Sleep Deprivation Effects on Growth Factor Expression in Neonatal Rats: A Potential Role for BDNF in the Mediation of Delta Power

Ilana S. Hairston, Christelle Peyron, Daniel P. Denning, Norman F. Ruby, Judith Flores, Robert M. Sapolsky, H. Craig Heller, and Bruce F. O’Hara. Sleep deprivation effects on growth factor expression in neonatal rats: a potential role for BDNF in the mediation of delta power. J Neurophysiol 91: 1586 –1595, 2004. First published December 10, 2003; 10.1152/jn.00894.2003. The sleeping brain differs from the waking brain in its electrophysiological and molecular properties, including the expression of growth factors and immediate early genes (IEG). Sleep architecture and homeostatic regulation of sleep in neonates is distinct from that of adults. Hence, the present study addressed the question whether the unique homeostatic response to sleep deprivation in neonates is reflected in mRNA expression of the IEG cFos, brain-derived nerve growth factor (BDNF), and basic fibroblast growth factor (FGF2) in the cortex. As sleep deprivation is stressful to developing rats, we also investigated whether the increased levels of corticosterone would affect the expression of growth factors in the hippocampus, known to be sensitive to glucocorticoid levels. At postnatal days 16, 20, and 24, rats were subjected to sleep deprivation, maternal separation without sleep deprivation, sleep deprivation with 2 h recovery sleep, or no intervention. mRNA expression was quantified in the cortex and hippocampus. cFos was increased after sleep deprivation and was similar to control level after 2 h recovery sleep irrespective of age or brain region. BDNF was increased by sleep deprivation in the cortex at P20 and P24 and only at P24 in the hippocampus. FGF2 increased during recovery sleep at all ages in both brain regions. We conclude that cortical BDNF expression reflects the onset of adult sleep-homeostatic response, whereas the profile of expression of both growth factors suggests a trophic effect of mild sleep deprivation.

INTRODUCTION

Neonatal sleep patterns differ from those of adults (reviewed in Davis et al. 1999). In adult rats, sleep deprivation of ≤12 h causes an increase in the proportion of the 1- to 4-Hz frequency range (delta power) in the electroencephalogram (EEG) during slow-wave sleep (SWS), whereby the level of increase is reliably predicted by the duration of preceding wake (Franken et al. 1991; Tobler and Borbély 1990). Conversely, neonatal rats do not show this correlation between delta power and wake history prior to postnatal day 20 (P20) but do display a compensatory increase in the amount of SWS (Frank et al. 1998). This suggests that the neonate’s sleep regulatory system detects sleep loss but uses a different compensatory strategy.

In adults, the expression of a number of genes in the cortex, such as immediate early genes (IEG) and growth factors, is higher during wake than during SWS (Cirelli 2002; O’Hara et al. 1993; Pompeiano et al. 1994; Terao et al. 2003). This study investigated whether the distinct homeostatic response to sleep deprivation observed in neonatal rats is reflected in the pattern of gene expression. Brain-derived growth factor (BDNF), basic fibroblast growth factor (FGF2), their receptors (TrkB and FGFR2, respectively), and cFos were measured after sleep deprivation and recovery sleep at P16, P20, and P24.

FGF2 mRNA increases with elevated corticosterone (CORT) levels in the hippocampus (Chao and McEwen 1994; Hansson et al. 2000; Molteni et al. 2001). Increased CORT levels are associated with a transient (3 h) increase (Marmigère et al. 2003) and long-term (>6 h) decrease of BDNF in the hippocampus (Nibuya et al. 1999; Ueyama et al. 1997). Conversely, sleep deprivation and selective paradoxical sleep (PS) deprivation have been shown to have no effect on BDNF expression in the hippocampus (Sei et al. 2000; Taishi et al. 2001). Consequently, as sleep deprivation has been previously shown to induce a stress response in neonatal rats (Hairson et al. 2001), we expected FGF2 expression in the hippocampus to increase, and BDNF expression to either increase or remain unchanged.

We found that the onset of the sleep-deprivation-induced increase in DP coincided with the onset of sleep deprivation induced-increase in BDNF in the cortex, i.e., P20. FGF2 expression was increased during recovery sleep irrespective of age and brain region consistent with the elevated levels of CORT.

METHODS

Animals

A total of 107 animals were used in this study. Long-Evans rats were bred in the lab and maintained on a 12:12 h light/dark cycle, at an ambient temperature of 22°C. Food and water were available ad libitum. Day of birth was designated postnatal day 0 (P0), and litter sizes were culled to 8–12 pups. Surgery, recording, and sleep-deprivation procedures were performed as previously described (Hairson et al. 2001). Briefly, at P9, six male and female pups per litter were anesthetized with methoxyflurane inhalant (Metofane, Mallinckrodt Veterinary). Four EEG electrodes (No. 000 stainless steel screws) were fastened bilaterally in frontal and parietal bones. Three electro-
myographic (EMG) electrodes (stainless steel wire) were inserted into the nuchal muscles. All electrodes were attached to a seven-pin electric socket that was fastened to the skull with dental acrylic and the incision was sutured. After surgery pups were left to regain consciousness on a heating pad for ~1 h and then returned to their litter.

EEG/EMG recording

EEG signals were recorded from either left or right hemispheres (fronto-parietal derivation) on a Grass 7 polygraph with one channel for differential EEG and one for differential EMG, per animal. EEG potentials were filtered at 0.3 and 35 Hz (1/2 max, 6 dB/octave), digitized at 100 Hz and stored in 10-s epochs on a personal computer. The EMG signal was full-wave rectified and integrated for each epoch. The EEG signal was Fourier transformed, and vigilance states were scored using a modified scoring algorithm (Frank and Heller 1997a). Epochs with high power in 1- to 4-Hz range (delta power) and low EMG signal were scored as SWS, epochs with low delta power and low EMG signal were scored as PS, epochs with low delta power and high EMG signal were scored as wake. To determine the effects of sleep deprivation, the amount of SWS was calculated as percent of total recording time after sleep deprivation at all ages. Vigilance state amounts were compared with time-matched values obtained from non-sleep-deprived (no-SD) animals of the same age. DP within SWS was calculated as the proportion of the mean DP in SWS during the dark phase, preceding the sleep deprivation.

Radioimmunoassay

The radioimmunoassay was based on a procedure developed by Jacobson et al. (1993). Briefly, triplicate samples of plasma (10 μl) were heat-denatured at 80°C. [3H]corticosterone (Sigma) and CORT antisera (Endocrine Sciences) were added to the samples that were incubated overnight. [3H]CORT was separated from nonradioiodinated CORT using Dextran T70 (Amersham Pharmacia Biotech AB)-coated charcoal, and quantified in a liquid scintillation counter (Beckman model LS 3801). Competition binding was assessed against a standard curve of CORT (Sigma) ranging from 0.01 to 5 ng/ml. A standard curve was produced by a nonlinear least-squares formula and best-fit analysis based on the F distribution. To maintain between-assay reliability, only assays in which slope coefficients were within 10% of each other’s error were used.

cDNA probes

We measured the mRNA expression of BDNF, TrkB, FGF2, FGF2r, cFos, and β-actin using the following cDNA probes: 1.127-bp rat BDNF cDNA (Rosenhall et al. 1991); full-length (~5 kb) rat TrkB cDNA (Middlemas et al. 1991) (courtesy of Dr E. Shooter); 465-bp mouse FGF2 cDNA (ATCC Cat. No. F43348); 2.5-kb mouse FGF2r cDNA (ATCC Cat. No. F63344); full-length (~2 kb) rat cFos cDNA (Curran et al. 1987); and 800-bp human β-actin (Hanukoglu et al. 1983). To extract inserts, plasmids were digested for 2 h at 37°C with HindIII and NarI for BDNF, HindIII and XbaI for TrkB, EcoRI and XbaI for FGF2, HindIII and EcoRI for FGF2r, EcoRI for cFos, and PstI for β-actin (Life Technologies, GIBCO BRL). Inserts were separated from vectors by electrophoresis on 1% low-melting-point agarose gel (Life Technologies, GIBCO BRL). The insert bands were cut from the gel, dissolved in 3 volumes of distilled water, and stored at ~20°C until use. 32P-radiolabeled random primed cDNA probes were made with an activity of 5–10 × 106 CPM/μl.

RNA extraction and Northern blotting

A total of 13 Northern blots were generated. Twelve blots contained total RNA from animals of the same age group and killed at the same time point and included: SD, recorded but not SD (no-SD), and littermate controls (controls). Corresponding sets for cortical and hippocampal tissue were made for each time point and age. An additional blot contained cortical tissue samples from no-SD animals from all three age groups, killed at the end of the sleep deprivation.

Total RNA was extracted from whole cortical and hippocampal samples using the TRIZOL protocol (Life Technologies, GIBCO BRL). Eight micrograms total RNA from each sample (1 animal/sample) were fractionated by electrophoresis (100 mV) on 1.2% formaldehyde/agarose gel, transferred to positively charged nylon membranes (Hybond-n+, Amersham Pharmacia Biotech), and cross-linked to the membrane by UV irradiation.

After prehybridization, membranes were hybridized overnight at 42°C in 5× SSC, 50% formamide, 50 mM sodium phosphate pH 6.8, 1% SDS, 1 mM EDTA, 2.5× Denhardt’s, 200 mg/ml herring sperm DNA, and a radiolabeled random prime probe (minimum: 7 × 107/μl). Membranes were washed in 1× SSC for 10 min at room temperature and then twice in 0.1× SSC, 0.2% SDS for 25 min at 55°C. Membranes were then wrapped in a plastic sheet and exposed on BIOMAX MS film (Kodak). Films were developed and the images were digitized.

For quantification, background densitometry measures were subtracted for each animal/lane, and an analysis of variance (ANOVA) with regression analysis. The optical density (O.D.) values for each gene of interest were divided by the values derived from the β-actin probe.

Experimental procedure

To habituate animals to handling during sleep deprivation, all pups were handled for 10 min in their home cages, without separation from dam or litter, at least twice on the 2 days prior to the experiment. In preparation for EEG recording, animals were lightly anesthetized, and flexible recording cables were connected to their head implants that were connected to slip-ring commutators. To minimize stress due to separation from the dam, we employed a method developed by Frank et al. (1997a) and based on van Oers et al. (1998) that enables separation of rat pups from their mother and litter for a significant period of time without inducing significant changes in CORT levels (Hairston et al. 2001). Briefly, P16 animals received enriched milk formula via a cannula inserted through their cheek. An automatic pump (Harvard Apparatus) delivered 53 μl every 45 min throughout the experiment. In addition, P16 animals had their anal-genital region gently stimulated with a Q-Tip to induce elimination reflexes. The purpose of these interventions was to mimic maternal care. P20 and P24 animals were fed rat chow and water. Pups were placed in 12.5 × 12.5 × 12.5-cm acrylic incubators containing bedding from their home cage. An age-appropriate thermoneutral environment was maintained by placing the incubators in an acrylic water bath heated to 30, 27, or 24°C for P16, P20, and P24 animals, respectively.

Figure 1 depicts the experimental groups. EEG recording started at lights off the evening prior to deprivation. At lights on the next day, animals were sleep deprived by gentle handling for 90, 140, or 180 min for P16, P20, or P24 animals, respectively.

Statistical analysis

Sleep measures were analyzed using a three-way ANOVA with age (P16, P20, P24), treatment (SD, no-SD), and proportion in each state (i.e., wake, SWS, or PS) in 30-min bins as a repeated measures factor. Plasma CORT levels were analyzed using a three-way ANOVA with factors age, sleep condition (REC, no-REC), and treatment (SD,
no-SD, controls). mRNA expression was determined from the Northern blots wherein for each animal the O.D. of the gene of interest was divided with the O.D. for β-actin. Due to differences in exposure and probe potency, no comparisons were made across RNA probes or blots. Comparisons for each gene, within single blots, were done using the χ² median test, and the standard residual formula was used to determine the source of the effect for each comparison.

RESULTS

Sleep measures

During the dark phase, a significant decrease in time spent asleep was associated with age coupled with an equivalent increase in the amount of time spent awake. In addition, there were large age differences in the overall amounts of PS and wake but not in SWS. These effects were most pronounced during the latter part of the dark phase (see Fig. 2). A two-way ANOVA with age as main factor and proportion time spent in each state within 30-min bins as a repeated measures factor was performed on the 7 h of recording from midnight to lights on. A significant decrease in time spent in PS [F(7,33) = 13.6, P < 0.0001], a main effect of age [F(2,39) = 20.1, P < 0.001] and an interaction of age by time [F(14,64) = 2.9, P = 0.02], were found. An equivalent increase in the amount of time spent awake [F(7,33) = 22.9, P < 0.0001], a main effect of age [F(2,39) = 24.4, P < 0.001], and an interaction of age by time [F(14,64) = 3.2, P < 0.001] were found. For SWS a main effect of time bins [F(7,33) = 14.0, P < 0.001] and an interaction with age [F(14,64) = 2.6, P = 0.005] were observed.

The amount of sleep during the sleep deprivation period was previously reported (Hairston et al. 2001). From lights on to the end of the sleep deprivation, no-SD animals slept 55–84% of recording time, with the highest amounts of sleep found in P20 animals. At all ages, sleep deprivation reduced total sleep time by 80–85% compared with no-SD animals. Recovery animals expressed an increase in the amount of SWS subsequent to the sleep deprivation (Fig. 3). A three-way ANOVA with age and sleep deprivation as main factors, and percent of SWS (in 30-min bins) as repeated-measures factor, yielded a main effect of sleep deprivation [F(1,45) = 12.5, P = 0.003], an effect of time [F(3,45) = 3.6, P = 0.021], and a two-way interaction of sleep deprivation by time [F(3,45) = 3.2, P = 0.033]. A similar analysis for the amounts of PS yielded a main effect of time [F(3,45) = 6.9, P < 0.001] and an interaction of sleep deprivation by time [F(3,45) = 3.2, P = 0.032; data not shown].

There was an age-dependent effect on DP during recovery SWS sleep. A three-way ANOVA with age and sleep deprivation as main factors and DP (in 30-min bins) as repeated-measures factor, yielded a main effect of sleep deprivation [F(1,45) = 44.7, P < 0.001], a main effect of age [F(2,45) = 15.4, P = 0.008], and an interaction of sleep deprivation with age [F(2,45) = 8.0, P = 0.028]. This interaction was due to a lack of difference between SD and no-SD animals at P16 [contrast analysis: t(1,14) = 2.15, P = 0.06], small increase in delta power at P20 in the SD group [contrast analysis: t(1,14) = 3.0, P = 0.104], and a large increase at P24 [contrast analysis: t(1,14) = 9.7, P = 0.008].

Effects on plasma CORT

A summary of the effects of sleep deprivation, age, and recovery sleep is shown in Table 1. The changes in CORT levels in no-REC animals were previously published (Hairston et al. 2001). Briefly, CORT levels were significantly higher in SD animals at all ages compared with age-matched controls [F(2,64) = 43.2, P < 0.0001]. A significant age-dependent increase in basal levels of CORT [F(2,64) = 6.6, P = 0.002] was observed. Similar analysis of the plasma collected after 2 h recovery sleep (REC) yielded no effect of sleep deprivation; however, the age-dependent increase was sustained [F(2,32) = 16.8, P < 0.0001]. P16 and P20, but not P24 no-SD animals had higher CORT levels than littermate controls [Dunnett’s t(2) = 2.31, P = 0.05; t(2) = 2.26, P = 0.05, P16 and P20, respectively].

Effects on gene expression

Expression of all mRNAs was detectable at all ages in both brain regions. Sleep deprivation had no effect on the expression of β-actin that was used as a control probe. The BDNF probe yielded two transcripts of 4.0 and 1.6 kb. Although some variation could be observed between the two, separate analysis
of each band yielded similar results. We thus averaged the O.D. of both bands and used this value for further analyses.

Sleep deprivation effects on gene expression in cortex

cFos expression was increased in SD animals at all ages (P16: $\chi^2 = 6.2, P = 0.04$, P20: $\chi^2 = 8.5, P = 0.01$, P24: $\chi^2 = 10.2, P = 0.01$). There were no detectable changes in cFos, BDNF, and FGF2r expression at any age (see Table 2 for all P values).

Sleep deprivation effects on gene expression in hippocampus

cFos expression was increased in SD animals at all age groups (P16: $\chi^2 = 6.0, P = 0.05$, P20: $\chi^2 = 7.4, P = 0.03$, P24: $\chi^2 = 8.9, P = 0.03$).

FIG. 2. The distribution of arousal states at the different ages during the dark phase, starting at midnight. Percent of 30 min bins spent in each state [paradoxical sleep (PS), slow-wave sleep (SWS), wake] were calculated for each animal during the dark phase before the sleep deprivation, and averaged across age groups. A–C: the means ± SE of the percent in each state within each age group. There were age-dependent changes in the amounts of wake and PS with no change in SWS, expressed as a main effect of age in PS and wake [$F(2,39) = 30.1, P < 0.001; F(2,39) = 24.4, P < 0.001$, respectively], and an interaction of the age factor with all 3 sleep states [PS: $F(14,64) = 2.9, P = 0.02$; SWS: $F(14,64) = 2.6, P = 0.005$; wake: $F(14,64) = 3.2, P < 0.001$]. In addition, the distribution of states within the dark phase revealed the emergence of diurnal regulation. At P16, the 3 states are evenly distributed, whereas in the older animals, an increase in the percent time spent awake increases across the dark phase.

FIG. 3. Percent time spent in SWS and delta power (DP) within SWS expressed in equivalent 30 min time bins during recovery sleep (i.e., starting from the end of the sleep deprivation). The percent of 30 min bins spent in SWS (no-SD; Δ, SD) were calculated for each animal and averaged across age group. Superimposed are the 1- to 4-Hz integrated values (no-SD; Δ, SD), corrected to the mean value derived from the baseline night recording. There were no age-dependent differences in the amount of SWS during recovery. A 3-way ANOVA with age and sleep deprivation as main factors and percent of SWS (in 30 min bins) as repeated-measures factor, yielded a main effect of sleep deprivation [$F(1,45) = 12.5, P = 0.003$] with no effect of age. There were age-dependent differences in DP, wherein a similar analysis for DP yielded a main effect of sleep deprivation [$F(1,45) = 44.7, P < 0.001$], a main effect of age [$F(2,45) = 15.4, P = 0.001$], and an interaction of sleep deprivation with age [$F(2,45) = 8.0, P = 0.028$].

FIG. 3. Percent time spent in SWS and delta power (DP) within SWS expressed in equivalent 30 min time bins during recovery sleep (i.e., starting from the end of the sleep deprivation). The percent of 30 min bins spent in SWS (no-SD; Δ, SD) were calculated for each animal and averaged across age group. Superimposed are the 1- to 4-Hz integrated values (no-SD; Δ, SD), corrected to the mean value derived from the baseline night recording. There were no age-dependent differences in the amount of SWS during recovery. A 3-way ANOVA with age and sleep deprivation as main factors and percent of SWS (in 30 min bins) as repeated-measures factor, yielded a main effect of sleep deprivation [$F(1,45) = 12.5, P = 0.003$] with no effect of age. There were age-dependent differences in DP, wherein a similar analysis for DP yielded a main effect of sleep deprivation [$F(1,45) = 44.7, P < 0.001$], a main effect of age [$F(2,45) = 15.4, P = 0.001$], and an interaction of sleep deprivation with age [$F(2,45) = 8.0, P = 0.028$].
TABLE 1. Effects of sleep deprivation and recovery sleep on plasma CORT levels

<table>
<thead>
<tr>
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<th>P16</th>
<th>P20</th>
<th>P24</th>
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<tr>
<td></td>
<td>No-REC</td>
<td>REC</td>
<td>No-REC</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>n</td>
<td>8</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>No-SD</td>
<td>7</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>SD</td>
<td>6</td>
<td>5</td>
<td>5</td>
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</table>

Values are means ± SE in μg/dL of corticosterone (CORT) derived from the radioimmunoassay. *, a significant difference between the group and control, †, difference from non-sleep-deprived (no-SD) animals. There was an age-dependent increase in the amount of CORT [F(2, 64) = 6.6, P = 0.002], and at all ages, SD animals had high levels of CORT compared to no-SD and controls [F(2, 64) = 43.2, \( P < 0.0001 \)]. After 2 h recovery sleep (REC), CORT levels were similar to no-SD and control sibling killed at the same time point. In addition, at P16 and P20, but not at P24, no-SD animals had significantly higher CORT levels than their littermate controls (Dunnett’s \( n(2) = 2.31, P = 0.05; n(2) = 2.26, P = 0.05 \), respectively).

P24: \( \chi^2 = 8.9, P = 0.01 \) (Fig. 5). BDNF levels were increased at P24 (\( \chi^2 = 9.1, P = 0.01 \)). TrkB levels were increased in SD animals at P20 (\( \chi^2 = 6.5, P = 0.04 \)) and at P24 (\( \chi^2 = 5.8, P = 0.05 \)); data not shown). FGF2 was significantly increased in SD and no-SD animals compared with control at P16 (\( \chi^2 = 9.0, P = 0.01 \)). There were no detectable changes in FGF2r at any age (data not shown).

Age-dependent effects on the expression of BDNF in the cortex (Fig. 6)

To eliminate the possibility that constitutively high levels of BDNF expression at the younger age may explain the lack of change in BDNF after sleep deprivation in P16 animals, a blot containing cortical sample of no-SD animals from all three age groups was probed for BDNF and \( \beta \)-actin. There were no detectable changes in BDNF expression between the age groups (\( \chi^2 = 3.5, P = 0.17 \)).
TABLE 2.  Gene expression after 2 h recovery sleep

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cortex</th>
<th>Hippocampus</th>
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<tr>
<td></td>
<td>P16</td>
<td>P20</td>
</tr>
<tr>
<td>BDNF</td>
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</tr>
<tr>
<td>SD</td>
<td>0.382 ± 0.07</td>
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<tr>
<td>No-SD</td>
<td>0.346 ± 0.10</td>
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<tr>
<td>Control</td>
<td>0.329 ± 0.13</td>
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<tr>
<td>TrkB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.770 ± 0.07</td>
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<tr>
<td>No-SD</td>
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<td>Control</td>
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<tr>
<td>FGF2</td>
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<tr>
<td>FGF2r</td>
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<td>cFos</td>
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<tr>
<td>Control</td>
<td>0.494 ± 0.24</td>
<td>0.653 ± 0.21</td>
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Gene expression after 2 h recovery sleep. Values are ±SE and the results of the χ² median test for all mRNAs shown as the ratio to β-actin expression. †, a significant difference between the SD, and no-SD and/or control animals. BDNF, brain-derived neural growth factor; FGF2, basic fibroblast growth factor.
We assessed changes in gene expression after sleep deprivation in neonatal rats. The main findings were an age- and brain-region-independent increase of cFos after sleep deprivation; an age-dependent increase in BDNF expression in the cortex and hippocampus after sleep deprivation, which correlated with the age when increased DP after sleep deprivation was observed; and an age- and brain-region-independent increase in FGF2 expression during recovery sleep.

The age-dependent effects on sleep states and response to sleep deprivation were largely consistent with previous reports (Frank and Heller 1997a,b; Frank et al. 1998). After sleep deprivation, there were no changes in DP at P16, a small increase at P20, and a large increase at P24. Notably, Frank et al. (1998) did not show an increase in DP at P20. This divergence from previous published findings is probably due to natural variance in maturation rate between cohorts.

In this study, the sleep-deprivation duration was titrated to the different ages, based on the assumption that the ability to endure sleep-deprivation increases with age. This assumption is substantiated by our previously reported observation that the amplitude of CORT increase, due to sleep deprivation, decreased with age (Hairston et al. 2001). Within 2 h of recovery sleep, CORT levels were similar to no-SD animals, indicating that the stress-response duration was similar across age groups. The conditions used for recording sleep in neonatal rats, given the right parameters (i.e., increased ambient temperature and stimulated elimination reflexes), caused a small increase in CORT levels in P16 and P20 no-SD animals, suggesting that maternal separation may have induced a mild stress response in younger animals. As CORT increases FGF2 expression (Chao and McEwen 1994), this observation may explain the mild increases in FGF2 in P16 recorded animals compared with littermate controls.

The sleep-deprivation effects on cFos expression were consistent with the effects in adults. cFos levels were increased independent of age and brain region, despite varying duration of sleep deprivation and different sleep patterns between age groups. In adult rats, cFos is highly expressed both during sleep deprivation and during spontaneous wake (Pompeiano et al. 1994), suggesting that its expression reflects the arousal state and not the stress of sleep deprivation.

Sleep-deprivation effects on the two growth factors and their receptors were markedly different. In the cortex, BDNF expression was increased in SD P20 and P24 but not P16, whereas TrkB increases were observed in P24 SD animals after recovery sleep only. In the hippocampus, P24 SD animals demonstrated increased BDNF, whereas TrkB expression was increased in SD animals at P20 and P24. FGF2 expression was increased after recovery sleep, independent of age and brain region, whereas FGF2r levels were unaffected by sleep deprivation.

The time delay between mRNA synthesis and BDNF protein translation is unknown. However, Nanda and Mack (2000) demonstrated elevated levels of BDNF protein (>100% of control) in the cortex 1 h after whisker stimulation, reaching peak values within 4 h. If a similar time course of transcription-translation occurs post sleep deprivation, it is possible that the correlation between the developmental emergence of wake-induced expression of BDNF with the emergence of wake-dependent DP indicates a functional relationship between BDNF and DP augmentation.

The age groups in this study represent a developmental period characterized by increased synaptic connectivity of the noradrenergic and cholinergic systems to neocortical regions. Activity in both the noradrenergic (NA) and cholinergic systems is associated with wake maintenance and arousal quality (Berridge and Waterhouse 2003; Sarter and Bruno 1999). NA projections to the cortex are present and innervating the appropriate cortical layers by P3 (Foote and Morrison 1987; Levitt and Moore 1979). However, during the third week of postnatal development (i.e., P15–21), an increase in noradrenaline and tyrosine hydroxylase content in the neocortex occurs, reaching adult levels at the beginning of week four (Foote and Morrison 1987; Levitt and Moore 1979; Loizou 1971). Similar to the NA system, cortical cholinergic afferents increase in density throughout weeks three to six (Coyle and Yamamura 1976).

Cirelli and co-workers have shown that BDNF and TrkB transcription in the cortex is mediated by ascending NA fibers from the locus coeruleus (Cirelli 2002; Cirelli and Tononi 2000; Tononi and Cirelli 2001). Additionally, BDNF is synthesized by excitatory neurons located in projection fields of basal forebrain cholinergic neurons (Thoenen et al. 1991), suggesting a cholinergic role in the expression of BDNF. Mitzoguchi et al. (2002) have recently demonstrated that BDNF caused sustained increases of intracellular Ca\(^{2+}\) and Ca\(^{2+}\)-activated potassium currents in cortical neurons, both of which are believed to mediate cellular mechanisms underlying slow-wave oscillations observed during SWS (Bazhenov et al. 2002; Massimini and Amzica 2001). The potential role for BDNF in activity-dependent increase of DP is further indicated by the observations that dark rearing reduces DP from visual cortex derivation (Miyamoto et al. 2003) as well as locally attenuates BDNF expression in the same region (Tropea et al. 2001).

Given the developmental changes in neuromodulator excitatory input to the cortex, the involvement of BDNF in intracellular Ca\(^{2+}\) and Ca\(^{2+}\)-activated potassium currents, and the

**DISCUSSION**

**FIG. 6.** Age-dependent effects on the expression of BDNF in the cortex of no-SD animals, indicating that age did not affect the level of BDNF expression. A: mean and SE for each age group. The values for BDNF are the average of the 2 bands. B: Northern blot probed for of BDNF (4.0 kb, upper; 1.6 kb, lower), and β-actin (1.9 kb). No age-dependent differences in cortical BDNF mRNA were found.
relationship between sensory input, DP development, and BDNF expression, we propose the following sequence of events: neuromodulator activity during wake increases BDNF expression in the adult cortex; BDNF, in turn, facilitates inhibitory signaling in cortical neurons via its effects on intracellular Ca\(^{2+}\); increased inhibition of cortical neurons results in the augmented power in delta oscillations. At P16, BDNF signaling is attenuated, thus slow-wave activity during SWS would not reflect wake history. Notably, Kushikata et al. (1999) reported that intracerebroventricular administration of BDNF did not increase DP, whereas an increase in SWS amount was observed. Combined with our findings, this may suggest that BDNF is necessary but not sufficient to mediate an increase in DP.

Sleep deprivation resulted in elevated levels of CORT, especially in younger animals. Repeated and prolonged increases in CORT levels are associated with reduced levels of hippocampal BDNF (Chao and McEwen 1994; Hansson et al. 2003; Nibuya et al. 1999), although BDNF expression can be increased after a short-duration of stress (i.e., 3 h) (Marmigere et al. 2003) and by excitatory cholinergic (da Penha Berzaghi et al. 1993; Thoenen et al. 1991) or glutamatergic activity (Thoenen et al. 1991). Thus BDNF expression in the hippocampus can be increased under conditions associated with either decrease or increase in CORT levels.

In this study, BDNF increased in the hippocampus only of SD P24 animals and remained elevated after recovery sleep. As the hippocampus is anatomically mature by P16 and pruning constitutes the main developmental event henceforth (Gaarskaer 1985), it is unlikely that our results can be explained by an age-dependent increase of excitatory input. Moreover, our findings are inconsistent with previous studies in adults in which BDNF mRNA or protein levels in the hippocampus were unaffected by total sleep deprivation (Taishi et al. 2001) or selective paradoxical sleep deprivation (Sei et al. 2000). On the other hand, it is possible that our findings reflect the time course of BDNF transcription after stress. In a recent study by Marmigere et al. (2003), BDNF mRNA levels were measured after immobilization stress of varying duration. These authors showed that BDNF levels increased within 3 h and then proceeded to decline, to below control levels, after 5 h of immobilization stress. Thus after 3 h of sleep deprivation, our P24 animals would be exactly at the peak of the stress-induced BDNF increase.

FGF2 expression increased in both brain regions only during recovery sleep. FGF2 expression is mediated by intracellular Ca\(^{2+}\), cAMP, β-adrenergic receptor activation, and activation of cholinergic nicotinic receptors (Belluardo 1999; Belluardo et al. 1999; Follesa and Mocchetti 1993; Riva et al. 1996, 1997; Roceri et al. 2000), suggesting that its transcription is activity dependent. The time course observed for β-adrenergic induced transcription of FGF2 was 4–5 h in adult animals (Follesa and Mocchetti 1993; Hansson et al. 2003). Hence, we conclude that the observed increase in FGF2, during recovery sleep, was in response to the stimulation of the sleep deprivation. It is unlikely that FGF2 plays a role in inducing compensatory sleep patterns as it does not seem to correlate with age, and it has been previously shown that FGF2 administration had no effect on any sleep parameter (Knefati et al. 1995).

In conclusion, this study addressed the changes in gene expression after sleep deprivation in neonatal rats. It has been established that marked changes in sleep architecture, homeostatic, and circadian regulation of sleep occur throughout development. While the mechanism underlying DP increase after prolonged wake is unknown, it has been proposed to reflect experience- or use-dependent processes (Meerlo et al. 1997; Vyazovskiy et al. 2000) and not only the time spent awake. Our findings suggest BDNF signaling as a candidate for facilitating DP increase after prolonged wake or enriched experience.

We also demonstrated that sleep deprivation increased FGF2 expression during subsequent recovery sleep. FGF2 stimulates proliferation and neuronal fate (Bennharroch and Birnbaum 1990; Palmer et al. 1999) and is increased after exposure to wheel-running (Gomez-Pinilla et al. 1997) and enrichment (Schneider et al. 2001). Thus the consequence of mild sleep deprivation may have long-term trophic effects. This contrasts with reduced proliferation after 96 h of sleep deprivation observed by Guzman-Marín et al. (2003) in adult rats but agrees with the neuroprotective and synaptogenic role of BDNF increases after short periods of sleep deprivation.

Although it remains to be determined whether sleep is necessary for the downstream action of growth-related proteins, our observations contribute to growing evidence implicating waking experience in sleep quality. Early experiences such as prenatal stress, postnatal handling, and enriched environment have long-lasting effects on sleep patterns and stress management (Dugovic et al. 1999; Meaney et al. 1991; Mirmiran et al. 1982) and have long-term implications for the quality of aging (Fuxe et al. 1996; Johansson et al. 1999; Mohammed 1993). It is possible that changes in sleep quality are a necessary component of the cellular processes underlying these neuroprotective effects.

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