Exploratory Behavior, Cortical BDNF Expression, and Sleep Homeostasis

Reto Huber, PhD; Giulio Tononi, MD, PhD; Chiara Cirelli, MD, PhD

Department of Psychiatry, University of Wisconsin-Madison, Madison, WI

Study Objective: Slow-wave activity (SWA; 0.5-4.0 Hz) during non-rapid eye movement (NREM) sleep is a reliable indicator of sleep need, as it increases with the duration of prior wakefulness and decreases during sleep. However, which biologic process occurring during wakefulness is responsible for the increase of sleep SWA remains unknown. The aim of the study was to determine whether neuronal plasticity underlies the link between waking activities and the SWA response.

Design: We manipulated, in rats, the amount of exploratory activity while maintaining the total duration of waking constant. We then measured the extent to which exploration increases cortical expression of plasticity-related genes (BDNF, Arc, Homer, NGFI-A), and the SWA response once the animals were allowed to sleep.

Setting: Basic neurophysiology and molecular laboratory.

Participants: Male Wistar Kyoto rats (250-300g; 2-3 month old).

Interventions: None.

Results: We found that, within the same animal, the amount of exploratory behavior during wakefulness could predict the extent to which BDNF was induced, as well as the extent of the homeostatic SWA response during subsequent sleep.

Conclusions: This study suggests a direct link between the synaptic plasticity triggered by waking activities and the homeostatic sleep response and identifies BDNF as a major mediator of this link at the molecular level.

Keywords: Cerebral cortex, BDNF, novelty, sleep deprivation, synaptic plasticity

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INTRODUCTION

SLEEP IS HOMEOSTATICALLY REGULATED AS A FUNCTION OF PRIOR WAKEFULNESS IN SPECIES RANGING FROM DROSOPHILA MELANOGASTER1 TO HUMANS:2 the longer one stays awake, the longer and more intensively one sleeps. In mammals, a well-established electrophysiologic correlate of sleep homeostasis is the amount of slow-wave activity (SWA; power density in the electroencephalogram [EEG] between 0.5 and 4.0 Hz) during non-rapid eye movement (NREM) sleep.2 NREM SWA (from now on referred to simply as SWA) increases in proportion to the time spent awake and decreases during sleep (e.g., Huber et al).3 Thus, SWA is thought to reflect the accumulation of sleep need during wakefulness and its release during sleep. Unfortunately, the biologic process responsible for the increase of SWA as a function of prior wakefulness, and possibly underlying the need for sleep, remains unknown.

Intriguingly, novel experimental evidence suggests that, if a given brain region is strongly activated by a specific waking activity, that region will show the strongest SWA response during subsequent sleep.4,6 Most relevantly, in a study with high-density EEG in humans, it was shown that SWA was locally increased in a specific cortical region after a visuomotor learning task involving that region, but not after a kinematically equivalent motor task that did not require learning.7 On the basis of this and other evidence, it has been suggested that the homeostatic SWA response is a direct consequence of the amount of synaptic plasticity, notably synaptic potentiation, occurring during the prior waking period.8 According to this hypothesis, sleep SWA should depend not just on the duration or quantity of prior waking, but also on the kind or quality of waking.

In animal models, the exposure to novel and enriched environments enhances exploratory behavior, and induces widespread plasticity in cortical and hippocampal circuits.9,10 At the cellular level, enrichment enhances synaptic density,11 and induces the expression of brain-derived neurotrophic factor (BDNF),12 activity-regulated cytoskeleton-associated protein (Arc),13 Homer,14 and nerve growth factor-induced gene A (NGFI-A).14 To date, the most compelling evidence linking gene expression with synaptic plasticity, and most specifically synaptic potentiation, has been provided for BDNF. For instance, the whisker stimulation-induced synaptogenesis in the barrel cortex is impaired in BDNF-deficient adult mice,15 and BDNF signaling regulates spine maintenance in the adult mouse visual cortex.16 Furthermore, BDNF in the rat parietal cortex is required for the acquisition of a fear-motivated task,17 and, in the insular cortex, it may enhance the retention of a conditioned taste aversion task.18 Finally, in the human motor cortex, a BDNF polymorphism has been recently associated with changes in experience-dependent plasticity.19 Thus, BDNF is emerging as a major mediator of synaptic plasticity in vivo, in adult animals, and specifically in the cerebral cortex. Arc,20 Homer,21 and NGFI-A22 have also been to some extent linked to synaptic plasticity, although not as directly as BDNF.

To determine whether neuronal plasticity underlies the link between waking activities and the SWA response, we manipulated in rats the amount of exploratory activity while maintaining the total duration of waking constant. We then measured the extent to which exploration increases the expression in the cerebral cortex of BDNF, Arc, Homer, and NGFI-A, as well as the SWA response once the animals were allowed to sleep. We predicted that, for the same amount of wakefulness, increased exploratory activity should be associated with an increase in the expression of BDNF and other markers of neuronal activity and plasticity. More im-

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Address correspondence to: Chiara Cirelli, MD, PhD, University of Wisconsin-Madison, Department of Psychiatry, 6001 Research Park BLVD, Madison WI 53719; Tel: (608) 263-9236; Fax: (608) 263-9340; E-mail: ccirelli@wisc.edu


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portantly, we predicted that, if SWA depends on how much synapic plasticity occurred during prior waking, the SWA response should be higher if rats spend more time exploring and molecular markers of synaptic plasticity are more strongly induced. Most stringently, the amount of exploratory behavior during wakefulness should be able to predict the extent to which plasticity-related genes such as BDNF are induced, as well as the extent of the SWA response during subsequent sleep. As described below, we found that the SWA response is positively correlated with both the amount of waking exploratory behavior and the increase in the cortical expression of BDNF.

METHODS

Experimental Animals, Surgery, and Sleep Deprivation

Male Wistar Kyoto (WKY) rats (250-300g; 2-3 month old) were implanted for chronic polygraphic recordings with epidural EEG electrodes over the right occipital cortex, the right parietal cortex, and the right frontal cortex and a reference electrode over the cerebellum. In addition, gold wires were inserted into the neck muscle and used to record the electromyogram (EMG). The electrodes were connected to stainless-steel wires that were soldered to a plug and anchored to the skull with dental cement. Each animal was individually housed in a Plexiglas cage (36×25×45cm) inside a single sound-proof chamber. Food and water were available ad libitum and replaced daily at 10 am. Each day, from 10 to 10:30 AM, all rats were gently handled and exposed to a new object to become familiar with the sleep-deprivation procedure (these objects were not reused during sleep deprivation). Novel objects included nesting and bedding material from other rat cages, wooden blocks, small rubber balls, plastic, metallic, wooden, or paper boxes and tubes of different shape and color. The boxes often had holes through which the rat could reach for a palatable food pellet or a paper towel. The room temperature was maintained at 22°C to 24°C.

Experiment 1

At least 1 week after surgery, all rats (n = 10) underwent the following experimental protocol: (1) 24-hour baseline recording in 12-hour:12-hour light:dark (LD) conditions (lights on at 10 am, 100 to 200 lux as measured 2 cm above the floor level, < 1 lux during the dark period), followed immediately by (2) 24-hour recording in LD including 6 hours of sleep deprivation (starting at 10 am) followed by 18 hours of recovery sleep; (3) 24-hour baseline recording in constant dark (DD) conditions (starting at 10 am, when lights remained off) followed, 3 to 4 days later, by (4) 24-hour recording in DD conditions (starting at 10 AM, when lights remained off) including 6 hours of sleep deprivation (starting at 10 AM) followed by 18 hours of recovery sleep. During the 3 to 4 days between baseline in DD and sleep deprivation in DD, rats went back to their regular 12:12 LD schedule. Sleep deprivation in LD and DD were spaced at least 1 week apart. Half of the animals were sleep deprived in LD first, and the other half in DD first. Rats were sleep deprived by introducing novel objects in their cages. Every new object was delivered just following the first signs of synchronization in the EEG signal or any behavioral sign of sleep. During sleep deprivation in DD, enough light for the experimenter to see the rat was still coming from the screen of the computer where the online EEG data were being collected. The monitor was located approximately 2 to 3 meters from the cages, oriented with the back toward the rat cages. In DD, light intensity at cage floor level was always less than 1 lux.

Experiment 2

Three groups of animals not previously used (n = 5 per group) and kept during baseline in 12-hour:12-hour LD conditions were deprived of sleep for 6 hours starting at 10 AM with lights on, or deprived of sleep for 6 hours starting at 10 AM with lights off, or allowed to sleep at libitum for 6 hours with lights on starting at 10 AM. The percentages of behavioral states in sleeping animals for the last 6 hours before sacrifice were as follows (mean ± SEM): wakefulness = 21.3 ± 1.2, NREM sleep = 63.8 ± 1.4, REM sleep = 14.9 ± 0.6. All 15 rats were then immediately decapitated at 4 PM under isoflurane anesthesia. Brains were quickly removed, and the cerebral cortex from the right side carefully dissected out and divided into 3 regions, frontal (including all cortical areas rostral to Bregma (B) = 0), parietal (from ~B = 0 to ~B = -4), and occipital (including all cortical areas caudal to B = -4). The dissected tissues were stored at -80°C until used for quantitative polymerase chain reaction (PCR).

Experiment 3

Rats not previously used (n = 10) and kept during baseline in 12-hour:12-hour LD conditions were deprived of sleep for 5 to 6 hours starting at 10 am with lights on and then immediately decapitated under isoflurane anesthesia as soon as they completed 10 minutes of NREM sleep. In all animals, behavior was continuously assessed using EEG, EMG, and video recordings. Brains were quickly removed, and the whole right cerebral cortex carefully dissected out and stored at -80°C until used for quantitative PCR. All animal protocols followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were in accordance with institutional guidelines.

EEG and Behavior Analysis

Rats were connected by means of a flexible cable and a commutator (Airflyte, Bayonne, NJ) to a Grass electroencephalograph (mod. 15LT, Astro-Med. Inc., West Warwick, RI). The EEG and EMG signals were amplified, conditioned by analogue filters (high-pass filter: -3 dB at 0.016 Hz; low-pass filter: -3 dB at 40 Hz; less than -35 dB at 128 Hz), sampled at 512 Hz, digitally filtered (EEG: high-pass filter 0.3 Hz; low-pass FIR filter 25 Hz; EMG: band-pass FIR filter 20-50 Hz) and stored with a resolution of 256 Hz. Sleep stages were scored visually based on 4-second epochs (SleepSign, Kissei). Epochs that could not be scored for technical or logistical reasons (e.g., cleaning of the cages) accounted for 1.0% ± 0.4% of the total 24-hour baseline recording time in LD and 0.1% ± 0.1% in DD. Epochs that were scored but contained EEG artifacts were marked and omitted from further analysis of the spectra (number of artifacts during baseline, expressed as percentage of total 24-hour recording time: LD 8.4 ± 4.0, DD 7.4 ± 3.9; more than 85% were waking artifacts). EEG power spectra of consecutive 4-second epochs (fast Fourier transform routine, Hanning window) were calculated for the parietal or occipital derivations within the frequency range of 0.25 to 25.0 Hz. The results refer to the occipital derivation in Experiment 1 and to the parietal derivation in Experiment 3 because they provided the
best data quality in all rats for the entire experiment. In agreement with previous studies (e.g., Vyazovskiy et al), SWA values were higher in the frontal versus parietal and/or occipital derivation, but we found no systematic differences when we compared SWA in parietal versus occipital derivations of individual rats. The final analysis did not focus on the frontal derivation because of the lack of reliable recordings in some of the animals.

Individual real-time video recordings using infrared cameras were obtained for the first 6 hours (10 AM–4 PM) of the 4 experimental days in a subgroup of the animals in Experiment 1 (5 out of 10 during baseline, 6 out of 10 during sleep deprivation), and in all animals for Experiment 3 (n = 9). Waking behavior for each 10-second epoch was classified into 3 categories: (1) exploring; (2) grooming, including eating and drinking; and (3) quiet waking (the rat had open eyes, maintained the head in the upright position, but showed no body movements). Waking behaviors were similarly distributed across the 6 hours of recordings in all animals. Examples of the scoring of waking behavior is provided in Supplementary Figures 1 and 2 (available at www.journalsleep.com), which show that EMG activity during active waking was highly variable, and it was impossible to predict, based solely on neck EMG values, whether the rat was engaged in active exploratory behavior or in more automatic behaviors such as grooming, eating, and drinking.

The duration and frequency of episodes was computed based on minimal interruption criteria previously used in rats. Specifically, the minimum episode duration for waking, NREM sleep, and REM sleep was 32, 24, and 4 seconds, respectively. Brief awakenings were defined as uninterrupted waking episodes shorter than 16 seconds and computed per hour of total sleep. Differences were analyzed by 3-way analysis of variance (ANOVA) with factors ‘condition’ (LD vs DD), ‘day’ (baseline vs recovery), and ‘time interval’. Contrasts were tested by post-hoc two-tailed t-tests only if the main factor or interaction of the ANOVA reached significance. We performed multiple regression analysis (Statistica) to dissect individual contributions of correlation factors. β Coefficients were calculated to reveal the relative contribution of each independent variable (i.e., amount of exploratory, wakefulness, sleep) in the prediction of the dependent variable (i.e., SWA during the first hour after sleep deprivation).

**Real-time Quantitative PCR**

Real-time quantitative PCR was performed essentially as described, with small modifications. In Experiment 2, reverse transcription reactions were carried out in parallel on DNase I-digested pooled total RNA from sleep and sleep-deprived rats (n = 5 rats per pool) and cleaned using the Qiagen RNeasy Kit RNA Cleanup protocol. In Experiment 3, reverse-transcription reactions were carried out in parallel on DNase I-digested total RNA from the pool of sleeping rats (same pool as in Experiment 2) and from individual sleep-deprived rats (n = 9). Reverse-transcription reactions were as follows: 50 ng total RNA, 6.25 µL oligo dT16 (100 µM), and H2O to 28.75 µL total. Samples were incubated at 70°C for 10 minutes, put briefly on ice, and then incubated at 42°C for 2 to 5 minutes. Mix #2 (10 µL of 5X Superscript III First Strand Buffer, 5 µL of 0.1 M DTT, 5 µL dNTPmix (10 mM each dNTP), and 1.25 µL Superscript III RNAse H–Reverse Transcriptase) was added (21.25 µL for each reverse-transcription reaction) and mixed, and samples were immediately returned to incubate at 42°C for 1 hour. Reactions were stopped by incubation at 70°C for 15 minutes. PCR reactions to measure levels of rat ribosomal protein S12 cDNA were done to confirm uniformity of reverse transcription between samples, and differences in concentration were adjusted for. Each PCR reaction contained specific forward and reverse primers (200-750 nM final concentration), 2X SYBR Green Master Mix, reverse transcription product in a concentration of 50 ng/µL, and 1.25 µL of 5X SYBR Green Master Mix. Real-time quantitative PCR was performed essentially as described, with small modifications. In Experiment 2, reverse transcription reactions were carried out in parallel on DNase I-digested pooled total RNA from sleep and sleep-deprived rats (n = 5 rats per pool) and cleaned using the Qiagen RNeasy Kit RNA Cleanup protocol. In Experiment 3, reverse-transcription reactions were carried out in parallel on DNase I-digested total RNA from the pool of sleeping rats (same pool as in Experiment 2) and from individual sleep-deprived rats (n = 9). Reverse-transcription reactions were as follows: 50 ng total RNA, 6.25 µL oligo dT16 (100 µM), and H2O to 28.75 µL total. Samples were incubated at 70°C for 10 minutes, put briefly on ice, and then incubated at 42°C for 2 to 5 minutes. Mix #2 (10 µL of 5X Superscript III First Strand Buffer, 5 µL of 0.1 M DTT, 5 µL dNTPmix (10 mM each dNTP), and 1.25 µL Superscript III RNAse H–Reverse Transcriptase) was added (21.25 µL for each reverse-transcription reaction) and mixed, and samples were immediately returned to incubate at 42°C for 1 hour. Reactions were stopped by incubation at 70°C for 15 minutes. PCR reactions to measure levels of rat ribosomal protein S12 cDNA were done to confirm uniformity of reverse transcription between samples, and differences in concentration were adjusted for. Each PCR reaction contained specific forward and reverse primers (200-750 nM final concentration), 2X SYBR Green Master Mix, reverse transcription product in a concentra-
Figure 2a,c—Effects of lighting conditions on the time course of non-rapid eye movement (NREM) sleep electroencephalogram (EEG) power density in the slow-wave activity frequency range (SWA, 0.5-4 Hz) during baseline (A) and after 6 hours of sleep deprivation (B, n = 10). Curves connect 1-h mean values (± SEM) of SWA expressed for every individual animal as a percentage of the 24-h baseline mean (= 100%) for rats kept under lights-on (LD) and the lights-off (DD) conditions. Triangles indicate differences between the LD and the DD conditions (p < .05, 2-tailed paired t test after significance in ANOVA). Diamonds indicate differences between intervals after sleep deprivation and the corresponding baseline intervals for LD (open) and DD (filled) conditions (p < .05, 2-tailed paired t test after significance in ANOVA). Note that, in the second part of the dark period, SWA values are sometimes lower in the DD than in the LD condition, despite the slightly higher percentage of time spent in NREM sleep in DD. b,d Effects of lighting conditions on EEG power density during NREM sleep during baseline (B) and after 6 hours of sleep deprivation (D, n=10). Curves connect means (± SEM) of relative power density in hours 3-4 during baseline and during the first 2 hours after sleep deprivation expressed as percentage of power in the same frequency bin during the 24-h baseline. Bars indicate frequency bins for which power in rats kept under DD was significantly different from power in rats kept under LD conditions (unpaired t test, p < .05).
The SWA Response Increases After Acute Dark Exposure

Figure 2a depicts the changes in the time course of NREM SWA during baseline. Under both lighting conditions, as expected, SWA peaked shortly after the onset of the subjective light phase and decreased thereafter. However, SWA reached higher values when lights remained off compared to when they were on. This occurred despite comparable 24-hour and 12-hour values of waking, NREM sleep, and REM sleep in LD and DD (Table; Figure 3). Figure 2b shows the effects of lighting conditions on the entire EEG power spectrum in NREM sleep during the early part of the major sleep phase, ie, when SWA differed between LD and DD. It is evident that the higher EEG power density observed when lights were off relative to when they were on is specific for the SWA frequency range, and it does not extend to other frequency bands.

The Table shows that the exposure to novel objects kept rats awake for more than 96% of the time with lights both on or off. Moreover, during the first 5 to 6 hours after sleep deprivation, rats in both lighting conditions showed a similar increase in NREM sleep.

Table 1—Amounts of Wakefulness, NREM Sleep, and REM Sleep in Rats Kept Under Lights-on (LD) or Lights-off (DD) Conditions

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Wakefulness</th>
<th>NREM sleep</th>
<th>REM sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD</td>
<td>DD</td>
<td>LD</td>
</tr>
<tr>
<td>1-24</td>
<td>63.2 ± 3.4</td>
<td>62.8 ± 4.5</td>
<td>29.0 ± 2.6</td>
</tr>
<tr>
<td>1-12</td>
<td>50.3 ± 4.1</td>
<td>53.7 ± 5.4</td>
<td>38.0 ± 3.0</td>
</tr>
<tr>
<td>13-24</td>
<td>76.2 ± 3.2</td>
<td>71.9 ± 3.8</td>
<td>19.9 ± 2.8</td>
</tr>
<tr>
<td>1-6</td>
<td>41.3 ± 3.9</td>
<td>46.3 ± 4.0</td>
<td>45.3 ± 2.8</td>
</tr>
<tr>
<td>7-12</td>
<td>59.4 ± 4.7</td>
<td>61.1 ± 7.1</td>
<td>30.7 ± 3.3</td>
</tr>
<tr>
<td>13-18</td>
<td>79.0 ± 3.0</td>
<td>73.9 ± 3.7</td>
<td>17.1 ± 2.7</td>
</tr>
<tr>
<td>19-24</td>
<td>72.4 ± 3.6</td>
<td>63.8 ± 3.6</td>
<td>22.7 ± 3.1</td>
</tr>
<tr>
<td>6-h sleep deprivation</td>
<td>96.5 ± 1.5</td>
<td>98.9 ± 0.6</td>
<td>3.3 ± 1.4</td>
</tr>
<tr>
<td>9-12</td>
<td>41.7 ± 3.6b</td>
<td>38.9 ± 4.8b</td>
<td>45.5 ± 3.1b</td>
</tr>
<tr>
<td>13-18</td>
<td>75.3 ± 4.5</td>
<td>61.4 ± 4.8as</td>
<td>18.6 ± 3.7</td>
</tr>
<tr>
<td>19-24</td>
<td>68.0 ± 5.0</td>
<td>61.1 ± 3.7</td>
<td>23.8 ± 3.9</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM and refer to 6-h, 12-h, and 24-h intervals during baseline and 6-h intervals during and after sleep deprivation. All values are expressed as percentage of total recording time.

Table shows that the exposure to novel objects kept rats awake for more than 96% of the time with lights both on or off. Moreover, during the first 5 to 6 hours after sleep deprivation, rats in both lighting conditions showed a similar increase in NREM sleep.

RESULTS

Exploratory Behavior Increases After Acute Dark Exposure

Rats are nocturnal animals, spending most of the dark period awake and most of the light period asleep. In pilot studies, however, we noticed that if we failed to turn on the lights at the onset of the light phase (10 AM), in the following 6 hours, rats spent more time exploring and less time in quiet waking, although they were still awake only approximately 20% to 25% of the total recording time (Figure 1a). A similar phenomenon occurred when rats were sleep deprived during the first 6 hours of the light phase by exposing them to novel objects. In this case, the animals spent most of the time exploring, rather than grooming, but, as during baseline, the time spent exploring increased when sleep deprivation occurred with lights off (DD condition) relative to when it occurred with lights on (LD condition; 33.0 ± 9.0 more minutes exploring in DD vs LD, Figure 1b). As during baseline, time spent in quiet waking decreased with lights off, whereas grooming time was unaffected (Figure 1b).

REFERENCES

The Table shows that the exposure to novel objects kept rats awake for more than 96% of the time with lights both on or off. Moreover, during the first 5 to 6 hours after sleep deprivation, rats in both lighting conditions showed a similar increase in NREM sleep.
Sleep amount and a similar decrease in wakefulness amount relative to baseline (Table, Figure 3). As expected, SWA after sleep deprivation was also significantly increased relative to baseline, and this occurred under both lighting conditions (Figure 2c). Consistent with the findings during baseline, however, the increase in SWA was larger when rats were sleep deprived with lights off compared to when they were sleep deprived with lights on (Figure 2c). Moreover, the increase in SWA relative to baseline persisted longer in DD than in LD (Figure 2c). Figure 2d shows the effects of lighting conditions on the entire EEG power spectrum in NREM sleep during the first 2 hours of recovery after sleep deprivation. As during baseline, the increase was selective for the SWA. The entire EEG power spectrum in REM sleep was not affected by lighting condition (data not shown).

Sleep homeostasis as measured by SWA is tightly related to sleep continuity. Specifically, the increase in SWA after sleep deprivation is correlated with the decrease in the number of brief awakenings. We assessed sleep continuity by calculating the number of brief awakenings (< 16 seconds) as well as the number and duration of NREM and REM sleep episodes. During baseline, none of these measures differed when rats were kept in LD relative to when they were exposed to DD (Supplemental Figure 3 available at www.journalsleep.com). After sleep deprivation, rats showed a similar increase in sleep continuity during the first several hours after sleep deprivation. This was reflected by an increase in the duration of both NREM (Supplemental Figure 3a) and REM (Supplement Figure 3b) sleep episodes and by a decrease in the number of brief awakenings (Supplement Figure 3e). However, the decrease in the number of brief awakenings was more pronounced and longer lasting when rats were sleep deprived with lights off, resulting in a significant difference, compared to lights on, in the second to the fourth 2-hour interval after sleep deprivation (Supplement Figure 3e). In summary, acute dark exposure, which increases the time spent exploring without affecting total waking duration, affects the intensity of the following sleep without affecting total sleep duration.

Exploratory Behavior and Sleep SWA Are Positively Correlated Across Different Lighting Conditions

To test the hypothesis that the quality of wakefulness has a major impact on the homeostatic sleep response, we regressed the percentage of time spent exploring during the 6-hour sleep-deprivation period against the percentage increase of SWA during subsequent recovery sleep. The analysis was performed in a subgroup of animals (6 out of 10) in which individual real-time
video recordings were available for the first 6 hours (10 AM - 4 PM) of all 4 experimental days. As shown in Figure 4, we found that the amount of exploratory behavior during sleep deprivation was positively correlated with SWA during the first hour of sleep after sleep deprivation ($r = 0.85$, $p < .0001$). The percentage of time spent grooming during sleep deprivation, on the other hand, did not correlate with SWA ($r = -0.06$), whereas the percentage of time spent in quiet waking, not surprisingly, was negatively correlated with SWA ($r = -0.69$; the total waking time was scored as exploring, grooming, or quiet waking— see Methods). Also, there was no correlation between the amount of wakefulness or NREM sleep during the first hour after sleep deprivation and the amount of exploratory behavior during sleep deprivation (wakefulness: $r = -0.08$, $p > .5$; NREM sleep: $r = 0.16$, $p > .2$).

In 5 out of 6 rats, more exploratory behavior was present when lights were off than when they were on, and the initial SWA response was also stronger in DD than in LD. One rat, however, showed less exploratory behavior in DD than in LD. In this case, the SWA increase also was smaller in DD than in LD, suggesting that the correlation between exploratory behavior and sleep SWA may persist even across animals tested in the same lighting condition. Indeed, when we considered separately LD and DD values, we found that the SWA response was still positively correlated with the amount of exploratory behavior, although not as significantly as before (LD only, $r = 0.96$, $p < .01$; DD only, $r = 0.83$, $p < .05$), likely due to the lower number of experiments.

As mentioned before, during sleep deprivation, rats were almost continuously awake, independent of lighting condition. Sleep deprivation with lights off, however, was slightly more effective than with lights on (when in DD, rats slept 8 minutes less than when in LD). Thus, to determine the contribution of this and other factors, we performed multiple regression analysis with SWA during the first hour after sleep deprivation as the dependent variable. We found that only the amount of exploratory behavior ($β = 0.85$, $p < .005$), but not the amount of wakefulness or NREM sleep during sleep deprivation, predicted the initial increase in SWA during the first hour of recovery sleep (wakefulness: $β = 0.11$, NREM sleep: $β = 0.17$, $p > .5$). Thus, a slightly more effective sleep deprivation cannot explain why the SWA response was larger in DD than in LD.

**BDNF and Other Plasticity-Related Genes Show Stronger Induction After Acute Dark Exposure**

In the cerebral cortex, both mRNA and protein levels of BDNF are higher during spontaneous waking or sleep deprivation than during sleep.26,27 Arc, Homer (1a-c), and NGFI-A expression is also higher in waking than in sleep.26,27 The expression of the same genes increases in rats exposed to a novel enriched environment, as compared with handled-only or undisturbed animals, although these experiments did not control for the amount of waking (e.g., Cancedda et al).32 We predicted therefore that the expression of these genes should be higher when rats were exposed to DD and spent more time exploring and less time in quiet waking, than when they were kept in LD. Quantitative PCR was used to measure cortical levels of these genes in 3 independent groups of rats that had not been used for the previous SWA analysis. One group of animals was sleep deprived via exposure to novel objects with lights on, the other with lights off. A third group of rats was instead allowed to sleep ad libitum for the last 6 hours before sacrifice (see Methods). As in the previous experiment, rats that were sleep deprived with lights off showed more exploratory behavior than rats that were sleep deprived with lights on (DD 86.2% ± 1.4%, LD 74.7% ± 1.0% exploration, $p < .005$). Previous studies26,27 had measured BDNF, Arc, Homer (1a-c), and NGFI-A expression in samples taken from the entire cerebral cortex. In the current study, the cerebral cortex was instead divided into 3 regions—frontal, parietal, and occipital (see Methods)—and PCR experiments focused on the 2 more rostral regions. This is because acute exposure to light per se induces the expression of BDNF28 and NGFI-A in the visual cortex,27 making it difficult to assess any specific effect of waking behavior on gene expression in occipital areas. As shown in Figure 5, in both frontal and parietal regions, mRNA levels of all genes were higher in rats sleep deprived with lights off than in rats sleep deprived with lights on. For Homer, both the short (1a) and long (1bc) isoforms were measured, and, in agreement with previous results,27 both increased their expression after sleep deprivation relative to sleep, and Homer 1bc did so less than Homer1a (see also Figure 5 and legend). We also measured mRNA levels of the gene coding for minoxidil (aryl) sulfotranserase, a class II sulfotransferase linked to the catabolism of catecholamines and in the detoxification of drugs. The expression of this gene has not been related to the induction of synaptic plasticity but increases with the duration of wakefulness.29 Aryl sulfotransferase transcript levels increased in the frontal (but not in the parietal) regions of the cortex after sleep deprivation relative to sleep, as previously described using whole cortex.26,27 However, rats that were sleep deprived with lights on or off showed similar increases in aryl sulfotransferase mRNA levels relative to sleep (percentage increase in frontal regions in sleep deprivation relative to sleep, LD = 63%, DD = 58%). Thus, relative to when they are kept in LD, rats exposed to acute DD conditions spend more time exploring and show a stronger induction of BDNF and other plasticity-related genes.

**Exploratory Behavior and Sleep SWA Response Are Correlated Across Animals in the Same Lighting Condition**

The data presented so far suggest that exploratory behavior, induction of plasticity-related genes, and SWA response may be linked. However, in the first series of experiments, a change in lighting conditions was used to induce a change in exploratory behavior. Light per se can have an EEG desynchronizing effect, which could ultimately lower the SWA response.30 Thus, we could not completely rule out that the blunted SWA response in LD was due, at least in part, to the presence of light rather than to a decrease in exploratory activity. Furthermore, in the second series of experiments, more exploration was associated with a stronger induction of BDNF and other plasticity-related genes, but we did not measure SWA in the same animals. Thus, a direct link between markers of synaptic plasticity and measures of sleep intensity could not be demonstrated.

To overcome these limitations, we took advantage of the significant interindividual variability in the amount of exploratory activity during sleep deprivation (Figure 4). Independent of lighting condition, some rats explore more, others less, so that the time spent exploring ranges from 40% to 70% of the total...
wake time. We thus performed a third series of experiments in which, as before for the LD condition, rats were sleep deprived with lights on during the first half of the light period, allowed to sleep for 10 minutes afterward, and then sacrificed. In all animals, behavior was continuously assessed using EEG, EMG, and video recordings. As shown in Figure 6, we found that the amount of exploratory behavior during sleep deprivation with lights on was positively correlated with the SWA response measured during the first 10 minutes of recovery sleep ($r = 0.83$, $p < .01$; open circles).

Ten minutes of sleep is a long enough period to reliably measure the SWA response because the first 10-minute SWA values are strongly correlated with the mean SWA values, as measured during the entire first hour of recovery sleep ($r = 0.90$, $p < .0005$). When we included in the regression the 10-minute SWA values for those 6 rats whose 1-hour SWA values are shown in Figure 4, the correlation was even more significant ($r = 0.82$, $p < .0002$; open squares in Figure 6). Thus, the amount of exploratory behavior reliably predicts the SWA response across animals subjected to the same lighting condition.

**BDNF Expression Is Correlated With Both Exploratory Behavior and Sleep SWA Across Animals in the Same Lighting Condition**

At the end of sleep deprivation, the rats shown in Figure 6 (open circles) were killed as soon as they completed 10 minutes of NREM sleep so that the amount of exploratory behavior, cortical mRNA levels of plasticity-related genes, and SWA could be measured in the same animal. While 10 minutes of sleep is a long enough period to reliably measure the SWA response, it is short enough to avoid significant effects of sleep on waking-induced gene expression even for Arc, whose induction is the most transient among the genes tested here. As shown in Figure 7a, the amount of exploratory behavior was correlated with mRNA levels of BDNF measured in the entire cerebral cortex ($r = 0.74$, $p < .02$; Figure 7a). Furthermore, cortical BDNF expression was also strongly correlated with the SWA response ($r = 0.86$, $p < .005$; Figure 7b). In contrast, the expression of Arc, Homer, and NGFI-A was not significantly correlated with either exploratory behavior (Arc, $r = 0.51$; Homer1a, $r = -0.04$; Homer1bc, $r = -0.38$; NGFI-A, $r = -0.04$) or with the SWA response (Arc, $r = 0.31$; Homer1a, $r = 0.35$; Homer1bc, $r = 0.09$; NGFI-A, $r = -0.06$).

**DISCUSSION**

The present results show that SWA during sleep depends not just on the amount of prior wakefulness, but also on its quality. Specifically, the higher the level of exploratory behavior during waking, the larger the SWA response during sleep. This occurred whether the animals were kept awake for a few hours by exposure to novel objects or were spontaneously awake, thereby ruling out nonspecific effects of sleep deprivation. Moreover, the correlation between exploratory behavior and SWA response was also...
long-term depression, respectively. Most relevant here, in vivo animal studies show that BDNF in the cerebral cortex strongly affects mature synapses and facilitates their potentiation by regulating spine maintenance, by allowing synapse formation after sensory stimulation, and by preventing the induction of long-term depression. Intriguingly, the same study found that cortical BDNF induction partially depends on NMDA activity. Another developmental study found that sleep deprivation in rats is followed by an increase in sleep SWA only when sleep loss occurs at postnatal day 24 but not at postnatal day 16 or 20. Intriguingly, the same study also found that cortical BDNF was induced by sleep deprivation only at postnatal day 24 but not at earlier developmental times. The authors did not speculate about the mechanisms responsible for BDNF induction but pointed out that the fourth week of postnatal development coincides with the maturation of cortical noradrenergic and cholinergic fibers (see below). On the other hand, Kushikata et al. found that intracerebroventricular injections of BDNF increased NREM sleep in rabbits and rats without increasing SWA. They concluded that BDNF may not play a major role in regulating sleep SWA, seemingly at odds with the conclusions from Hairston et al. and our study. In the latter 2 studies, however, the increase in sleep SWA was preceded by the intracortical increase in BDNF levels, which may not have occurred after intracerebroventricular injections of BDNF. Finally, our data support previous experiments showing that sleep intensity also depends on the nature of the waking experience. Specifically, Meerlo et al. found that sleep deprivation after social-defeat stress produced a larger and/or longer-lasting SWA increase than did sleep deprivation by gentle handling. Since the effect could not be explained by a stronger stress response after social defeat, present when the lighting condition was kept constant, thus ruling out nonspecific effects of light. We also found that SWA was negatively correlated with quiet waking. This, however, simply reflects the fact that the percentages of time spent in exploring, grooming, and quiet waking are not independent variables and does not imply that prolonged quiet waking should decrease, rather than increase, SWA.

Animals with higher levels of exploratory behavior, for the same amount of wakefulness, also showed stronger induction of BDNF, Arc, Homer, and NGFI-A, all genes that to some extent have been linked to brain plasticity. Among them, BDNF is the one with the most established role as a mediator of synaptic plasticity and, most specifically, synaptic potentiation. BDNF is involved in the induction of long-term potentiation in the hippocampus and in the cerebral cortex, where increases and decreases in BDNF secretion parallel the induction of long-term potentiation and

![Figure 7a](image-url)  
Correlation between the amount of exploratory behavior during sleep deprivation (SD) with lights on in the first half of the light period and cortical BDNF mRNA levels, expressed as fold increase after sleep deprivation relative to same sleeping “control” group used in Figure 5.  

![Figure 7b](image-url)  
Correlation between cortical BDNF mRNA levels (whole cerebral cortex) and the increase in slow-wave activity (SWA, parietal derivation) during the first 10 minutes of non-rapid eye movement sleep. SWA is expressed as a percentage of the 24-h baseline mean (B includes only 9 animals because, for 1 of the rats shown in panel A, SWA analysis was not possible due to electroencephalogram recording artifacts).
the authors suggested that the first condition was associated with “increased cerebral activity,” an interpretation consistent with the possibility that social conflict induces more plastic changes than gentle handling.

While the increase in cortical BDNF expression during sleep deprivation was positively correlated with the amount of exploratory activity and the SWA response during sleep, the increased expression of the other genes we tested was not. It is unlikely that BDNF (and SWA) mediates a special form of plasticity. Experimental paradigms that have been shown to increase synaptic plasticity and BDNF expression (e.g., exposure to enriched environment or hippocampal long-term potentiation) also induce at least some of the other genes (e.g., 12-14). By contrast, we are not aware of any experimental situation in which the induction of BDNF is the only molecular marker of an increase in synaptic plasticity. In fact, in the hippocampus, BDNF-mediated synaptic potentiation has been hypothesized to act, at least in part, through Arc induction.44 There are several possible reasons why the induction of Arc, Homer, and NGFI-A did not correlate with either exploratory behavior or sleep SWA. The dynamics of the induction of these genes differs from that of BDNF and may have not been fully captured in our study, in which gene expression could only be measured at 1 time point. After seizure, for instance, cortical Arc mRNA levels peak earlier and more transiently than BDNF mRNA levels,31,45 and Arc induction mainly reflects the neuronal activation of the last 20 to 40 minutes before sacrifice.46 Furthermore, the induction of these genes may not be linked to synaptic plasticity as directly as BDNF or may reflect aspects of synaptic plasticity that do not directly affect sleep SWA. According to a recent hypothesis, a direct link between sleep SWA and synaptic potentiation is to be expected because strengthened synapses would favor the synchronization of sleep slow oscillations in cortical circuits, thereby leading to higher SWA in EEG recordings. Although BDNF activation can directly affect synaptic size and glutamatergic receptors,47 the induction of scaffolding and cytoskeleton-associated proteins such as Homer and Arc may only indirectly reflect changes in synaptic strength.44 Similarly, the induction of the transcription factor NGFI-A may simply reflect the strong neuronal activation usually associated with synaptic plasticity.22

What are the mechanisms by which increased exploratory behavior increases SWA homeostasis? Although several neurotransmitters or neuromodulators may be involved, the release or activity of these factors on the EEG power spectrum must be restricted to NREM or REM duration, as shown in this study. Finally, the effects of these factors on the EEG power spectrum must be restricted to SWA. An intriguing possibility is that one of such mechanisms involves an increased release of norepinephrine. It is well known that the activity of the noradrenergic system of the locus coeruleus is high during wakefulness and low during sleep (refs in48). Moreover, locus coeruleus activity increases in response to salient external stimuli and whenever an animal makes behaviorally relevant choices.48 Importantly, locus coeruleus lesions dampen waking-induced BDNF expression,23 which we now have shown to be linked to sleep SWA. If the activity of the locus coeruleus is causally involved in mediating SWA homeostasis during subsequent sleep, noradrenergic lesions should also dampen the SWA response. Indeed, in a recent study in which the central noradrenergic system was chronically lesioned using the neurotoxin DSP-4, we found that SWA homeostasis was markedly reduced relative to controls after both spontaneous wakefulness and sleep deprivation.23 As in the present study, changes in sleep SWA occurred in the absence of changes in sleep duration, suggesting that these parameters can be regulated independently.

In summary, this study suggests a direct link between the synaptic plasticity triggered by waking activities and the homeostatic sleep response and identifies BDNF as a major mediator of this link at the molecular level.

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