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Sleep Does Not Enhance the Recovery of Deprived Eye Responses in Developing Visual Cortex

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Abstract

Monocular deprivation (MD) during a critical period of visual development triggers a rapid remodeling of cortical responses in favor of the open eye. We have previously shown that this process is enhanced by sleep and is inhibited when the sleeping cortex is reversibly inactivated. A related but distinct form of cortical plasticity is evoked when the originally deprived eye (ODE) is reopened, and the non-deprived eye is closed during the critical period (reverse monocular deprivation (RMD)). Recent studies suggest that different mechanisms regulate the initial loss of deprived eye responses following MD and the recovery of deprived eye responses following RMD. In this study we investigated whether sleep also enhances RMD plasticity in critical period cats. Using polysomnography combined with microelectrode recordings and intrinsic signal optical imaging in visual cortex we show that sleep does not enhance the recovery of ODE responses following RMD. These findings add to the growing evidence that different forms of plasticity *in vivo* are regulated by distinct mechanisms and that sleep has divergent roles upon different types of experience-dependent cortical plasticity.

Keywords

development; nonREM; synaptic remodeling; activity-dependent; ocular dominance; V1; critical period

Several findings in humans and animals strongly suggest that sleep may promote synaptic plasticity. Sleep is reported to have beneficial effects on processes dependent upon synaptic remodeling, such as learning and memory (Smith, 1995; Stickgold et al., 2000; Huber et al., 2004; Wagner et al., 2004). Sleep is also associated with a number of neurophysiological events that may facilitate synaptic remodeling (Benington and Frank, 2003; Steriade and Timofeev, 2003; Tononi and Cirelli, 2006). For example, neuronal reactivation of wake-active circuits during sleep has been hypothesized to contribute to memory consolidation (Wilson and McNaughton, 1994), and several plasticity-related genes and proteins are upregulated in sleep (Ribeiro et al., 2002; Basheer et al., 2005; Cirelli, 2005). However, the specific role of sleep

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in memory consolidation remains controversial (Stickgold and Walker, 2005; Vertes and Siegel, 2005) and the precise mechanisms governing sleep-dependent synaptic plasticity are unknown (Benington and Frank, 2003).

We have shown previously an important role for sleep in a canonical model of *in vivo* synaptic remodeling known as ocular dominance plasticity (Frank et al., 2001, 2006; Jha et al., 2005). During a critical period of development, blockade of patterned visual input to one eye, or monocular deprivation (MD), results in a reduction of visual cortical responses to the deprived eye (Wiesel and Hubel, 1963; Hubel and Wiesel, 1970; Mioche and Singer, 1989). This form of plasticity is enhanced by sleep, but is inhibited by sleep deprivation or when the sleeping visual cortex is reversibly silenced (Frank et al., 2001, 2006; Jha et al., 2005). These findings demonstrate that there is an activity-dependent process in sleep that promotes the loss of function in deprived eye pathways following MD. To further identify the underlying mechanisms involved in sleep-dependent plasticity, we examined the effects of sleep in a related type of plasticity that involves a gain of function in a recently deprived eye.

Recovery of deprived eye responses following MD can be induced during the critical period either by opening the deprived eye, termed binocular recovery (Giffin and Mitchell, 1978; Malach et al., 1984; Mitchell et al., 2001; Kind et al., 2002), or by reversing the eyelid suture, termed a reverse monocular deprivation (RMD) (Blakemore and Van Sluyters, 1974; Antonini et al., 1998). Recent findings have shown that the initial MD and recovery may trigger distinct plasticity mechanisms. For example, the loss of deprived-eye responses produced by MD requires CREB function and protein synthesis (Mower et al., 2002; Taha and Stryker, 2002), but the recovery of deprived eye responses does not (Liao et al., 2002; Krahe et al., 2005). Therefore, an examination of the role of sleep in RMD plasticity will help identify the specific cellular pathways involved in sleep-dependent plasticity.

Experimental Procedures

Experimental Design

To determine whether sleep enhances the recovery of deprived eye responses following RMD, critical period cats were assigned to one of three experimental groups (Figure 1): MD (imaging, $n=7$; units, $n=6$), RMD (imaging and units, $n=7$), and RMDS (RMD + sleep) (imaging, $n=8$; units, $n=6$) groups. Cats in the MD group provided a baseline comparison (MD only) for groups RMD and RMDS. Cats in the RMD and RMDS groups were surgically prepared for electroencephalogram/electromyogram recordings (see below for details). These two groups of cats would be used to measure the amount of recovery induced by experience alone (RMD), and if this recovery was enhanced by a subsequent period of sleep (RMDS). After 3–5 days post-operative recovery, polygraphic recordings were made in these two groups to verify that the cats had similar sleep/wake histories before the RMD procedure (Frank et al., 2001).

One day after the baseline registration of sleep and wake, cats in all three groups underwent an identical 24 h period of right eye MD to induce a robust, but sub-maximal shift in visual cortical responses towards the non-deprived eye (hereafter referred to as the originally non-deprived eye or ONDE). Following the 24 h MD, cats in the MD group were immediately anesthetized and prepared for intrinsic signal optical imaging and extracellular, electrophysiological recordings to assess ocular dominance changes as described previously (Frank et al., 2001). In RMD and RMDS cats, the ONDE was then closed and the originally deprived eye was opened (hereafter referred to as the ODE). During this period, the cats were kept awake for 6 h in the light (verified by polygraphic recordings) to ensure that similar amounts of visual input were provided to the ODE in both groups. Immediately thereafter, cats in the RMD group were anesthetized and prepared for physiological assessment of ocular dominance. The RMDS cats were treated identically to the RMD cats, except that they were

permitted to sleep *ad lib.* in complete darkness for an additional 6 h before optical imaging and electrophysiological measurements. For logistical purposes (as shown in Figure 1), the start and stop times of each segment of the experiment were different between groups. However, all cats spent equal amounts of the MD period in lighted portions of the light-dark cycle (approximately 12 hours). As discussed in Frank et al., 2001 kittens have very weak (or no) circadian sleep/wake regulation and there is no evidence for circadian influences on ocular dominance plasticity. Therefore differences in start and stop times across groups were not considered important factors in the experimental design.

Housing Conditions and Constitution of Groups

Cats from our colony were raised with littermates and queens on a 12:12h light/dark cycle at 20–22 °C ambient room temperature (food and water *ad lib.*). Critical period cats were assigned to experimental groups such that each group consisted of cats from at least 5 different litters to minimize litter sampling bias. All cats were of similar age at the start of the 24 h MD (mean $\pm$ SEMs postnatal age in days: MD, 32.4 ± 1.4 , RMD, 31.1 ± 1.1 , RMDS, 29.3 ± 0.9).

Electroencephalogram/Electromyogram (EEG/EMG) Implant Surgeries

RMD and RMDS group cats underwent EEG/EMG implant surgeries at postnatal days (P) 21–29 as described previously (Frank et al., 2001). Briefly, under anesthesia, the skull was exposed and an EEG/EMG implant consisting of 6 EEG electrodes was placed bilaterally in the frontal and parietal bones surrounding bregma. An additional 8–12 anchor screws were placed bilaterally in frontal and parietal bones lateral to the EEG electrodes. Three EMG electrodes were placed deep in the nuchal muscles. All 9 EEG/EMG electrodes were soldered to a Microtech (Microtech Inc., Boothwyn, PA) electrical socket. The EEG/EMG implant was affixed to the skull using dental acrylic. The incision surrounding the EEG/EMG electrical socket was closed with 4-0 vicryl. Analgesia was administered immediately upon extubation and PRN through the following morning. Animals were returned to their queens and littermates as soon as they were ambulating normally and vocalizing spontaneously. Animals received postoperative antibiotics (5mg/kg IM enrofloxacin SID) daily for at least 5 days following surgery. Animals were monitored daily for adequate weight gain, normal behavior, and comfort, and the surgical implant and incision were examined daily. Animals were permitted at least three full days of recovery prior to recording sleep data.

Monocular Deprivation (MD) Procedure

Cats were anesthetized with isoflurane/O₂ inhalational anesthetic. The fur around the right eyelid was gently trimmed with iris scissors. Bacitracin neomycin polymyxin B sulfate ophthalmic antibiotic ointment was placed in the right eye prior to suturing the eyelids with 3 mattress sutures of 4-0 vicryl. 2% lidocaine jelly was placed on the sutures and animals were returned to their queens and littermates once they were vocalizing spontaneously, and ambulating normally. Monocular deprivation of the right eye was maintained for 24 h and sutures were inspected to insure that they remain intact. Cats were housed with their queens and littermates throughout the 24 h duration of the right eye MD (see above, under Housing conditions and constitution of groups, for colony housing conditions).

Reverse Monocular Deprivation (RMD) Procedure

In RMD and RMDS cats, the ODE was opened and ONDE (left) eye was closed using the same surgical procedures as described above for the right eye MD. Immediately following the reverse eyelid suture, an electrical recording cable was affixed to the EEG/EMG implant, the animals were returned to the sleep recording chamber and kept awake for 6 hours. This was accomplished by small stepwise radial movements of the cage floor, as well as by soft noise and gentle handling (Frank et al., 2001).

EEG/EMG Recordings and Sleep/Wake Analysis

EEG/EMG signals were routed to an electrical cable connected to a counter-balanced, slip-ring commutator. The circular, sleep recording chamber was grounded by a surrounding Faraday cage, illuminated, and maintained at ambient room temperatures comparable to colony conditions. During each experiment, animals were fed KMR PO every 4–6 hours. Using a Grass 7PCB polygraph machine, differential EEGs and EMGs were captured, filtered at 1 and 35 Hz and 10 and 75 Hz (high and low pass), respectively, and amplified (sample polygraphic traces are shown in Figure 2). These signals were digitized, continuously collected and displayed on a computer monitor in 10-second epochs. EEG and EMG files were saved and analyzed offline to assess sleep architecture as described previously (Frank et al., 2001). Six hour baseline recordings in the RMD and RMDs groups began at ~ 14:00. Following each experiment, we inspected the 12–18 hours of digitized polygraph EEG/EMG data epoch by epoch and deleted artifacts, usually consisting of less than 5% of the total record. Custom software (courtesy of J. Bennington) was used to score vigilance states (NREM, REM, or Wake) presented as percentages of total recording time (%TRT) for the baseline, reverse MD period, and *ad lib.* sleep recordings. Mean values of state amounts ($\pm$ SEMs) were calculated and compared using t-tests (GraphPad Prism, San Diego, CA).

Intrinsic Signal Optical Imaging and Analysis

Optical imaging was used to measure ocular dominance plasticity in the visual cortex of anesthetized cats according to methods described previously (Frank et al., 2001). Briefly, a 12 $\times$ 12 mm craniotomy was performed bilaterally over the visual cortex in anesthetized cats (isoflurane followed by barbiturate). Neuromuscular blockade was induced and maintained with pancuronium bromide (0.08–0.2 mg/kg/hr IV) and cats were ventilated at a rate and volume that maintained end-tidal %CO₂ near 4.0%. Contact lenses were used to focus the eyes upon a computer monitor placed 40 cm in front of the cat and optic discs were mapped to adjust the screen height and location to center it on the area centralis representation. The cortex was stabilized with a solution of 3% agarose in saline and covered with a glass coverslip.

The ORA 2000 system (Optical Imaging, Inc., Germantown, NY) was used to measure intrinsic signal optical responses (Crair et al., 1997). The reflectance of 610 nm light from the visual cortex in response to the visual stimuli described below was measured through a CCD Camera (Princeton Instruments, NJ) focused 500 μ m beneath the pial surface. The intrinsic signal response through one eye to a given orientation was normalized by the average intrinsic signal response through that eye to all eight orientations. Images were high-pass filtered using a 1.2 mm kernel but were not smoothed. A total of 20 visual stimuli, consisting of 8 orientations of drifting high-contrast square gratings (0.2 cycles/degree moving at 2 cycles/second) presented to one eye or the other eye plus 4 blank (gray) screen stimuli presented binocularly, were pseudorandomly presented a total of eight times, comprising one imaging episode. A total of six such imaging episodes were obtained and the resulting data averaged. A template was chosen for a given map as an area containing minimal or no vascular or edge artifact. The area within this template was analyzed for ocular dominance using custom software written in IDL (Research Systems, INC., Boulder CO; Issa et al.).

Scalar measures of ocular dominance were measured with an optical Contralateral Bias Index (OCBI) as described previously (Issa et al., 1999). Briefly, this involved the calculation of OD ratios where at a given pixel, the maximum response through the dominant eye to all eight orientations was compared to the response through the other eye at that same preferred orientation. A distribution of such pixel-by-pixel ocular dominance ratios was created and the average of this distribution normalized to the range of 0 to 1 is the OCBI (Issa et al., 1999). The optical shift index (OSI) was computed as the difference between the OCBI of the hemisphere ipsilateral (IPSI) to the ODE and that of the hemisphere contralateral (CONTRA)

to the ODE. The computation of OCBI and OSI is similar to that used for single or multiple-unit physiology (Issa et al., 1999). OCBI (average value is 0.55 in normal animals) (Crair et al., 1997) and OSI (value is 0 in normal animals) were statistically evaluated using one (MD vs RMD) or two-tailed (all other comparisons) Mann-Whitney U tests (GraphPad Prism, San Diego, CA) and the bootstrap analysis described below.

We also constructed polar optical maps (from the same sampled areas) which show the preferred orientation of each pixel by hue and the strength and selectivity of the response by brightness (Frank et al., 2001). The polar map responses were quantified by calculating the vector sum of the optical responses to all orientations, multiplied by 2 to map 180 degrees of orientation into the unit circle. Average values of response strength and selectivity were computed in this way for the response of each hemisphere to each eye and then compared using the Mann-Whitney U test as described above.

Microelectrode Recordings and Analysis

Following intrinsic signal optical imaging, ocular dominance plasticity was measured in anesthetized animals with extracellular electrophysiological recordings. Visual stimuli consisted of 20 conditions (8 orientations to either eye, 4 blanks to both eyes) presented in a pseudorandom order for a total of five repetitions per recording site. Each orientation presentation consisted of a drifting high-contrast square grating (0.2 cycles/degree moving at 2 cycles/second). Recordings were made with tungsten microelectrodes (FHC, Bowdoinham, ME) which were advanced tangentially along the medial bank in 50–100 μm steps. Two to three random penetrations were made in each hemisphere and the responses of single or multiple-units at each recording site were isolated using a window discriminator.

For each recording site, we used custom software (PCV, M.P.S.) to objectively assess changes in ocular dominance based on the electrophysiological recordings. At each recording site, we compared the mean response at the preferred orientation in the dominant eye to the response in the other eye at the same orientation, following correction for background firing in both eyes (preferred response - response to blank screen). Using custom software (PCV, M. P. S.), the resulting ocular dominance ratio (ODR) was then normalized to a value between 0 and 1 and converted into ocular dominance scores (ranging from 1 to 7) traditionally used for subjective assessments of unit responses (Hubel and Wiesel, 1962, 1970). Ocular dominance scores were then used to calculate a unit contralateral bias index (CBI) and shift index (SI) as described in Issa et al. (1999). Unit CBIs and SIs were statistically evaluated using the Mann-Whitney U tests as described above (GraphPad Prism, San Diego, CA) and the bootstrap analysis described below.

Unit recordings were obtained in 12 hemispheres of 6 cats in the MD group, 13 hemispheres of 7 cats in the RMD group, and 12 hemispheres of 6 cats in the RMDS group. In all hemispheres in which units were obtained, optical imaging data was also obtained. We did not obtain unit data in three cats (1 MD cat, 2 RMDS cats), nor in the IPSI hemisphere of 1 RMD cat for which we obtained imaging data. A minimum of 21 recording sites per hemisphere was established as a criterion by which to compute a CBI, however, the majority of hemispheres contained at least 40 recording sites.

We also verified that the overall balance of responses to the two eyes, as measured by the CBI, can be assessed accurately from multiple-unit recordings. This was accomplished with computer simulations of both single and multi-unit responses. In each simulated penetration, 120 single units were assigned spike rates uniformly distributed between 0 and 20 spikes/second in the right eye, between 0 and 10 spikes/second in the left eye, and with spontaneous activity between 0–1 spike/second. The CBI was calculated for 50 such penetrations using the algorithm that was applied to the real data. Each simulated penetration was then converted to

a 30-site multiple-unit penetration by combining the spikes from each successive group of 4 single units, and CBIs were again calculated for the multiple-unit data. The multiple-unit CBIs were nearly identical to the single-unit CBIs for almost all penetrations, with the average difference equal to 0.02 with a standard deviation of 0.02. Therefore, the use of multiple-unit rather than single-unit data for the calculation of CBIs does not affect any conclusion that we draw from the microelectrode recordings.

Bootstrap Statistical Analysis

Our initial scalar measurements of ocular dominance showed clear trends in the data; however statistical power (given our sample sizes) was generally low in RMD vs. RMDS comparisons. For example power values for optical CBI and SI comparisons varied from 0.18–0.26 and microelectrode recording CBI and SI comparisons varied from 0.31–0.61 (power values for all MD vs. RMD or MD vs. RMDS comparisons were at or exceeded 0.8).

As an additional statistical test between the groups, a bootstrap analysis was applied to the data. For the micro-electrode recordings, this involved repeated random samplings with replacement of the ocular dominance scores taken from the ocular dominance histograms. Each sampling produced a new set of ocular dominance scores of the same size as the original distribution, from which a CBI was calculated. This process was repeated many times to produce a distribution of CBIs which allowed us to evaluate a confidence interval. For a given animal group, this was performed on data pooled across both hemispheres (TOTAL) and separately from data from each hemisphere (IPSI and CONTRA). Each histogram plot of probability density versus CBI involved a bootstrap of 100,000 samplings. The mean of each histogram corresponds to the CBI of the real data set, and the lower and upper bounds of the 95% confidence intervals of the distribution of CBIs can be measured from the histogram.

For optical data, the bootstrap analyses required constructing a honeycomb-like template consisting of adjacent hexagons, each hexagon having an area of 850 μ m (representing the ocular dominance column size typical of cats in the lab colony). This allowed us to randomly divide an original optical imaging template into several non-overlapping, adjacent hexagonal sub-templates. Sub-templates smaller than 50% of a full hexagon's area were discarded from the analysis. The OCBI values of the hexagonal sub-templates were measured. These OCBI values were then randomly sampled, with replacement, to produce a new set of OCBI values of the same size as the original set of sub-templates and the average of these values was computed. We repeated this process 100,000 times to produce a histogram of probability distribution versus mean OCBI. This was performed for both the IPSI and CONTRA hemispheres of the three experimental groups. We also applied a bootstrap analysis to the OSI. In this case, each hexagonal sub-template was matched to one in the opposite hemisphere of the same animal to calculate an OSI as the difference between OCBI of the two hemispheres. These were then bootstrapped in a similar fashion and histograms were plotted of probability density versus mean OSI.

Results

The goal of this study was to determine if sleep enhanced the recovery of cortical responses to an eye visually deprived and then reopened during the critical period. To this end, we compared the strength of responses to the two eyes in three groups of cats: MD, which provides the starting point for all groups from which recovery begins; RMD, studied immediately after a period of reverse suture; and RMDS, like RMD but followed by an additional period of sleep.

Vigilance States

Baseline Period, RMD, and *Ad lib.* Sleep: During the baseline EEG/EMG recording session NREM sleep and REM sleep amounts were similar between the RMD and RMDS groups ($p=0.28$, NREM; $p=0.69$, REM; two-tailed unpaired t-test). This demonstrated that the cats were at similar developmental stages with respect to their sleep/wake architecture (Figure 3). In addition, EEG/EMG recordings confirmed that cats in the RMD and RMDS groups were all kept awake in the light during the RMD period, thus verifying that both groups of cats received similar amounts of visual input to the ODE ($p=0.32$, Wake; two-tailed unpaired t-test).

In RMDS cats, there was a significant increase in sleep amounts following the RMD period compared to baseline values (Figure 3). NREM sleep significantly increased from $37 \pm 3\%$ total recording time (%TRT $\pm$ SEM) during the baseline session to $47 \pm 4\%$ TRT during the rebound period ($p<0.01$, two-tailed paired t-test), while REM sleep significantly increased from $27 \pm 3\%$ TRT during the baseline session to $38 \pm 2\%$ during the *ad lib.* sleep session ($p<0.03$, two-tailed paired t-test). The amounts of sleep in the post-RMD period, or NREM and REM sleep considered separately, were not significantly correlated with changes in ocular dominance as measured by units or optical imaging (data not shown).

The Effect of Sleep on the Recovery of ODE Responses Studied by Microelectrode Recording

Microelectrode recordings showed that a 1d MD caused a dramatic loss of responses through the deprived eye (Figure 4A). A subsequent period of RMD partially reversed this plasticity as evidenced by an increase in the number of neurons responding well to the ODE (Figure 4B). The effect of the reverse suture was present in both hemispheres, but appeared to be greater in the hemisphere contralateral to the ODE (See Figure 4B; MD $n=6$, 12 hemispheres, RMD $n=7$, 13 hemispheres (6 IPSI, 7 CONTRA)). The recovery of ODE responses elicited by RMD was not enhanced by a subsequent period of sleep. Instead, the total ocular dominance histogram of the RMDS group (Figure 4C; 6 IPSI, 6 CONTRA) was intermediate between those of the MD and RMD groups. The effects of sleep were similar in both hemispheres, but most evident in the hemisphere contralateral to the ODE. Chi-square analyses showed significant differences between the following OD histograms: Total, MD vs. RMD and RMDS, RMD vs. RMDS; IPSI, MD vs. RMD and RMDS; CONTRA, MD vs. RMD, RMD vs. RMDS ($p<0.05$).

Intrinsic Optical Imaging Confirms that Sleep Reduces the Recovery of ODE Responses following RMD

The ocular dominance changes observed by microelectrode recordings were also evident in the intrinsic signal optical maps. Polar maps of a representative animal from each group (Figure 5) show that ODE responses are reduced in the MD group, especially in the IPSI hemisphere. Following brief RMD, these ODE responses recover to a considerable extent in both hemispheres as measured by mean response strengths in the polar maps (IPSI: $p<0.01$, MD vs. RMD; CONTRA: $p<0.05$, MD vs. RMD; MD, $n=7$, RMD, $n=7$; two-tailed Mann-Whitney U test). This gain in ODE responses was not further enhanced by subsequent sleep in the RMDS group (IPSI: $p=0.69$, RMD vs. RMDS; CONTRA: $p=0.40$, RMD vs. RMDS; RMD, $n=7$, RMDS, $n=8$; two-tailed Mann-Whitney U test). In contrast to the ODE, there was no appreciable change in ONDE responses across the different groups (MD vs RMD vs RMDS, all comparisons, $p \geq 0.05$).

In Figure 6, we show a comparison of ocular dominance between optical imaging and microelectrode recordings from the same representative cats illustrated in Figure 5. In the MD cat (Figure 6A), consistent with the ocular dominance histograms from microelectrode recordings, ONDE responses dominate the map. In the RMD cat (Figure 6B), ODE responses recover and occupy larger areas of the map, especially in the hemisphere contralateral to the

ODE. In the RMDS cat (Figure 6C), sleep appears to reduce rather than enhance this relative gain in ODE responses.

Scalar Measures and Bootstrap Analyses of Ocular Dominance in MD, RMD and RMDS Groups

Scalar measures of ocular dominance showed significant differences between the MD and RMD groups (Figures 7 and 8) and unit and optical measures were highly correlated (Pearson $r=0.79$, $p<0.0001$, data not shown). As shown in Figures 7 and 8, sleep after RMD did not enhance and appeared to reduce the recovery of responses to the ODE. This reduction was not significant based on conventional scalar assessments, but the bootstrap analyses showed that the RMDS group was significantly different from both the RMD and the MD groups (Figures 9–10). As shown in Figure 9A, the overall unit CBI from the RMDS group lies between the values obtained from the RMD and MD groups (RMD vs RMDS, MD vs RMD, RMDS, $p<0.001$). Similar differences were found in both the IPSI and CONTRA hemispheres (Figure 10A–B; RMD vs. RMDS, MD vs RMD, RMDS, $p<0.01$). Similar results were obtained when the bootstrap analysis was applied to the imaging data. With the exception of hemispheres IPSI to the ODE in the RMD and RMDS groups, all comparisons between groups were significant (Figures 9B, 10C–D; RMD vs. RMDS, MD vs RMD, RMDS, $p<0.05$).

Discussion

We have previously shown that sleep enhances the loss of deprived eye responses following MD and that this process requires cortical activity in the sleeping brain (Frank et al., 2001, 2006; Jha et al., 2005). In this study, we examined the role of sleep upon a related form of plasticity triggered by RMD that engages cellular mechanisms distinct from those triggered by MD. Surprisingly, and in contrast to our earlier results with short-term MD, our data show unequivocally that an *ad lib.* sleep period does not enhance plasticity triggered by short-term RMD. Our data also suggest that sleep may actually impede or impair the plasticity triggered by RMD. These results in conjunction with our earlier studies demonstrate that sleep has strikingly divergent effects on different types of cortical plasticity *in vivo*.

The fact that an *ad lib.* sleep period does not enhance and may partially reverse a preceding period of RMD might be explained in several ways. These include time-dependent processes that do not require sleep, or sleep-dependent processes that impede plasticity mechanisms triggered by RMD. These possibilities are discussed in greater detail in the following sections.

Processes Not Dependent On Sleep

One simple explanation of our results is that during the *ad lib.* sleep period of the RMDS group, there is a gradual, time-dependent deterioration of the cortical changes elicited by RMD. For example, the effects of MD slowly degrade in anesthetized, paralyzed cats with or without continued visual input (Kameyama et al., 2005). However, this seems an unlikely explanation for the following reasons. The time-dependent reversal of MD effects requires at least 24–48 hours (vs. 6 hours of *ad lib.* sleep), and there is no evidence that a similar reversal occurs after RMD. Indeed, in unanesthetized cats time in total darkness enhances the effects of RMD (Ramachandran and Ary, 1982) or related forms of plasticity (i.e. gain of function in a previously inexperienced eye) (Pettigrew and Garey, 1974; Peck and Blakemore, 1975). These latter findings are somewhat difficult to interpret since vigilance states were not measured in these studies, but they do support the idea that a simple time-dependent degradation can not readily explain our results.

An alternative explanation is that the combination of MD, RMD and sleep results in a deterioration in both visual pathways. For example, it is possible that changes in cortical

plasticity in the RMDS group reflect a sleep-dependent enhancement of MD in both eyes (i.e. the ODE and the newly deprived ONDE). This also seems highly unlikely because, as shown in Figure 5, polar optical maps demonstrate that ONDE responses do not change across MD, RMD and RMDS conditions, while ODE responses are significantly stronger after RMD. This is also evident in the optical ocular dominance maps. After sleep there is a retraction of ODE cortical territories that had expanded following RMD (see Figure 6). Nor can a simple weakening of both visual pathways produce a reversal of the effects of RMD. A weakening in both visual pathways, followed by a comparison of responses in the two eyes would show them to be more similar. But as shown in Figures 4 and 6, sleep after RMD partially reverses the recovery of responses to the ODE and shifts cortical responses towards the ONDE. Therefore, our results are best explained by a sleep-dependent process that selectively modulates the recovery of responses in the ODE, and not by a general deterioration of vision in both pathways.

Sleep-dependent Processes

One interesting interpretation of our results is that sleep promotes certain plasticity mechanisms, but not others. For example, the cortical plasticity elicited by MD (but not by RMD) requires CREB-dependent transcription and protein synthesis (Mower et al., 2002; Krahe et al., 2005). As suggested by Krahe et al., it is possible that MD plasticity requires transcription of genes at CREB sites and this pathway may have to be inactivated in order for rapid recovery to ensue (Pham et al., 1999; Liao et al., 2002; Krahe et al., 2005). Since sleep, and in particular NREM sleep is associated with increased cerebral protein synthesis (Ramm and Smith, 1990; Nakanishi et al., 1997), it is plausible that sleep specifically facilitates protein synthetic pathways which are mediated by CREB-dependent transcription, a process that would antagonize recovery.

Alternatively, it has been suggested that sleep promotes LTD-like processes either through Hebbian or non-Hebbian mechanisms (Benington and Frank, 2003; Tononi and Cirelli, 2006). The loss of function in deprived eye pathways may also involve LTD-like events, but a gain of function may engage different Hebbian processes (Malach and Van Sluyters, 1989; Rittenhouse et al., 1999; Heynen et al., 2003). Therefore, if indeed sleep promotes LTD in some fashion, then sleep after RMD might impede recovery of function in the deprived eye.

The notion that sleep might impair the recovery of function in a previously deprived visual pathway may seem counter-intuitive. After all, visual pathways do eventually recover following RMD or the restoration of binocular vision (if performed during the critical period) (Blakemore and Van Sluyters, 1974; Movshon and Blakemore, 1974; Mitchell and Gingras, 1998; Mitchell et al., 2001; Kind et al., 2002; Faulkner et al., 2006). However, it is possible that the changes we report after sleep are part of a larger process that eventually produces adaptive changes in cortical circuitry. An example of such a process is suggested by recent findings in song birds. Deregnacourt et al. showed that the structure of a learned song deteriorates after a period of sleep, but birds that show the greatest amount of post-sleep deterioration are more able to emulate the tutor song over time (Deregnacourt et al., 2005). A similar paradoxical retraction of cortical representations after sleep followed by enhanced performance on motor learning tasks has also been reported in humans and primates (Strata et al., 2001; Fischer et al., 2005).

In conclusion, the findings of this study demonstrate that sleep has divergent roles in different forms of developmental, experience-dependent cortical plasticity. In particular, the present results in conjunction with our earlier findings suggest that sleep promotes or acts downstream of CREB and protein synthesis dependent pathways involved in synaptic remodeling. These findings provide an additional clue to the mystery of sleep function by identifying candidate cellular processes that might be enhanced or impaired by sleep. They are also consistent with

previous investigations that have shown that sleep may be especially important for the developing brain (Oksenberg et al., 1996; Shaffery et al., 2002; 2006).

This study adds to the growing evidence that loss and recovery of deprived eye responses may be regulated by different mechanisms. More than one percent of children undergo surgery early in life for correction of conditions that would lead to stimulus deprivation amblyopia, for whom early intervention is essential to prevent permanent visual impairment (Mitchell and MacKinnon, 2002; Arora et al., 2005). During this same period of early postnatal development the brain is predominantly in a state of sleep. Therefore, understanding the mechanisms that regulate experience-dependent plasticity of visual cortical circuits during early postnatal development, and specifically the role that sleep may have in these processes, may inform our decisions regarding appropriate therapeutic interventions.

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Abbreviations used in this manuscript

IPSI	ipsilateral
CONTRA	contralateral
CBI	contralateral bias index
CREB	cAMP/Ca ²⁺ response element-binding protein
MD	monocular deprivation
NREM	

	non-rapid-eye-movement
ODE	originally deprived eye
ONDE	originally non-deprived eye
REM	rapid-eye-movement
RMD	reverse monocular deprivation
SI	shift index
% TRT	percent total recording time

Figure 1.

Experimental design. Critical period cats were divided into three groups, MD, RMD, and RMDS, to investigate the effect of sleep on the recovery of ODE responses. The animals in all three groups first underwent a 24h MD while being housed in a 12:12h light/dark cycle (gray fill). The RMD and RMDS cats underwent a 6h RMD immediately following the 24h MD. During the RMD period, animals were kept awake in the light (white fill). The RMDS cats were treated identically to the RMD cats, but were provided a 6 hour *ad lib.* sleep period (in the dark) following the 6h RMD (actual start and stop times for each group are shown in insets). The arrowheads represent the start time of assessment of ocular dominance plasticity via intrinsic optical imaging and microelectrode recordings. (See Experimental Procedures for details).

Figure 2.

Representative EEG/EMG Polygraphic Trace. Examples of NREM sleep, REM sleep, and Wake EEG/EMG traces are shown for a cat from the RMDS group acquired during the *ad lib.* sleep recording session. Each trace represents 30 s.

Figure 3.

Mean amounts of NREM sleep, REM sleep, and wake for the RMD and RMDS groups for the baseline, RMD, and *ad lib.* sleep periods. Data are expressed as percentages of the total recording time (%TRT $\pm$ SEM; RMD, n=7; RMDS, n=8). There were no significant differences in sleep/wake amounts between the groups in any of the periods. Sleep time was significantly greater in the post-RMD *ad lib.* sleep period compared to baseline (in the RMDS animals).

Figure 4.

Ocular dominance histograms for the MD Group (A), RMD Group (B), and RMDS Group (C) for unit recordings pooled from both hemispheres (TOTAL) and for units recorded in hemispheres ipsilateral (IPSI) and contralateral (CONTRA) to the ODE. Following MD (A), visual cortical neurons respond predominantly to the ONDE and lose responsiveness to the ODE. ODE responses recover following RMD in the light (B) and this gain in response decreases following subsequent sleep in the dark (C). In each histogram, ocular dominance distributions are presented as percentages of the total number of recording sites. For TOTAL histograms, an ocular dominance score of 1 represents cells driven entirely by the ONDE, a score of 7 represents cells driven entirely by the ODE, and a score of 4 represents cells driven equally by both eyes. Histograms for IPSI and CONTRA hemispheres are ranked according to the traditional Hubel and Wiesel 7-point scale (Hubel and Wiesel, 1970). CBI values and number (n) of recording sites are shown for each histogram.

Figure 5.

Polar optical maps from a representative animal from each of the MD, RMD, and RMDS groups are presented for the CONTRA and IPSI hemispheres. For each hemisphere, the upper panel represents visual cortical responses to ONDE stimulation and the lower panel represents visual cortical responses to ODE stimulation. The hue of a given pixel represents the orientation of the drifting stimulus bar that best activates the pixel. The brighter a given pixel, the greater is the strength and selectivity of its response to its preferred orientation. 24 hours of MD induces a marked reduction in cortical responses to ODE stimulation. ODE responses partially recovered in the RMD animal, whereas in the RMDS animal, ODE responses are at levels intermediate between those of the MD and RMD cats. White bar represents 1 mm.

Figure 6.

Ocular dominance maps and corresponding ocular dominance histograms of a representative animal from the MD Group (A), the RMD Group (B), and the RMDS group (C). Ocular dominance data from the hemisphere ipsilateral (IPSI) to the ODE is presented on the left of the figure whereas ocular dominance data from the hemisphere contralateral (CONTRA) to the ODE is presented on the right. Darker pixels represent regions of the map which respond preferentially to the ODE and brighter pixels represent regions of the map which respond preferentially to the ONDE. Boundaries between the areas of dark and bright pixels are shown in red. Ocular dominance maps are constructed using the preferred stimulus orientation at each pixel. For each ocular dominance map, the optical CBI value is shown in the lower left-hand

side of the map's panel. Corresponding ocular dominance histograms of microelectrode data recorded from the IPSI and CONTRA hemispheres are presented below the ocular dominance maps. The optical SI and unit SI for each animal are shown to the right of the ocular dominance maps and unit histograms, respectively. The grayscale bar represents 1 mm.

Figure 7.

Scalar measurements of ocular dominance plasticity across both hemispheres for the MD, RMD, and RMDS groups. The mean ($\pm$ SEM) unit shift index (SI) is shown for all three groups in (A) (** $p < 0.01$, MD vs. RMD, † $p < 0.05$, MD vs. RMDS; Mann Whitney U test). The mean ($\pm$ SEM) optical SI is shown for all three groups in (B) (** $p < 0.01$, MD vs. RMD; Mann Whitney U test). The SI for normal binocular animals is 0.

Figure 8.

Contralateral bias indices (CBI) for unit and optical recordings in the MD, RMD, and RMDS groups. In (A), the mean ($\pm$ SEM) unit CBI (top panel) and optical CBI (bottom panel) from hemispheres ipsilateral (IPSI) to the ODE is shown for all three groups (* $p < 0.05$, MD vs. RMD; †† $p < 0.01$ MD vs. RMDS). In (B), the mean ($\pm$ SEM) unit CBI (top panel) and optical CBI (bottom panel) from hemispheres contralateral (CONTRA) to the ODE is shown for all three groups (** $p < 0.01$, MD vs RMD; * $p < 0.05$, MD vs RMD).

Figure 9.

Bootstrap statistical analysis of unit and optical imaging data pooled across both hemispheres. For all histograms, black fill represents the MD group, white fill represents the RMD group, and grey fill represents the RMDS group. The probability distribution of unit recording CBIs is shown for the three groups in A and the probability distribution of optical SIs is shown in B. The mean and 95% confidence intervals are presented as brackets above each probability distribution. The probability density is the probability per bin and each histogram consists of 500 bins.

Figure 10.

Bootstrap statistical analysis of unit and optical imaging data in hemispheres ipsilateral (IPSI) and contralateral (CONTRA) to the ODE. For all histograms, black fill represents the MD group, white fill represents the RMD group, and grey fill represents the RMDS group. Panels A and C show the probability distribution of unit and optical CBIs is shown for the three groups for data obtained from hemispheres IPSI to the ODE. Panels B and D show the probability distribution of unit and optical CBIs for data obtained from hemispheres CONTRA to the ODE. The mean and 95% confidence intervals are presented as brackets above each probability distribution. The probability density is the probability per bin and each histogram consists of 500 bins.