REM sleep promotes bidirectional plasticity in developing visual cortex in vivo

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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 awake 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.

1. Introduction

Sleep enhances a classic model of developmental plasticity in vivo (ocular dominance plasticity: ODP) (Frank et al., 2001). 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 (NDE). 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 function (Espinosa and Stryker, 2012; Hensch, 2005; Tropea et al., 2009).

The precise role of rapid-eye-movement (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 NDE (Dumoulin et al., 2015; Zhou et al., 2020). The types of plasticity governing this shift are unclear. In cats, sleep strengthens responses to the NDE, but the specific role of REM sleep in this process was not investigated (Dumoulin et al., 2015; Aton et al., 2009). In mice, REM sleep weakens responses to the deprived eye (DE), but this analysis was restricted to one type of neuron (apical dendrites from layer 5 neurons) (Zhou et al., 2020). Considering that excitatory neurons in cortical layers 2/3 are among the first to exhibit ODP (Trachtenberg et al., 2000), 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 NDE. This supports the idea that sleep promotes bidirectional changes in plasticity depending on the cell and circuit (Seibt and Frank, 2019).

2. Methods

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

2.1. Virus injection

At P0–P3, equal numbers of male and female CaMKIIalpha-Cre mice (B6.G-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 μl/min) with 1 μl of

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AAV9-CAG-Flex-GCaMP6s (titer: ~ 1 × 10e13 genomes/mL, Penn Vector core) and 1 µl of AAV1-CAG-tdTomato (titer: ~ 1 × 10e13 genomes/mL, Penn Vector core). The procedure lasted around 10–15 min (Kim et al., 2014). As shown previously, the former injection leads to selective expression of GCaMP6 in CaMKII + neurons (Chen et al., 2013). 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.

2.2. EEG & EMG, cranial window surgery and baseplate positioning

At P18-21, mice were prepared for GCaMP6s and EEG & EMG recording as described previously (Ingiosi et al., 2020). Briefly, after dura removal, a 3 mm diameter cranial window was positioned over binocular (right hemisphere) V1 using previously described coordinates (Kalatsky and Stryker, 2003). 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 3.1 mm diameter miniature connector (Omnnetics 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.

2.3. 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 h head restraint one to two times each day for at least 5 days prior to visual testing (Zhou et al., 2020). 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 h to induce ODP as previously performed in cats (Frank et al., 2001). 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 h (MD + S), while the other group was REM sleep deprived (MD + RSD) for the first 3 h post-MD followed by 3 h ad lib recovery sleep. The post-MD sleep and RSD periods were performed in dim red light to control for visual input. Between 2-photon measurements, polosomographic 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 (Zhou et al., 2020). This latter approach results in >90% reduction in REM sleep with non-significant changes in nonREM sleep in CP mice (Zhou et al., 2020).

2.4. 2-Photon signal processing and visual testing

2-photon microscopy in non-anesthetized head restrained mice was performed as previously described (Ingiosi et al., 2020). 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°, and grey screen, spatial and temporal frequency as described in (Cang et al., 2005), 3 s each separated by 6 s of black screen presentation). The grey screen was set at equal luminance and zero contrast. Visual stimuli were generated using Matlab 2016a and psychtoolbox. Visual responses were recorded between ~150 µm and ~200 µm 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 (Ingiosi et al., 2020).

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., vascularule 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 ΔF/F values (ΔF/F=(Fstim−Fshot)/Fshot) where $F_{stim}$ = peak signal during 3 s visual stimulation (+2 s of subsequent black screen) and $F_{shot} = $ peak signal during the last 2 s of the preceding black screen presentation. This calculation ensured that slow GCaMP6s responses to a visual stimulus were also captured (Fig. 2). 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.

2.5. 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 Kruskal Wallis test.

3. Results

We used a design adopted from previous investigations of sleep and ODP (Frank et al., 2001; Zhou et al., 2020). After verifying that we could selectively express GCaMP6s in V1 CaMKII neurons (Fig. 1), we then obtained baseline measurements of visual responses to each eye. The eye contralateral to the measured V1 was then closed and the mice were kept awake for 6 h to induce ODP. We then determined the effects of subsequent 6 h ad lib sleep or 3 h of RSD (+3 h of recovery sleep) 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 = 0.51±0.02 (std. error) vs. baseline 0.44±0.02: paired Student’s t-test, p < 0.001; MD + RSD: 0.48±0.02; vs. baseline 0.47±0.02, p = 0.37; paired Student’s t-test). The effects of sleep on ODP involved both a loss of response to the DE and a gain of response to the NDE (Fig. 2). Averaged across neurons, both changes were enhanced after sleep compared to baseline values. These plastic changes did not occur in RSD mice. After RSD, changes in the NDE response were not different from baseline, and DE responses were instead enhanced (Fig. 3).

4. 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 h) 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 ODP (Gordon and Stryker, 1996), and sleep only promotes weakening of DE circuits (Zhou et al., 2020). We discuss aspects of these findings below.
4.1. 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 DE circuits while strengthening responses to the NDE (Aton et al., 2009, 2013). 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 (Zhou et al., 2020; Aton et al., 2009). 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 weakening (Zhou et al., 2020).

One explanation for this apparent discrepancy is that sleep-dependent plasticity varies depending on the type of neuronal circuit (Seibt and Frank, 2019; Yang et al., 2014). For example, in adult mice motor learning followed by sleep increases dendritic spine number in motor cortex, but only on some dendritic branches (Yang et al., 2014). Similarly, sleep-dependent hippocampal (morphological) plasticity is highly determined by region (CA1 vs. CA3) and spatial location on a given dendrite (reviewed in Franklin, 2021; Raven et al., 2017). Our previous study in mice examined changes in spine number and GCaMP signaling in layer 5 neuron apical dendrites (Zhou et al., 2020). 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 spines (Tan et al., 2020). This suggests that more heterogeneous plastic changes may occur in layer 2/3 neurons compared to layer 5 neurons in sleep-dependent plasticity. In addition, some differences between the two studies may be due to slight methodological differences (e.g., the use of GCaMPS vs. GCaMPF, measures of signal amplitude and spine number vs. events, etc.).

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 enhances visual responses in a manner that favors the open eye (Turrigiano, 2017). In support of this idea, in mice the loss of response to the deprived eye is mediated by Hebbian long-term depression (Cooke and Bear, 2013) (and see Hensch, 2005)). However, the increased response to the non-deprived eye is governed by TNFα (Kaneko et al., 2008); a cytokine that promotes homeostatic synaptic up-scaling and is at its highest brain concentrations during sleep (Kaneko et al., 2008; Krueger, 2008). Although the role of sleep in homeostatic V1 plasticity is complex (Torrado Pacheco et al., 2021; Hengen et al., 2013, 2016), the above findings are consistent with previous suggestions that under
following post-MD. This weakening does not occur in RSD mice; instead, responses to the DE are weaker after sleep (Kruskal-Wallis, \( p < 0.001 \)).

Non-Hebbian mechanisms. In addition to TNF-α, enzymes implicated in Hebbian synaptic plasticity (ERK and mTOR) are also key mediators of ODP (Tropea et al., 2009). In CP cats, both ERK and mTOR are activated in V1 during REM sleep (Dumoulin et al., 2015; Renouard et al., 2018), and inhibition of both kinases during post-MD sleep reduces ODP in a manner similar to RSD (Dumoulin et al., 2015; Seibt et al., 2012). 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 (Ravassard et al., 2009; Tudor et al., 2016), which suggests that these mechanisms may be evolutionarily conserved.

Hebbian plasticity may also explain our peculiar observation that RSD increased responsiveness to the DE. Similar paradoxical shifts in favor of the DE can occur under certain conditions. Reversible silencing of V1 combined with MD also causes a shift in favor of the DE. This has been explained as a Hebbian process where the more active pre-synaptic (NDE) inputs are punished when post-synaptic neuronal firing is out of phase (Hata and Stryker, 1994; Hata et al., 1999). Therefore, it is possible that in the absence of REM sleep, spontaneous V1 activity becomes decorrelated with pre-synaptic inputs, leading to a similar condition. While speculative, this idea is supported by the observation that cortical inhibition is enhanced during REM sleep by heightened activity of parvalbumin- (PV) GABAergic neurons (Niethard et al., 2017). PV neurons play essential roles in ODP by influencing the opening and closing of the CP and gating excitation and (possibly) spike-timing-dependent plasticity in pyramidal neurons following MD (Hensch, 2005; Honks and Chen, 2020; Yazaki-Sugiyama et al., 2009). Removing REM sleep, and these specific brain conditions, may thus disorganize the balance of cortical excitation and inhibition necessary for normal effects of MD.

CRediT authorship contribution statement

Leslie Renouard: Conceptualization, Data curation, Software, Validation, Formal analysis, and, Methodology. Investigation, and, Writing – original draft. Christopher Hayworth: Conceptualization, Data curation, Software, Validation, Formal analysis, and, Methodology. Project administration. Michael Rempe: Conceptualization, Data curation, Software, Validation, Formal analysis, and, Methodology. Will Clegern: Methodology. Jonathan Wisor: Conceptualization, Data curation, Software, Validation, Formal analysis, and, Methodology. Marcos G. Frank: Project administration, Supervision, Visualization, Writing – review & editing, Funding acquisition, and, Resources.

Declaration of competing interest

The authors have no conflicts of interest.

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References

Aton, S.J., et al., 2009. Mechanisms of sleep-dependent consolidation of cortical plasticity. Neuron 61, 454–466.
Aton, S.J., et al., 2013. 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.
Cang, J., Kalatsky, V.A., Lowel, S., Stryker, M.P., 2005. Optical imaging of the intrinsic signal as a measure of cortical plasticity in the mouse. Vis. Neurosci. 22, 685–691.
Chen, T.-W., et al., 2013. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499, 295–300.
Cooke, S.F., Bear, M.F., 2013. How the mechanisms of long-term synaptic potentiation and depression serve experience-dependent plasticity in primary visual cortex. Phil. Trans. Biol. Sci. 368.
Dumoulin Bridi, M.C., et al., 2015. Rapid eye movement sleep promotes cortical plasticity in the developing brain. Sci. Adv. 1, e1500105.
Dumoulin, M.C., et al., 2015. Extracellular signal-regulated kinase (ERK) activity during sleep consolidates cortical plasticity in vivo. Cereb. Cortex 25, 507–515.
Espinosa, J.S., Stryker, M.P., 2013. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499, 295–300.
Frank, M.G., 2012. Erasing synapses in sleep: is it time to be SHY? Neural Plast. 2012.
Frank, M.G., 2021. Renormalizing synapses in sleep: the clock is ticking. Biochem. Pharmacol. 191, 11453.
Frank, M.G., Insa, N.P., Stryker, M.P., 2001. Sleep enhances plasticity in the developing visual cortex. Neuron 30, 275–287.

Gordon, J.A., Stryker, M.P., 1996. Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J. Neurosci. 16, 3274–3286.

Hata, Y., Stryker, M.P., 1994. Control of thalamocortical afferent rearrangement by postsynaptic activity in developing visual cortex. Science 265, 1732–1735.

Hata, Y., Tsutsumo, T., Stryker, M.P., 1999. Selective pruning of more active afferents when cat visual cortex is pharmacologically inhibited. Neuron 22, 375–381.

Hengen, Keith B., Lambo, Mary E., Van Hooser, Stephen, D., Katz, Donald B., Turrigiano, Gina G., 2013. Firing rate homeostasis in visual cortex of freely behaving rodents. Neuron 80, 335–342.

Hengen, K.B., Torrado Pacheco, A., McGregor, J.N., Van Hooser, S.D., Turrigiano, G.G., 2016. Neuronal firing rate homeostasis is inhibited by sleep and promoted by wake. Cell 165, 180–191.

Hensch, T., 2005. Critical period plasticity in local cortical circuits. Nat. Rev. Neurosci. 6, 877–888.

Hooks, B.M., Chen, C., 2020. Circuitry underlying experience-dependent plasticity in the mouse visual system. Neuron 106, 21–36.

Ingiosi, A.M., et al., 2020. A role for astroglial calcium in mammalian sleep and sleep regulation.Curr. Biol. 30, 4373–4383 e4377.

Kalatsky, V.A., Stryker, M.P., 2003. New paradigm for optical imaging: temporally encoded maps of intrinsic signal. Neuron 38, 529–545.

Kaneko, M., Stellwagen, D., Malenka, R.C., Stryker, M.P., 2008. Tumor necrosis factor-alpha mediates one component of competitive, experience-dependent plasticity in developing visual cortex. Neuron 58, 673–680.

Kim, J.Y., Gronke, S.D., Levites, Y., Golde, T.E., Jankowsky, J.L., 2014. Intracerebroventricular viral injection of the neonatal mouse brain for persistent and widespread neuronal transduction. JoVE, 51863.

Krueger, J.M., 2008. The role of cytokines in sleep regulation. Curr. Pharmaceut. Des. 14, 3408–3416.

Niethard, N., Burgalosi, A., Born, J., 2017. Plasticity during sleep is linked to specific regulation of cortical circuit activity. Front. Neural Circ. 11.

Ravassard, P., et al., 2009. 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.

Raven, F., Van der Zee, E.A., Meerlo, P., Havekes, R., 2017. The role of sleep in regulating structural plasticity and synaptic strength: implications for memory and cognitive function. Sleep Med. Rev. (in press).

Renouard, L., Bridi, M.C.D., Coleman, T., Arkens, L., Frank, M.G., 2018. Anatomical correlates of rapid eye movement sleep-dependent plasticity in the developing cortex. Sleep 41.

Seibt, J., Frank, M.G., 2019. Primed to sleep: the dynamics of synaptic plasticity across brain states. Front. Syst. Neurosci. 13, 2.

Seibt, J., et al., 2012. Protein synthesis during sleep consolidates cortical plasticity in vivo. Curr. Biol. 22, 676–682.

Tan, H.L., Roth, R.H., Graves, A.R., Cudmore, R.H., Huganir, R.L., 2020. Lamina-specific AMPA receptor dynamics following visual deprivation in vivo. Elife 9.

Torrado Pacheco, A., Bottorff, J., Gao, Y., Turrigiano, G.G., 2021. Sleep promotes downward firing rate homeostasis. Neuron 109, 530–544 e536.

Trachtenberg, J.T., Trepel, C., Stryker, M.P., 2000. Rapid extragranular plasticity in the absence of thalamocortical plasticity in the developing primary visual cortex. Science 287, 2029–2032.

Tropoa, D., Van Wart, A., Sur, M., 2009. Molecular mechanisms of experience-dependent plasticity in visual cortex. Phil. Trans. Biol. Sci. 364, 341–355.

Tudor, J.C., et al., 2016. Sleep deprivation impairs memory by attenuating mTORC1-dependent protein synthesis. Sci. Signal. 9, ra41.

Turrigiano, G.G., 2017. The dialectic of Hebb and homeostasis. Phil. Trans. Biol. Sci. 372, Yang, G., et al., 2014. Sleep promotes branch-specific formation of dendritic spines after learning. Science 344, 1173–1178.

Yazaki-Sugiyama, Y., Kang, S., Catania, H., Fukai, T., Hensch, T.K., 2009. Bidirectional plasticity in fast-spiking GABA circuits by visual experience. Nature 462, 218–221.

Zhou, Y., et al., 2020. REM sleep promotes experience-dependent dendritic spine elimination in the mouse cortex. Nat. Commun. 11, 4819.