REM sleep promotes bidirectional plasticity in developing visual cortex *in vivo*

Leslie Renouard¹, Christopher Hayworth¹, Michael Rempe, Will Clegern, Jonathan Wisor, Marcos G. Frank^{1*}

¹ Department of Translational Medicine and Physiology, Elson S. Floyd College of Medicine, Washington State University, HSB 280M, Spokane, WA, 99223 USA

*corresponding author: marcos.frank@wsu.edu

Abstract

Sleep is required for the full expression of plasticity during the visual critical period (CP). However, the precise role of rapid-eye-movement (REM) sleep in this process is undetermined. Previous studies in rodents indicate that REM sleep weakens cortical circuits following MD, but this has been explored in only one class of cortical neuron (layer 5 apical dendrites). We investigated the role of REM sleep in ocular dominance plasticity (ODP) in layer 2/3 neurons using 2-photon calcium imaging in non-anesthetized CP mice. In contrast to findings in layer 5 neurons, we find that REM sleep promotes changes consistent with synaptic strengthening and weakening. This supports recent suggestions that the effects of sleep on plasticity are highly dependent upon the type of circuit and preceding waking experience.

Key words: ocular dominance plasticity, monocular deprivation, development, synapse, ontogeny, remodeling

Introduction

Sleep enhances a classic model of developmental plasticity *in vivo* (ocular dominance plasticity: ODP)¹. ODP is induced by monocular deprivation (MD) during a critical period (CP) of development, resulting in a shift of cortical response to the non-deprived eye. ODP is considered physiological as it involves adaptive changes in response to sensory input and normally governs the proper development of binocular vision. ODP is also considered a canonical form of plasticity as many of the underlying mechanisms discovered in this system govern diverse forms of plasticity elsewhere in the brain. Therefore, what we learn about sleep in ODP may reveal general rules about sleep function²⁻⁴.

The precise role of REM sleep in ODP is unknown. Previous studies indicate that REM sleep is necessary for ODP, as REM sleep deprivation (RSD) in CP cats and mice reduce shifts in cortical response to the non-deprived eye^{5,6}. The types of plasticity governing this shift are unclear. In cats, sleep strengthens responses to the non-deprived eye, but the specific role of REM sleep in this process was not investigated^{5,7}. In mice, REM sleep weakens responses to the deprived eye, but this analysis was restricted to one type of neuron (apical dendrites from layer 5 neurons)⁶. Considering that excitatory neurons in cortical layers 2/3 are among the first to exhibit ODP⁸, this raises the possibility of a more heterogeneous response.

To determine the role of REM sleep more precisely in ODP, we used a combination of 2-photon fluorescence microscopy and polysomnography in CP mice expressing GCaMP6s in layer 2/3 CaMKII+ neurons. We find that short periods of MD and sleep lead to more complex changes than previously reported, including a gain of response to the

non-deprived eye. This supports the idea that sleep promotes bidirectional changes in plasticity depending on the cell and circuit⁹.

We used a design adopted from previous investigations of sleep and ODP^{1,6}. Following baseline measurements of visual responses to each eye, the eye contralateral to the measured V1 was closed and the mice were kept awake for 6 hours to induce ODP. We then determined the effects of subsequent 6 hours *ad lib* sleep or 3 hours of RSD on ODP. Undisturbed sleep after MD (MD+S) led to a shift in the ocular dominance index (ODI) which did not occur in the MD+RSD group (ODI MD+S = 0.52 +/- 0.02 (std. error) vs. baseline 0.47 +/- 0.02: paired Student's t-test, $p < 0.018$; MD+RSD: 0.48 +/- 0.02, vs. baseline 0.47 +/- 0.02, $p = 0.374$, paired Student's t-test). The effects of sleep on ODP involved both a loss of response to the deprived eye and a gain of response to the non-deprived eye (Figure 1). Across neurons, the gain of response to the non-deprived eye was significantly enhanced and the response to the deprived eye significantly weakened after sleep compared to baseline values. These plastic changes did not occur in RSD mice (Figure 2). After RSD, changes in the non-deprived eye response were not different from baseline, and deprived eye responses were slightly enhanced.

Discussion

We investigated the role of REM sleep in a canonical model of experience-dependent plasticity (ODP). We find that undisturbed sleep after short periods of MD (6 hours) induce bidirectional plastic changes in V1 neurons as measured by 2-photon microscopy in awake mice. These results are surprising because previous studies of

ODP in mice indicate that several days of MD are required for measurable changes in ODP¹⁰, and sleep only promotes weakening of deprived eye circuits⁶. We discuss aspects of these findings below.

Sleep-dependent ODP

The roles of different sleep states in CP plasticity are undefined. Studies in cats demonstrate that sleep enhances ODP by maintaining depression in deprived eye circuits while strengthening responses to the non-deprived eye^{7,11}. Cortical activity in sleep is required for both changes—as intracortical inhibition of NMDA receptors during post-MD sleep abolishes signs of circuit weakening and strengthening^{6,7}. Our findings in mice are similar in some respects. First, like cats, we find that sleep is required for similar changes in both visual pathways as they are abolished by RSD during the first few hours of the post-MD period. However, in contrast to the present study, our earlier study in mice showed that MD followed by undisturbed sleep only produced evidence consistent with synaptic weakening⁶.

One explanation for this apparent discrepancy is that sleep-dependent plasticity varies depending on the type of neuronal circuit^{9,12}. For example, in adult mice motor learning followed by sleep increases dendritic spine number in motor cortex, but only on some dendritic branches¹². Similarly, sleep-dependent hippocampal (morphological) plasticity is highly determined by region (CA1 vs. CA3) and spatial location on a given dendrite (reviewed in^{13,14}). Our previous study in mice examined changes in spine number and GCaMP signaling in layer 5 neuron apical dendrites⁶. In this study, we

instead examined somatic GCaMP signaling in layer 2/3 excitatory neurons. AMPA receptor trafficking (which is a proxy for changes in synaptic efficacy) varies in different V1 neurons, with more dynamic changes occurring in layer 2/3 dendritic spines compared to layer 5 apical dendritic spines¹⁵. This suggests that more heterogeneous plastic changes may occur in layer 2/3 neurons compared to layer 5 neurons in sleep-dependent plasticity.

Changes in ODP across the sleep-wake cycle may be explained by a Hebbian process that promotes synaptic weakening when visual experience is severely reduced and a homeostatic scaling mechanism in sleep that restores and enhances responses to the open eye¹⁶. In support of this idea, in mice the loss of response to the deprived eye is mediated by Hebbian long-term depression¹⁷ (but see³). However, the increased response to the non-deprived eye is governed by $\text{TNF}\alpha$ ¹⁸; a cytokine that promotes homeostatic synaptic up-scaling and is at its highest brain concentrations during sleep^{18,19}. Although the role of sleep in homeostatic V1 plasticity is complex²⁰⁻²², the above findings are consistent with previous suggestions that under certain conditions, sleep may promote homeostatic synaptic upscaling²³.

REM sleep mechanisms in ODP

This study extends and confirms previous work in cats and mice demonstrating that REM sleep is required for the full expression of ODP. In both species, RSD after MD reduces ODP based on a variety of measures including extracellular recording, intrinsic signal imaging, and 2-photon microscopic measures of spine morphology and

intracellular calcium^{5,6}. The underlying molecular mechanisms are not entirely known but may involve both Hebbian and non-Hebbian mechanisms. In addition to TNF α , enzymes implicated in Hebbian synaptic plasticity (ERK and mTOR) are also key mediators of ODP⁴. In CP cats, both ERK and mTOR are activated in V1 during REM sleep^{5,24}, and inhibition of both kinases during post-MD sleep reduces ODP in a manner similar to RSD^{25,26}. Whether similar events occur in rodent ODP is unknown, but sleep deprivation in adult mice inhibits mTOR (total sleep deprivation) and ERK (RSD) in the hippocampus^{27,28}, which suggests that these mechanisms may be evolutionarily conserved.

Methods

All procedures were approved by the Washington State University Institutional Animal Care and Use Committee.

Virus injection: At P0-P3, equal numbers of male and female CaMKIIalpha-Cre mice (B6.Gg-Tg(CaMKIIalpha-Cre)T29-1Stl/J stock005359, Jackson Laboratories) were anesthetized under isoflurane 2-3% and injected bilaterally intraventricularly (stereotaxic coordinate from lambda) with a Hamilton syringe mounted to a syringe pump (0.5 μ l/min) with 1 μ l of AAV9-CAG-Flex-GCaMP6s (titer: $\sim 1 \times 10^{13}$ genomes/mL, Penn Vector core) and 1 μ l of AAV1-CAG-tdTomato (titer: $\sim 1 \times 10^{13}$ genomes/mL, Penn Vector core). The procedure lasted around 10 to 15 minutes²⁹. As shown previously, the former injection leads to selective expression of GCaMP6 in CaMKII+ neurons³⁰. tdTomato was used to create fiduciary marks for aligning frames. Following post-operative recovery on a heating pad, pups were returned to their home nest.

EEG & EMG, cranial window surgery and baseplate positioning: At P18-21, mice were prepared for GCaMP6 and EEG & EMG recording as described previously³¹. Briefly, after dura removal, a 3mm diameter cranial window was positioned over binocular (right hemisphere) V1 using previously described coordinates³². A baseplate that allows for head fixation for 2 photon imaging was also positioned. In the other hemisphere, three stainless-steel wire EEG (and one reference above the cerebellum) electrodes were implanted in frontal/parietal bone. Two EMG electrodes were inserted into the nuchal muscles. These electrodes were connected to a 3.1mm diameter miniature connector (Omnetics Connector Corp). The baseplate, socket and the head-restraint baseplate were secured with black (opaque) dental cement and the cranial window was protected from light with black tape.

MD and sleep procedures: At P23-25, male mice were placed in a Pinnacle systems (<http://www.pinnaclet.com/sleep-deprivation.html>) sleep-recording chamber and an EEG & EMG cable and miniaturized preamplifier were connected to the socket. Mice were acclimated to the chamber and cable for 3 days prior to the beginning of an experiment (12:12h light-dark cycle, food and water *ad lib*, ambient temperature, 24-25°C). In parallel with the preceding habituation, mice were also progressively habituated to 1 hour head restraint one to two times each day for at least 5 days prior to visual testing⁶. Following the acclimation period and beginning at zeitgeber time 0 (lights on), baseline visual responses in each eye were recorded. After this baseline measure, the eye contralateral to the hemisphere with the cranial window (left eye) was covered with a small piece of light-proof tape held by a metallic tab secured to the head bar. The mice were then kept awake with gentle handling for 6 hours to induce ODP as previously

performed in cats ¹. We then determined the effects of post-MD *ad lib* sleep or RSD on ODP in separate groups of mice. Following the above MD procedures, one group was allowed to sleep *ad lib* for 6 hours (MD+S), while the other group was REM sleep deprived (MD+RSD) for the first 3 hours post-MD followed by 3 hours *ad lib* recovery sleep. The post-MD sleep and RSD periods were conducted in dim red light to control for visual input. Between 2-photon measurements, polysomnographic signals were continuously monitored by an investigator with experience studying REM sleep in rodents (L.R.) to ensure that all mice were awake during the MD, that all mice slept during *ad lib* sleep periods, and that RSD mice were awakened during REM sleep, as previously described ⁶. This latter approach results in >90% reduction in REM sleep with non-significant changes in nonREM sleep in CP mice ⁶.

2-photon signal processing and visual testing: 2-photon microscopy in non-anesthetized head restrained mice was performed as previously described ³¹. For visual testing, the mouse was positioned approximately 25 cm in front of a 120Hz LED monitor and each eye was presented with drifting gratings in 4 orientations (4 times per orientation at 0, 45, 90, 135 degrees, and grey screen, spatial and temporal frequency as described in ³³, 3 seconds each separated by 6 seconds of black screen presentation). The grey screen was set at equal luminance, and zero contrast. Visual stimuli were generated using Matlab2016a and psychtoolbox. Visual responses were recorded between ~150µm and ~200µm below the window surface (visual cortical layer 2/3). A Kalman and bleaching filter (in ImageJ) was used to reduce noise and correct linear trends in the signal ³¹.

The preferred orientation (the orientation that on average produced the largest response) was determined for each neuron and used for all further calculations. Only neurons that were visually responsive in the baseline recordings (those with a mean peak grating response > mean peak response grey screen) were included in analyses. In addition, we only analyzed neurons that could be recorded across the baseline and post-MD periods. This was determined by using fiduciary marks (e.g. vasculature maps and TdTomato signals) to position the microscope over the same region at different points of the experiment, followed by image registration in the X and Y planes to align frames from different sets of recordings. Calcium signals obtained during visual testing were converted into modified $\Delta F/F$ values ($\Delta F/F = F_{stim} - F_{spon}$; where F_{stim} = peak signal during 3 second visual stimulation (+2 seconds of subsequent black screen) and F_{spon} = peak signal during the last 2 seconds of the preceding black screen presentation. This calculation ensured that slow gCaMP6s responses to a visual stimulus were also captured (Figure 1). Overall changes in ocular dominance were measured using an ocular dominance index (ODI) calculated as $ODI = NDE / (DE + NDE)$, where DE = deprived eye, NDE = non-deprived eye. To determine specific changes in the response to each eye, each neuron's responses were normalized to the overall mean of the baseline + post sleep or post-RSD values, respectively. The number of mice and ROIs per group were as follows: MD+S, 4, 136; MD+RSD, 4, 101.

Statistical analysis: IBM SPSS Statistics (version 28) and Systat Sigmaplot 11 were used for statistical analysis. Data were first tested for normality. Parametric data were analyzed using Student's t-test (paired, where applicable). Nonparametric data were assessed with the Mann Whitney U test.

Figure 1. Sleep promotes bidirectional plasticity in developing visual circuits. (A)

Yellow circles surround representative neuronal cell bodies in V1 identified by GCaMP6s 2-photon microscopy in an awake mouse during the critical period for visual plasticity. Only those neurons recorded across all experimental conditions were included for analyses. Following baseline recordings of visual responses, the mouse was subjected to 6 hours of monocular deprivation (MD) while awake + 6 hour *ad lib* sleep (+S). **(B)** 2 representative V1 neurons with different responses to MD and sleep (shown in inset box in **A**). **(C)** Calcium signal from the two neurons shown in **B**. Note that the neuron indicated by the **blue asterisk** has a weakened response to the deprived eye (DE) after sleep with minimal change in the non-deprived eye (NDE) response. In contrast, the neuron indicated by the **orange asterisk** does not show a weakened response to the DE after MD+sleep. Instead, the response to the NDE becomes stronger. Grey bars indicate time when gratings on a LED monitor were presented to either the DE or NDE.

Figure 2. REM sleep deprivation inhibits bidirectional plasticity in developing visual circuits. Data are mean neuronal visual responses to the deprived eye (DE) and non-deprived eye (NDE) in critical period mice monocularly deprived while awake (6 hours) and then permitted *ad lib* sleep (+Sleep), or REM sleep deprived (+RSD). (A) Relative to baseline, responses to the deprived eye (DE) are weaker after sleep (Mann Whitney U, $p < 0.005$). This weakening does not occur in RSD mice; instead, responses to the DE are slightly enhanced (Mann Whitney U, $p < 0.05$) (B) Responses to the NDE become stronger following post-MD *ad lib* sleep (Mann Whitney U, $p < 0.001$); this effect is abolished by RSD (Mann Whitney U, *ns*). 5th and 95th percentiles are indicated by symbols. +Sleep = responses in sleeping group, +RSD=responses in RSD group. The number of mice and neurons per group are as follows: +Sleep, 4, 136; +RSD, 4, 101. * Indicates significant difference between groups.

Acknowledgements

The authors declare no conflicts of interest. This work was supported by NIH R21HD088829 (to MGF). The authors thank Dr. Wenbiao Gan for discussion and advice on 2-photon microscopy in awake mice.

References

- 1 Frank, M. G., Issa, N. P. & Stryker, M. P. Sleep enhances plasticity in the developing visual cortex. *Neuron* **30**, 275-287 (2001).
- 2 Espinosa, J. S. & Stryker, M. P. Development and plasticity of the primary visual cortex. *Neuron* **75**, 230-249 (2012).
- 3 Hensch, T. Critical period plasticity in local cortical circuits. *Nature Reviews Neuroscience* **6**, 877-888 (2005).
- 4 Tropea, D., Van Wart, A. & Sur, M. Molecular mechanisms of experience-dependent plasticity in visual cortex. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 341-355 (2009).
- 5 Dumoulin Bridi, M. C. *et al.* Rapid eye movement sleep promotes cortical plasticity in the developing brain. *Sci Adv* **1**, e1500105, doi:10.1126/sciadv.1500105 (2015).
- 6 Zhou, Y. *et al.* REM sleep promotes experience-dependent dendritic spine elimination in the mouse cortex. *Nature communications* **11**, 4819, doi:10.1038/s41467-020-18592-5 (2020).
- 7 Aton, S. J. *et al.* Mechanisms of sleep-dependent consolidation of cortical plasticity. *Neuron* **61**, 454-466, doi:10.1016/j.neuron.2009.01.007 (2009).
- 8 Trachtenberg, J. T., Trepel, C. & Stryker, M. P. Rapid extragranular plasticity in the absence of thalamocortical plasticity in the developing primary visual cortex. *Science* **287**, 2029-2032 (2000).
- 9 Seibt, J. & Frank, M. G. Primed to sleep: The dynamics of synaptic plasticity across brain states. *Frontiers in systems neuroscience* **13**, 2, doi:10.3389/fnsys.2019.00002 (2019).
- 10 Gordon, J. A. & Stryker, M. P. Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. *J Neurosci* **16**, 3274-3286 (1996).
- 11 Aton, S. J. *et al.* Visual experience and subsequent sleep induce sequential plastic changes in putative inhibitory and excitatory cortical neurons. *Proc Natl Acad Sci U S A* **110**, 3101-3106, doi:10.1073/pnas.1208093110 (2013).
- 12 Yang, G. *et al.* Sleep promotes branch-specific formation of dendritic spines after learning. *Science* **344**, 1173-1178 (2014).
- 13 Frank, M. G. Renormalizing synapses in sleep: The clock is ticking. *Biochem Pharmacol* **191**, 114533, doi:10.1016/j.bcp.2021.114533 (2021).

- 14 Raven, F., Van der Zee, E. A., Meerlo, P. & Havekes, R. The role of sleep in regulating structural plasticity and synaptic strength: implications for memory and cognitive function. *Sleep Medicine Reviews In Press* (2017).
- 15 Tan, H. L., Roth, R. H., Graves, A. R., Cudmore, R. H. & Huganir, R. L. Lamina-specific AMPA receptor dynamics following visual deprivation in vivo. *Elife* **9**, doi:10.7554/eLife.52420 (2020).
- 16 Turrigiano, G. G. The dialectic of Hebb and homeostasis. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372** (2017).
- 17 Cooke, S. F. & Bear, M. F. How the mechanisms of long-term synaptic potentiation and depression serve experience-dependent plasticity in primary visual cortex. *Philosophical Transactions of the Royal Society B: Biological Sciences* **369** (2013).
- 18 Kaneko, M., Stellwagen, D., Malenka, R. C. & Stryker, M. P. Tumor necrosis factor- α mediates one component of competitive, experience-dependent plasticity in developing visual cortex. *Neuron* **58**, 673-680, doi:S0896-6273(08)00377-2 [pii] 10.1016/j.neuron.2008.04.023 (2008).
- 19 Krueger, J. M. The role of cytokines in sleep regulation. *Curr Pharm Des* **14**, 3408-3416 (2008).
- 20 Torrado Pacheco, A., Bottorff, J., Gao, Y. & Turrigiano, G. G. Sleep promotes downward firing rate homeostasis. *Neuron* **109**, 530-544 e536, doi:10.1016/j.neuron.2020.11.001 (2021).
- 21 Hengen, K. B., Torrado Pacheco, A., McGregor, J. N., Van Hooser, S. D. & Turrigiano, G. G. Neuronal firing rate homeostasis is inhibited by sleep and promoted by wake. *Cell* **165**, 180-191, doi:10.1016/j.cell.2016.01.046 (2016).
- 22 Hengen, Keith B., Lambo, Mary E., Van Hooser, Stephen D., Katz, Donald B. & Turrigiano, Gina G. Firing rate homeostasis in visual cortex of freely behaving rodents. *Neuron* **80**, 335-342, doi:<https://doi.org/10.1016/j.neuron.2013.08.038> (2013).
- 23 Frank, M. G. Erasing synapses in sleep: is it time to be SHY? *Neural Plasticity* **2012**, doi:10.1155/2012/264378 (2012).
- 24 Renouard, L., Bridi, M. C. D., Coleman, T., Arckens, L. & Frank, M. G. Anatomical correlates of rapid eye movement sleep-dependent plasticity in the developing cortex. *Sleep* **41**, doi:10.1093/sleep/zsy124 (2018).
- 25 Dumoulin, M. C. *et al.* Extracellular signal-regulated kinase (ERK) activity during sleep consolidates cortical plasticity in vivo. *Cereb Cortex* **25**, 507-515, doi:10.1093/cercor/bht250 (2015).
- 26 Seibt, J. *et al.* Protein synthesis during sleep consolidates cortical plasticity in vivo. *Curr Biol* **22**, 676-682, doi:10.1016/j.cub.2012.02.016 (2012).
- 27 Ravassard, P. *et al.* Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. *Sleep* **32**, 227-240 (2009).
- 28 Tudor, J. C. *et al.* Sleep deprivation impairs memory by attenuating mTORC1-dependent protein synthesis. *Science Signaling* **9**, ra41 (2016).

- 29 Kim, J. Y., Grunke, S. D., Levites, Y., Golde, T. E. & Jankowsky, J. L. Intracerebroventricular viral injection of the neonatal mouse brain for persistent and widespread neuronal transduction. *J Vis Exp*, 51863, doi:10.3791/51863 (2014).
- 30 Chen, T.-W. *et al.* Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**, 295-300, doi:10.1038/nature12354 (2013).
- 31 Ingiosi, A. M. *et al.* A role for astroglial calcium in mammalian sleep and sleep regulation. *Curr Biol* **30**, 4373-4383 e4377, doi:10.1016/j.cub.2020.08.052 (2020).
- 32 Kalatsky, V. A. & Stryker, M. P. New paradigm for optical imaging: temporally encoded maps of intrinsic signal. *Neuron* **38**, 529-545 (2003).
- 33 Cang, J., Kalatsky, V. A., Lowel, S. & Stryker, M. P. Optical imaging of the intrinsic signal as a measure of cortical plasticity in the mouse. *Vis Neurosci* **22**, 685-691 (2008).