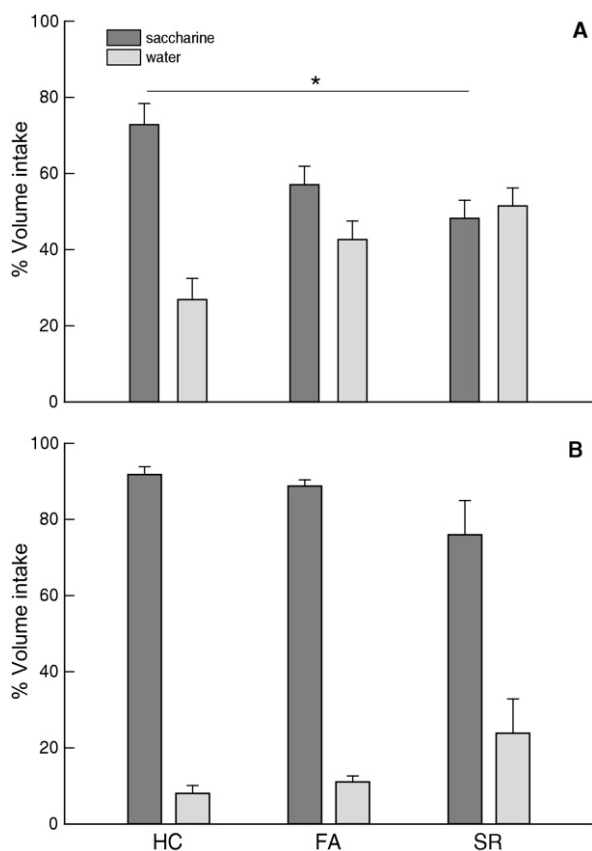


Fig. 2. Saccharine preference test on days 9 and 33 of the sleep restriction protocol (panels A and B, respectively). Rats were water deprived for 20 h and then subjected to a two-bottle choice preference task during the daily 4-h rest period. On day 9, home cage control rats displayed a clear preference for saccharine over water. In both sleep restricted and forced activity rats saccharine intake was comparable to water intake showing a decreased preference for sweet taste. Saccharine intake in sleep restricted rats was significantly lower than in home cage controls (* $P < 0.05$). On day 33 all groups displayed a strong preference for the saccharine over water and there was no longer any difference between the treatments.

Hippocampal volume

The volume of the dorsal hippocampus was estimated in NeuN stained sections (Fig. 4A). A number of animals had to be excluded from the volume analysis because of missing and/or damaged sections (one SR, two FA, and two HC). Chronic SR in adolescent rats caused a reduction in volume of the dorsal hippocampus of about 10% compared

to controls (Fig. 4B). One-way ANOVA revealed a significant treatment effect ($F_{2,16} = 9.63, P < 0.002$) and post-hoc Tukey test indicated a lower volume in SR rats as compared to HC controls ($P = 0.009$) and FA controls ($P = 0.003$). Hippocampal volume in FA rats was not changed compared to HC controls ($P = 0.871$).

The effect of SR did not appear to be restricted to a specific hippocampal subregion (Fig. 4D). One-way ANOVA revealed a trend toward a treatment effect on the volume of the GCL of the DG ($F_{2,16} = 2.95, P = 0.085$) and also a trend toward a treatment effect on the volume of the pyramidal cell layer of the CA1 region ($F_{2,16} = 2.75, P = 0.096$). Moreover, ANOVA revealed a significant treatment effect on the volume of the pyramidal cell layer of the CA2/3 region ($F_{2,16} = 5.91, P = 0.014$) with a significant reduction in SR rats compared to FA animals but not compared to HC rats (post-hoc Tukey $P = 0.011$ and $P = 0.023$, respectively).

To assess if the overall decrease in hippocampal volume might reflect a more global change in brain size, we determined the thickness of the neocortex in the sections used for the hippocampal measurements. On average, the cortical thickness was slightly lower in SR rats but this did not reach statistical significance.

DISCUSSION

The main finding of this study is that rats subjected to chronic SR during adolescence displayed a 10% reduction in dorsal hippocampal volume. This reduction in size of the hippocampus was not associated with significant changes in survival of newly generated BrdU-labeled cells or changes in DCX expression as a marker of young neurons. Therefore, the volume reduction is not likely explained by a reduction in neurogenesis. During the extended period of SR, the young rats displayed a temporary anhedonia as reflected in reduced saccharine preference, but this effect of SR had normalized at the end of the experiment. SR did not affect anxiety-like behavior in the open field test and elevated plus maze test.

In the current study, we did not quantify the exact amount of SR, although it may very well be that changes or lack of changes in some of the measures we took critically depend on the amount of sleep that is lost. Rats were subjected to a protocol of SR that only allowed them to sleep undisturbed for 4 h every day at the beginning of the light phase but it is not excluded that animals also had microsleeps even in the rotating wheels. Indeed, EEG

Table 2. Stress hormones concentrations after 7 and 25 days of sleep restriction. Plasma samples collected at the end of the daily 20 h sleep deprivation session (9 AM) and after 4 h rest (1 PM)

| | ACTH (pg/ml) | | | | Corticosterone (μg/dl) | | | |
|------------------|--------------|----------|----------|----------|------------------------|---------|---------|---------|
| | Day 7 | | Day 25 | | Day 7 | | Day 25 | |
| | 9 AM | 1 PM | 9 AM | 1 PM | 9 AM | 1 PM | 9 AM | 1 PM |
| Home cage | 42.8±6.7 | 35.8±2.6 | 35.9±2.8 | 33.7±2.6 | 1.2±0.4 | 0.8±0.1 | 1.1±0.6 | 1.6±0.4 |
| Forced activity | 3.8±2.0 | 33.7±3.3 | 33.6±2.0 | 27.3±1.7 | 1.4±0.3 | 7.0±3.9 | 3.2±1.1 | 1.3±0.4 |
| Sleep restricted | 47.0±6.6 | 32.5±3.8 | 38.8±4.0 | 26.0±2.5 | 10.5±6.9 | 2.2±1.0 | 3.7±0.9 | 1.1±0.1 |

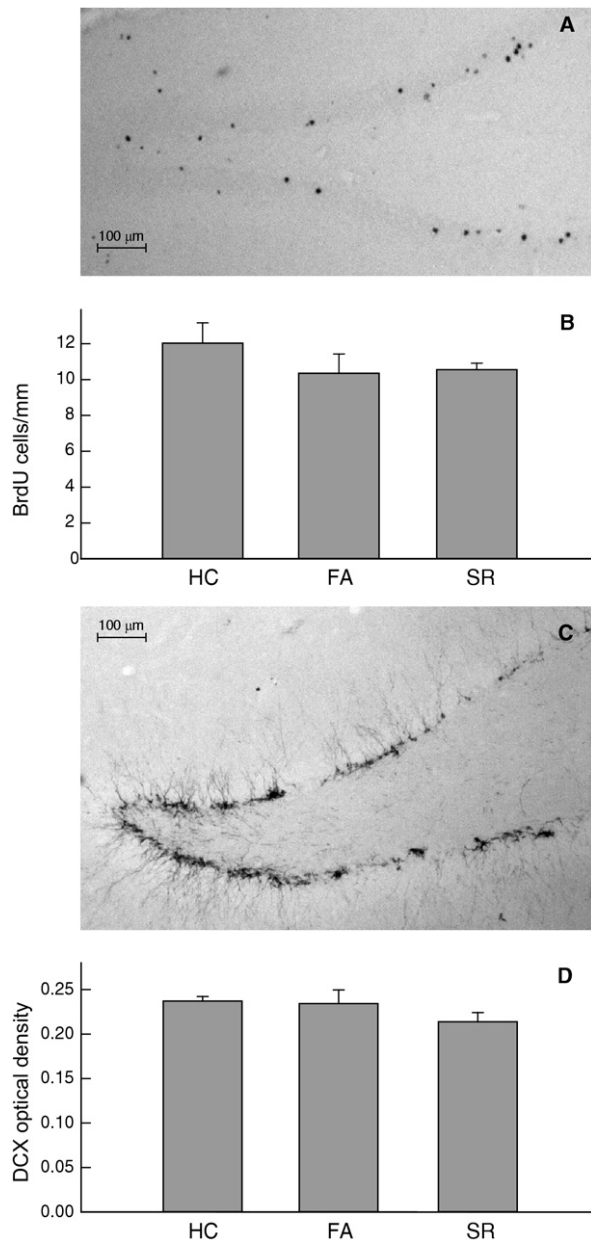


Fig. 3. Sleep restriction and hippocampal neurogenesis. (A) Photograph of BrdU-positive cells in the granular layer of the dentate gyrus. (B) Chronic sleep restriction did not affect the survival of newly formed cells in the dentate gyrus of the dorsal hippocampus. (C) Photograph of DCX expressing neurons in the dentate gyrus. (D) Chronic sleep restriction did not affect the optical density of DCX expressing new neurons in dentate gyrus of dorsal hippocampus.

recordings have shown that rats in the wheels have occasional brief sleep bouts that may add up to 10% of the time (Barf and Meerlo, unpublished observation). Furthermore, a recent study shows that after a long period without sleep, local clusters of cortical neurons may go offline while the rest of the brain is apparently awake (Vyazovskiy et al., 2011). The latter finding indicates that in general an exact quantification of sleep loss may not be as straightforward as usually thought. Importantly, rather than trying to

achieve total sleep deprivation or an exact amount of sleep deprivation, with our approach, we aimed to mimic chronically insufficient sleep as it often occurs in human society. While rats cope with our protocol of SR for prolonged periods of time without visible signs of illness, earlier studies had already shown that it gradually leads to neurobiological and neuroendocrine changes similar to what has

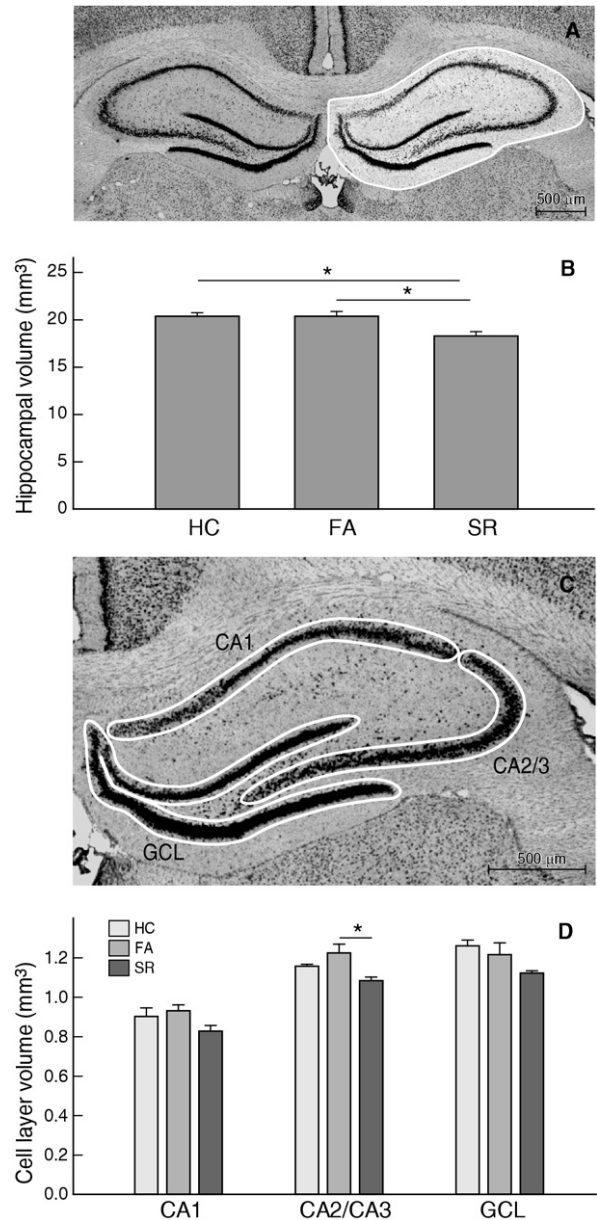


Fig. 4. Sleep restriction and hippocampal volume. (A) Photograph of NeuN staining in the dorsal hippocampus. The hippocampal area measured in each brain section is indicated on the right. (B) Volume of dorsal hippocampus was significantly reduced in sleep restricted rats compared to home cage and forced activity control groups. (C) Photograph indicating the cellular subregions that were measured. (D) On average the volume of the cellular subregions was lower in the sleep restricted rats than in control rats but this difference only reached statistical significance for the CA2/3 subregion in comparison with the forced activity control rats. * $P < 0.05$, see text for details.

been reported for depressed patients (Roman et al., 2005b; Novati et al., 2008).

In the present study, our analysis of SR effects on brain integrity was largely focused on the hippocampus and we only performed restricted control measurements in the cortex. The finding that cortical thickness was not significantly affected by SR suggests that the reduction in hippocampal volume was at least partly specific and did not simply reflect a more global change in brain morphology. On the other hand, this study certainly does not rule out the possibility that chronic SR affects other areas as well. Indeed, while the hippocampus appears to be particularly sensitive to sleep loss (Meerlo et al., 2009), other sensitive brain regions have been suggested, for example, the prefrontal cortex (Horne, 1993; Muzur et al., 2002).

Reductions in hippocampal volume have been found before in human sleep disorders such as primary insomnia (Riemann et al., 2007) and sleep apnea (Morrell et al., 2003). Also in psychopathologies such as major depression, reductions in hippocampal volume are commonly observed and have been implicated in specific symptoms of the disorders (Sapolsky, 2000; Czéh and Lucassen, 2007; Perera et al., 2008; Boldrini et al., 2009; Lucassen et al., 2010). Intriguingly, psychopathologies are often associated with disturbed sleep-wake patterns that may contribute to the development and aggravation of the disease (Tsunoo et al., 2005; Riemann and Voderholzer, 2003). Along these lines, also the reduction in hippocampal volume in psychopathologies might be partly a consequence of the sleep-wake disturbance. Indeed, the present reduction in hippocampal volume after experimental SR under controlled conditions in rats suggests that the hippocampal volume reduction observed in human sleep disorders and mood disorders may be a direct consequence of disrupted sleep rather than a nonspecific side-effect.

A variety of explanations have been proposed for a decrease in hippocampal volume in disease including neuronal death, neuronal shrinkage, lower dendritic arborization, reductions in neurogenesis, or decreases in glia numbers and production (Czéh and Lucassen, 2007). The lack of effects of SR on different markers of neurogenesis in the present study does not support the hypothesis that the smaller hippocampal size was related to a reduction in neurogenesis. In fact, even if SR would have fully suppressed neurogenesis it still could not have explained the magnitude of the hippocampal volume reduction we found. Moreover, measurements of the cellular subregions suggest that the volume reduction was not limited to the DG but may have included the non-neurogenic CA regions as well. Since there is little to no evidence for massive neuronal death even after prolonged total sleep deprivation (Cirelli et al., 1999; Eiland et al., 2002), it seems more likely that part of the volume reduction was caused by a decrease in the size of neuronal cell bodies and dendritic arborizations or by changes in the number and size of glia cells.

In the present study, we focused our analysis of neurogenesis on survival of new BrdU-labeled cells and differentiation of new cells into young DCX-expressing neurons.

While we did not specifically assess effects of SR on cell proliferation, a strong reduction in proliferation in the later part of the experiment most likely would have shown up in a reduced DCX expression as well since these DCX positive cells were born in the last 1 or 2 weeks of the experiment. This was clearly not the case. However, we cannot exclude that SR may have suppressed cell proliferation in the first half of the experiment, which would not be visible in DCX expression. Another limitation of our study is that we only assessed cell survival and differentiation in the dorsal limb of the hippocampus. One might argue therefore that SR possibly had an effect on neurogenesis in the ventral hippocampus that went unnoticed in our analysis. Yet, although one study on sleep deprivation in adult rats indeed showed a stronger suppression of cell proliferation in the ventral part of the hippocampus (Tung et al., 2005), most studies report a reduction of neurogenic measures in the dorsal limb as well (Guzman-Marin et al., 2007; Roman et al., 2005a). All together, the fact that our measures of neurogenesis were not affected by SR in young animals was somewhat unexpected. One explanation may lie in the fact that we sleep restricted rats during the transition from adolescence to adulthood, when neurogenesis rapidly declines toward the low levels that then persist in the mature brain throughout middle age and senescence (Heine et al., 2004; He and Crews, 2007; Cowen et al., 2008). Perhaps the additional impact of SR on neurogenesis is modest during a phase where neurogenesis already shows a strong and spontaneous decrease. Alternatively, the lack of an SR effect may be related to the social housing conditions in the current experiment. We chose to house the adolescent rats, two per cage or per SR drum because in this phase of their life social contact and play behavior is important for normal development. However, it might be that the enriched social housing condition has compensated for the adverse effects of SR. In support of this explanation are studies showing that environmental enrichment or exercise promote hippocampal neurogenesis (Kempermann et al., 1997; Van Praag et al., 2000) and may even decrease or reverse earlier brain deficits (Twiggs et al., 1978; Francis et al., 2002; Bredy et al., 2003; Nithianantharajah and Hannan, 2006; Naylor et al., 2008). Importantly, even if the present housing conditions modulated the consequences of SR and counteracted putative effects on neurogenesis, it clearly did not prevent the reduction in hippocampal volume.

Hippocampal atrophy as observed in various pathologies is often proposed to be a result of elevated concentrations of glucocorticoid stress hormones (Sapolsky, 2000; Czéh and Lucassen, 2007). However, smaller hippocampal size and cortisol levels do not always correlate (O'Brien et al., 2004). In the present study, SR did not lead to a major activation of the HPA axis. ACTH and CORT concentrations measured after 1 and 4 weeks of SR were similar to the levels in FA and HC controls. Obviously, given the restricted number of samples and time points, these data need to be considered with care. One might argue that samples collected at the end of the daily 20-h sleep deprivation session do not necessarily reflect HPA

axis activity during the initial hours of sleep deprivation. Yet, previous studies with our model have shown that, if anything, CORT levels are low at the beginning of sleep deprivation and gradually increase towards the end (Meerlo et al., 2002). Moreover, the results are in line with various other studies showing only mild effects of sleep deprivation on HPA axis activity (see Meerlo et al., 2008 for review), which often do not explain sleep deprivation-induced changes in hippocampal integrity and function (Guzman-Marin et al., 2007; Mueller et al., 2008; Tiba et al., 2008; Hagewoud et al., 2010). Together the data indicate that the reduction in hippocampal volume in our study is not easily explained by elevated HPA axis activity and glucocorticoid release.

Alternatively, a smaller hippocampus may have resulted from a reduction in trophic factors or a dysregulation in their signaling. One of the possible mechanisms involves altered expression of brain-derived neurotrophic factor (BDNF), which is highly expressed in the adult hippocampus (Schmidt-Kastner et al., 1996) and stimulates dendritic arborization (McAllister et al., 1995). Evidence suggests that low levels of BDNF may play a role in the hippocampal atrophy associated with depressive disorders (Shimizu et al., 2003). Literature further suggests BDNF levels can be affected by sleep deprivation, although the direction of the effect differs between studies and is so far difficult to interpret (Adrien, 2002; Fujihara et al., 2003; Cirelli, 2006; Guzman-Marin et al., 2006). In one study, 48 h of sleep deprivation resulted in decreased levels of BDNF in hippocampus (Guzman-Marin et al., 2006) while effects of longer periods of sleep deprivation or restriction on BDNF expression have not been reported. Should SR have a negative effect on BDNF expression, this may underlie hippocampal dendrite atrophy, which in turn could account for the reduction in hippocampal volume observed in this experiment. Future studies are needed to investigate the relationship between sleep, BDNF expression, and hippocampal integrity.

The reduction in hippocampal volume in our study was not associated with obvious changes in explorative activity or anxiety in an open field and elevated plus maze test. This is consistent with other studies in our laboratory that failed to find clear anxiety effects of acute and short sleep deprivation on the elevated plus maze test (Hagewoud et al., 2010). In fact, with a few exceptions (Silva et al., 2004), the majority of experimental studies show no effect or even indicate decreased anxiety in sleep-deprived rodents (Hicks and Moore, 1979; Moore et al., 1979; Suchecki et al., 2002; Martinez-Gonzalez et al., 2004; Tartar et al., 2009). While there is some inconsistency in the literature, this may be the result of a complex interaction between the duration and method of sleep deprivation, the anxiety test involved, species and strain differences, and perhaps other factors. Also in humans, some studies report no relationship between sleep loss and anxiety (Bonnet and Arand, 1998), while others suggest an association between lack of sleep and self-reported anxiety (Peeke et al., 1980; Sagaspe et al., 2006), and a correlation between sleep disturbance and the risk for anxiety disorders (Greg-

ory et al., 2005; Roth et al., 2006). Apparently, the relationship between restricted or disrupted sleep and anxiety is complex and requires further study.

While chronic SR in adolescent rats did not affect specific measures of anxiety, the treatment did have a temporary effect on the preference for a sweet saccharine solution, a commonly used measure of anhedonia (Willner et al., 1992; Moreau, 1997). Anhedonia refers to an inability to experience pleasure or to the loss of interest for normal aspects of life and is considered a psychological marker of depression (Schrader, 1997). After 1 week, sleep restricted rats did not display the normal preference for the saccharine solution that was seen in HC control animals. However, this lack of preference also occurred in the FA controls and therefore we cannot distinguish between effects of chronic sleep loss and forced locomotion. Also, this anhedonia did not persist but gradually disappeared in the course of the experiment and was no longer noticeable after 1 month, when all groups exhibited a clear preference for saccharine solution over water. Few other published records exist on chronic SR and anhedonic behavior. In several unpublished experiments, we have examined the effect of prolonged SR on saccharine or sucrose preference in adult animals with the same protocol as we used in the present study but the results have been inconsistent (Novati et al., unpublished observation). It could be that the preference test is not sophisticated enough to really detect anhedonic behavior or, alternatively, SR may only have a weak effect that is easily modulated or overruled by other factors we are yet unaware of.

In summary, the results of this experimental study suggest that lack of sleep during adolescence may reduce hippocampal volume, without affecting survival and differentiation of new neurons in the DG. The reduction in hippocampal volume was not associated with obvious changes in anxiety or persistent changes in anhedonic behavior. The mechanisms underlying the effect of SR on hippocampal volume require further investigation.

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REFERENCES

- Abrous DN, Koehl M, Le Moal M (2005) Adult neurogenesis: from precursors to network and physiology. *Physiol Rev* 85:523–569.
- Adrien J (2002) Neurobiological bases for the relation between sleep and depression. *Sleep Med Rev* 6:341–351.
- Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, Zhang WN, Pothuizen HH, Feldon J (2004) Regional dissociations within the hippocampus—memory and anxiety. *Neurosci Biobehav Rev* 28:273–283.

- Bast T (2007) Toward an integrative perspective on hippocampal function: from the rapid encoding of experience to adaptive behavior. *Rev Neurosci* 18:253–281.
- Birmaher B, Ryan ND, Williamson DE, Brent DA, Kaufman J, Dahl RE, Perel J, Nelson B (1996) Childhood and adolescent depression: a review of the past 10 years: part I. *J Am Acad Child Adolesc Psychiatry* 35:1427–1439.
- Bixler E (2009) Sleep and society: an epidemiological perspective. *Sleep Med* 10 (Suppl 1):s3–s6.
- Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J, Arango V (2009) Antidepressants increase neural progenitor cells in the human hippocampus. *Neuropsychopharmacology* 34:2376–2389.
- Bonnet MH, Arand DL (1998) The consequence of a week of insomnia. Part II: patients with insomnia. *Sleep* 21:359–368.
- Bredy TW, Humpalzoomian RA, Cain DP, Meaney MJ (2003) Partial reversal of the effect of maternal care on cognitive function through environmental enrichment. *Neuroscience* 118:571–576.
- Buysse DJ, Angst J, Gamma A, Ajdacic V, Eich D, Rössler W (2008) Prevalence, course, and comorbidity of insomnia and depression in young adults. *Sleep* 31:473–480.
- Chang PP, Ford DE, Mead LA, Cooper-Patrick L, Klag MJ (1997) Insomnia in young men and subsequent depression. The Johns Hopkins Precursors Study. *Am J Epidemiol* 146:105–114.
- Cirelli C (2006) Cellular consequences of sleep deprivation in the brain. *Sleep Med Rev* 10:307–321.
- Cirelli C, Shaw PJ, Rechtschaffen A, Tononi G (1999) No evidence of brain cell degeneration after long-term sleep deprivation in rats. *Brain Res* 840:184–193.
- Costello EJ, Pine DS, Hammen C, March JS, Plotsky PM, Weissman MM, Biederman J, Goldsmith HH, Kaufman J, Lewinsohn PM, Hellander M, Hoagwood K, Koretz DS, Nelson CA, Leckman JF (2002) Development and natural history of mood disorders. *Biol Psychiatry* 52:529–542.
- Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N, Bogdahn U, Winkler J, Kuhn HG, Aigner L (2005) Doublecortin expression levels in adult brain reflect neurogenesis. *Eur J Neurosci* 21:1–14.
- Cowen DS, Takase LF, Fornal CA, Jacobs BL (2008) Age-dependent decline in hippocampal neurogenesis is not altered by chronic treatment with fluoxetine. *Brain Res* 1228:14–19.
- Crowley SJ, Acebo C, Carskadon MA (2007) Sleep, circadian rhythms, and delayed phase in adolescence. *Sleep Med* 8:602–612.
- Curcio G, Ferrara M, De Gennaro L (2006) Sleep loss, learning capacity and academic performance. *Sleep Med Rev* 10:323–337.
- Czéh B, Abumaria N, Rygula R, Fuchs E (2010) Quantitative changes in hippocampal microvasculature of chronically stressed rats: no effect of fluoxetine treatment. *Hippocampus* 20:174–185.
- Czéh B, Lucassen PJ (2007) What causes the hippocampal volume decrease in depression? Are neurogenesis, glial changes and apoptosis implicated? *Eur Arch Psychiatry Clin Neurosci* 257: 250–260.
- Dagyte G, Van der Zee EA, Postema F, Luiten PG, Den Boer JA, Trentani A, Meerlo P (2009) Chronic but not acute foot-shock stress leads to temporary suppression of cell proliferation in rat hippocampus. *Neuroscience* 162:904–913.
- Dahl RE, Gunnar MR (2009) Heightened stress responsiveness and emotional reactivity during pubertal maturation: implications for psychopathology. *Dev Psychopathol* 21:1–6.
- Dayer AG, Ford AA, Cleaver KM, Yassaee M, Cameron HA (2003) Short-term and long-term survival of new neurons in the rat dentate gyrus. *J Comp Neurol* 460:563–572.
- Eiland MM, Ramanathan L, Gulyani S, Gilliland M, Bergmann BM, Rechtschaffen A, Siegel JM (2002) Increases in amino-cupric-silver staining of the supraoptic nucleus after sleep deprivation. *Brain Res* 945:1–8.
- Francis DD, Diorio J, Plotsky PM, Meaney MJ (2002) Environmental enrichment reverses the effects of maternal separation on stress reactivity. *J Neurosci* 22:7840–7843.
- Fujihara H, Sei H, Morita Y, Ueta Y, Morita K (2003) Short-term sleep disturbance enhances brain-derived neurotrophic factor gene expression in rat hippocampus by acting as internal stressor. *J Mol Neurosci* 21:223–232.
- Graves LA, Heller EA, Pack AI, Abel T (2003) Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learn Mem* 10:168–176.
- Gregory AM, Caspi A, Eley TC, Moffitt TE, O'Connor TG, Poulton R (2005) Prospective longitudinal associations between persistent sleep problems in childhood and anxiety and depression disorders in adulthood. *J Abnorm Child Psychol* 33:157–163.
- Guzman-Marin R, Bashir T, Suntsova N, Szymusiak R, McGinty D (2007) Hippocampal neurogenesis is reduced by sleep fragmentation in the adult rat. *Neuroscience* 148:325–333.
- Guzman-Marin R, Ying Z, Suntsova N, Methippara M, Bashir T, Szymusiak R, Gomez-Pinilla F, McGinty D (2006) Suppression of hippocampal plasticity-related gene expression by sleep deprivation in rats. *J Physiol* 575:807–819.
- Hagenauer MH, Perryman JI, Lee TM, Carskadon MA (2009) Adolescent changes in the homeostatic and circadian regulation of sleep. *Dev Neurosci* 31:276–284.
- Hagewoud R, Havekes R, Tiba P, Novati A, Hogenelst K, Weinreder P, van der Zee EA, Meerlo P (2010) Coping with sleep deprivation: shifts in regional brain activity and learning strategy. *Sleep* 19: 280–288.
- He S, Crews FT (2007) Neurogenesis decreases during brain maturation from adolescence to adulthood. *Pharmacol Biochem Behav* 86:327–333.
- Heine VM, Maslam S, Joëls M, Lucassen PJ (2004) Prominent decline of newborn cell proliferation, differentiation, and apoptosis in the aging dentate gyrus, in absence of an age-related hypothalamus-pituitary-adrenal axis activation. *Neurobiol Aging* 25:361–375.
- Hicks RA, Moore JD (1979) REM sleep deprivation diminishes fear in rats. *Physiol Behav* 22:689–692.
- Horne JA (1993) Human sleep, sleep loss and behaviour. Implications for the prefrontal cortex and psychiatric disorder. *Br J Psychiatry* 162:413–419.
- Kee N, Sivalingam S, Boonstra R, Wojtowicz JM (2002) The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis. *J Neurosci Methods* 115:97–105.
- Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. *Nature* 386:493–495.
- Lucassen PJ, Meerlo P, Naylor AS, Van Dam AM, Dayer AG, Fuchs E, Oomen CA, Czéh B (2010) Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: implications for depression and antidepressant action. *Eur Neuropsychopharmacol* 20:1–17.
- Martinez-Gonzalez D, Obermeyer W, Fahy JL, Riboh M, Kalin NH, Benca RM (2004) REM sleep deprivation induces changes in coping responses that are not reversed by amphetamine. *Sleep* 27:609–617.
- McAllister AK, Lo DC, Katz LC (1995) Neurotrophins regulate dendritic growth in developing visual cortex. *Neuron* 15:791–803.
- McDermott CM, LaHoste GJ, Chen C, Musto A, Bazan NG, Magee JC (2003) Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. *J Neurosci* 23:9687–9695.
- Meerlo P, Koehl M, Van der Borght K, Turek FW (2002) Sleep restriction alters the hypothalamic-pituitary-adrenal response to stress. *J Neuroendocrinol* 14:397–402.
- Meerlo P, Mistlberger RE, Jacobs BL, Heller HC, McGinty D (2009) New neurons in the adult brain: the role of sleep and consequences of sleep loss. *Sleep Med Rev* 13:187–194.

- Meerlo P, Overkamp GJ, Benning MA, Koolhaas JM, van den Hoofdakker RH (1996) Long-term changes in open field behaviour following a single social defeat in rats can be reversed by sleep deprivation. *Physiol Behav* 60:115–119.
- Meerlo P, Sgoifo A, Suchecki D (2008) Restricted and disrupted sleep: effects on autonomic function, neuroendocrine stress systems and stress responsivity. *Sleep Med Rev* 12:197–210.
- Meijer AM, Habekothé HT, Van-Den-Wittenboer GL (2000) Time in bed, quality of sleep and school functioning of children. *J Sleep Res* 9:145–153.
- Ming GL, Song H (2005) Adult neurogenesis in the mammalian central nervous system. *Annu Rev Neurosci* 28:223–250.
- Moore JD, Hayes C, Hicks RA (1979) REM sleep deprivation increases preference for novelty in rats. *Physiol Behav* 23:975–976.
- Moreau JL (1997) Validation of an animal model of anhedonia, a major symptom of depression. *Encephale* 23:280–289.
- Morrell MJ, McRobbie DW, Quest RA, Cummin AR, Ghiassi R, Corfield DR (2003) Changes in brain morphology associated with obstructive sleep apnea. *Sleep Med* 4:451–454.
- Mueller A, Pollock MS, Lieblich SE, Epp J, Galea LA, Mistlberger RE (2008) REM sleep deprivation can inhibit adult hippocampal neurogenesis independent of adrenal stress hormones. *Am J Physiol* 294:R1693–R1703.
- Muzur A, Pace-Schott EF, Hobson JA (2002) The prefrontal cortex in sleep. *Trends Cogn Neurosci* 6:475–481.
- Naylor AS, Bull C, Nilsson MK, Zhu C, Björk-Eriksson T, Eriksson PS, Blomgren K, Kuhn HG (2008) Voluntary running rescues adult hippocampal neurogenesis after irradiation of the young mouse brain. *Proc Natl Acad Sci U S A* 105:14632–14637.
- Nithianantharajah J, Hannan AJ (2006) Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nat Rev Neurosci* 7:697–709.
- Novati A, Roman V, Cetin T, Hagewoud R, Den Boer JA, Luiten PG, Meerlo P (2008) Chronically restricted sleep leads to depression-like changes in neurotransmitter receptor sensitivity and neuroendocrine stress reactivity in rats. *Sleep* 31:1579–1585.
- O'Brien JT, Lloyd A, McKeith I, Gholkar A, Ferrier N (2004) A longitudinal study of hippocampal volume, cortisol levels, and cognition in older depressed subjects. *Am J Psychiatry* 161:2081–2090.
- Paxinos G, Watson C (1986) *The rat brain in stereotaxic coordinates*, 2nd ed. Sydney: Academic Press.
- Peeke SC, Callaway E, Jones RT, Stone GC, Doyle J (1980) Combined effects of alcohol and sleep deprivation in normal young adults. *Psychopharmacology* 67:279–287.
- Pellow S, File SE (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacol Biochem Behav* 24:525–529.
- Perera TD, Park S, Nemirovskaya Y (2008) Cognitive role of neurogenesis in depression and antidepressant treatment. *Neuroscientist* 14:326–338.
- Rao MS, Shetty AK (2004) Efficacy of doublecortin as a marker to analyze the absolute number and dendritic growth of newly generated neurons in the adult dentate gyrus. *Eur J Neurosci* 19:234–246.
- Riemann D, Voderholzer U (2003) Primary insomnia: a risk factor to develop depression? *J Aff Disord* 76:255–259.
- Riemann D, Voderholzer U, Spiegelhalder K, Hornyak M, Buysse DJ, Nissen C, Hennig J, Perlis ML, van Elst LT, Feige B (2007) Chronic insomnia and MRI-measured hippocampal volumes: a pilot study. *Sleep* 30:955–958.
- Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, Merrow M (2007) Epidemiology of the human circadian clock. *Sleep Med Rev* 11:429–438.
- Roman V, Van der Borght K, Leemberg SA, Van der Zee EA, Meerlo P (2005a) Sleep restriction by forced activity reduces hippocampal cell proliferation. *Brain Res* 1065:53–59.
- Roman V, Walstra I, Luiten PG, Meerlo P (2005b) Too little sleep gradually desensitizes the serotonin 1A receptor system in rats. *Sleep* 28:1505–1510.
- Roth T, Jaeger S, Jin R, Kalsekar A, Stang PE, Kessler RC (2006) Sleep problems, comorbid mental disorders, and role functioning in the national comorbidity survey replication. *Biol Psychiatry* 60:1364–1371.
- Ruskin DN, Liu C, Dunn KE, Bazan NG, LaHoste GJ (2004) Sleep deprivation impairs hippocampus-mediated contextual learning but not amygdala-mediated cued learning in rats. *Eur J Neurosci* 19:3121–3124.
- Sagaspe P, Sanchez-Ortuno M, Charles A, Taillard J, Valtat C, Bioulac B, Philip P (2006) Effects of sleep deprivation on color-word, emotional, and specific Stroop interference and on self-reported anxiety. *Brain Cogn* 60:76–87.
- Sapolsky RM (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry* 57:925–935.
- Schmidt-Kastner R, Wetmore C, Olson L (1996) Comparative study of brain-derived neurotrophic factor messenger RNA and protein at the cellular level suggests multiple roles in hippocampus, striatum and cortex. *Neuroscience* 74:161–183.
- Schrader GD (1997) Does anhedonia correlate with depression severity in chronic depression? *Compr Psychiatry* 38:260–263.
- Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, Nakazato M, Watanabe H, Shinoda N, Okada S, Iyo M (2003) Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry* 54:70–75.
- Silva RH, Kameda SR, Carvalho RC, Takatsu-Coleman AL, Niigaki ST, Abílio VC, Tuffik S, Frussa-Filho R (2004) Anxiogenic effect of sleep deprivation in the elevated plus-maze test in mice. *Psychopharmacology (Berl)* 176:115–122.
- Sowell ER, Trauner DA, Gamst A, Jernigan TL (2002) Development of cortical and subcortical brain structures in childhood and adolescence: a structural MRI study. *Dev Med Child Neurol* 44:4–16.
- Suchecki D, Tiba PA, Tufik S (2002) Hormonal and behavioural responses of paradoxical sleep-deprived rats to the elevated plus maze. *J Neuroendocrinol* 14:549–554.
- Tartar JL, Ward CP, Cordeira JW, Legare SL, Blanchette AJ, McCauley RW, Strecker RE (2009) Experimental sleep fragmentation and sleep deprivation in rats increases exploration in an open field test of anxiety while increasing plasma corticosterone levels. *Behav Brain Res* 197:450–453.
- Tiba PA, Oliveira MG, Rossi VC, Tufik S, Suchecki D (2008) Glucocorticoids are not responsible for paradoxical sleep deprivation-induced memory impairments. *Sleep* 31:505–515.
- Tsuno N, Besset A, Ritchie K (2005) Sleep and depression. *J Clin Psychiatry* 66:1254–1269.
- Tung A, Takase L, Fornal C, Jacobs B (2005) Effects of sleep deprivation and recovery sleep upon cell proliferation in adult rat dentate gyrus. *Neuroscience* 134:721–723.
- Twigg DG, Popolow HB, Gerall AA (1978) Medial preoptic lesions and male sexual behavior: age and environmental interactions. *Science* 200:1414–1415.
- Van den Bulck J (2004) Television viewing, computer game playing, and Internet use and self-reported time to bed and time out of bed in secondary-school children. *Sleep* 27:101–104.
- Van der Werf YD, Altena E, Schoonheim MM, Sanz-Arigita EJ, Vis JC, De Rijke W, Van Someren EJ (2009) Sleep benefits subsequent hippocampal functioning. *Nat Neurosci* 12:122–123.
- Van Praag H, Kempermann G, Gage FH (2000) Neural consequences of environmental enrichment. *Nat Rev Neurosci* 1:191–198.

Vyazovskiy VV, Olcese U, Hanlon EC, Nir Y, Cirelli C, Tononi G (2011) Local sleep in awake rats. *Nature* 472:443–447.

Walker MA, Highley JR, Esiri MM, McDonald B, Roberts HC, Evans SP, Crow TJ (2002) Estimated neuronal populations and volumes of the hippocampus and its subfields in schizophrenia. *Am J Psychiatry* 159:821–828.

Willner P, Muscat R, Papp M (1992) Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci Biobehav Rev* 16:525–534.

Wolfson AR, Carskadon MA (2003) Understanding adolescents' sleep patterns and school performance: a critical appraisal. *Sleep Med Rev* 7:491–506.

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