Paradoxical somatodendritic decoupling supports cortical plasticity during REM sleep

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Rapid eye movement (REM) sleep is associated with the consolidation of emotional memories. Yet, the underlying neocortical circuits and synaptic mechanisms remain unclear. We found that REM sleep is associated with a somatodendritic decoupling in pyramidal neurons of the prefrontal cortex. This decoupling reflects a shift of inhibitory balance between parvalbumin–mediated somatic inhibition and vasoactive intestinal peptide–mediated dendritic disinhibition, mostly driven by neurons from the central medial thalamus. REM-specific optogenetic suppression of dendritic activity led to a loss of danger-versus-safety discrimination during associative learning and a lack of synaptic plasticity, whereas optogenetic release of somatic inhibition resulted in enhanced discrimination and synaptic potentiation. Somatodendritic decoupling during REM sleep promotes opposite synaptic plasticity mechanisms that optimize emotional responses to future behavioral stressors.

Decoupling of somatodendritic activities in cortical neurons during REM sleep

We first dissected dPFC microcircuit dynamics during REM sleep in head-restrained sleeping mice using simultaneous two-photon calcium imaging and electrophysiological recordings from chronic electroencephalogram (EEG) and electromyogram (EMG) electrodes (Fig. 1A). An adeno-associated virus encoding the calcium indicator GCaMP6 under the calmodulin kinase II promoter (AAV1-CaMKII-GCaMP6) was stereotactically injected into the dPFC area. The resulting GCaMP6 expression was specific to pyramidal (PYR) neurons of superficial layers (L2/3) (Fig. 1A and B). Simultaneous recordings revealed a significant decrease of PYR somatic activity during REM sleep as compared with non-REM (NREM) sleep or wakefulness (Fig. 1, C and D; movie S1; and fig. S1); see materials and methods for data analysis and motion artifact detection). This decrease was consistent across all animals tested (fig. S2) and was confirmed using deconvolution analytical methods (materials and methods and fig. S3, A to D). Although few pyramidal somas remained active during REM sleep (8%), a substantial fraction of PYR cells was preferentially active during either NREM sleep (31%) or wakefulness (61%) (Fig. 1E). Distribution of single-PYR cell ΔF/F0 values (where ΔF/F0 is the ratio of the change in fluorescence to the baseline fluorescence) indicated low activity during REM sleep as compared with NREM sleep and wakefulness (Fig. 1F). REM sleep was associated with a significant decrease in the frequency of calcium (Ca2+) events compared with those in other states (~18%; versus ~33% in NREM sleep or ~49% in wakefulness) (Fig. 1, G and H), although the integrals and amplitudes of Ca2+ events were similar across states (fig. S4).

We next tested whether apical dendrites of L2/3 dPFC PYR neurons were similarly modulated during REM sleep in mice coinjected with an AAV1-Syn-flex-GCaMP6 and an AAV1-CaMKII-Cre to obtain a sparse labeling of dendrites (materials and methods; Fig. 1, I and J; and movie S1). Spatial propagation of GCaMP6s activity along individual dendrites was quantified for all GCaMP6s-expressing dendrites. Each identified branch was segmented into 0.5-μm-wide rectangular regions of interest (ROIs), and ΔF/F0 traces of each ROI were extracted (materials and methods and Fig. 1, K and L). In contrast to PYR somas, PYR dendrites were highly active during REM sleep (Fig. 1M), as shown by the significant increase of the frequency of events spreading along the dendrites (>20 μm) compared with events confined to small portions of the dendritic branches (<20 μm). The average propagation of events along the dendrites was also higher during REM sleep (Fig. 1, N and O), providing evidence for a REM sleep–specific disinhibition of apical dendrites concomitant with a tonic inhibition of PYR somas.

Shift in excitation/inhibition balance during REM sleep in cortical microcircuits

To investigate the excitation/inhibition (E/I) balance during REM sleep, we recorded the activity of parvalbumin (PV), vasoactive intestinal peptide (VIP), and somatostatin (SST) dPFC interneurons from PV-IRES-Cre, VIP-IRES-Cre, or SST-IRES-Cre mice, respectively, transduced with AAV1-Syn-flex-GCaMP6 in sleeping mice (materials and methods, Fig. 2A, and movie S1). PV interneurons are strategically positioned to exert feed-forward inhibition by innervating principally the PYR somas (27). Accordingly, the activity of GCaMP6-expressing VIP interneurons was significantly increased during REM sleep (Fig. 2, B and C), an observation that is consistent with the elevated ΔF/F0 values of GCaMP6-expressing PV neurons during REM sleep (~72% of recorded neurons) (Fig. 2, D, E, and F).

VIP interneurons have been described to preferentially inhibit SST γ-aminobutyric acid (GABA)–releasing cells, which in turn synapse primarily onto the dendritic shaft of PYR neurons (28). GCaMP6-expressing VIP interneurons showed a significant enhancement of ΔF/F0 values during REM sleep, with a large portion of VIP neurons (~56%) displaying maximal activity during REM sleep (Fig. 2, G to K). By contrast, GCaMP6-expressing SST interneurons were mainly silent during REM sleep, except for a small subpopulation (~10%) (Fig. 2, L to P). These results were consistent across animals (fig. S5) and were confirmed with deconvolution analytical methods (fig. S3, E to H, for PV; fig. S3, I to L, for VIP; and fig. S3, M to P, for SST).

The activity of GCaMP6-expressing VIP and PV neurons progressively increased over NREM-to-REM sleep transitions, whereas GCaMP6-expressing PYR somas and SST interneurons both showed opposite responses (Fig. 2Q). Notably, the ΔF/F0 values of PYR somas during NREM sleep episodes were predictive of the next sleep-wake transition because ΔF/F0 values were lower in NREM sleep preceding REM sleep than ΔF/F0 values preceding awakening (fig. S6). Upon awakening, the ΔF/F0...
Fig. 1. Somatodendritic decoupling during REM sleep in dPFC PYR neurons. (A) Schematic of the experimental procedure. (B) (Left) Expression profile of L2/3 GCaMP6 PYR neurons. Scale bar, 1 mm. (Right) GCaMP6 PYR neurons. Scale bar, 20 µm. (C) EEG [red squares are magnifications of NREM sleep (gray), REM sleep (fuchsia), and wake (light blue) traces], EMG, hypnogram, color-coded power spectrum density, and color-coded and raw ΔF/F₀ activity profiles of a population of PYR somas. Freq., frequency. (D) Mean ± SEM (black line) ΔF/F₀ of PYR somas (n = 238 neurons; m = 6 mice) during wake, NREM, and REM sleep. ***P < 0.001; one-way repeated measures analysis of variance (RM ANOVA) with Tukey post hoc test. (E) Percentages of PYR somas with maximal values of ΔF/F₀ in wake (light blue), NREM (gray), and REM (fuchsia) sleep. (F) Line (top) and scatter (bottom) plots showing the distribution of ΔF/F₀ values during wake, NREM, and REM sleep. (G) Frequency of somatic Ca²⁺ events during wake, NREM, and REM sleep. *P < 0.05; one-way RM ANOVA with Tukey post hoc test. Black lines represent mean values. (H) Distribution of somatic Ca²⁺ events in wake, NREM, and REM sleep. (I) Schematic of the experimental strategy. (J) Expression profile of GCaMP6 in the superficial layers of the dPFC. Scale bar, 1 mm. (K) (Left) GCaMP6 PYR dendrites. Scale bar, 20 µm. (Right) Magnification of a segmented dendritic branch. Scale bar, 4 µm. (L) (Top) Hypnogram and ΔF/F₀ traces of ROIs from a single dendrite. (Bottom) Color-coded spreading event (left) and confined event (right) from the same dendrite. (M) Frequency ± SEM of dendritic Ca²⁺ confined (left) and spreading (right) events (n = 308 dendrites; m = 5 mice) during wake, NREM, and REM sleep. ***P < 0.001; one-way RM ANOVA with Tukey post hoc test. (N) Line (top) and scatter (bottom) plots showing the average propagation (in micrometers) of all (confined + spreading) Ca²⁺ events during wake, NREM, and REM sleep. (O) Mean propagation of all dendrites during wake, NREM, and REM sleep. ***P < 0.001; one-way RM ANOVA with Tukey post hoc test.

values of PYR and SST somas significantly increased, whereas those from VIP and PV somas progressively decreased (Fig. 2R). The activity of each population of neurons correlated with tonic (6 to 9 Hz) (Fig. 2S), but not phasic (9 to 13 Hz), REM sleep.

Thalamic control of PV neuron–mediated inhibition of PYR cell somas during REM sleep
We next sought to identify the dPFC synaptic inputs responsible for this REM sleep–specific shift in E/I balance within dPFC microcircuits using a rabies virus–based anatomical mapping and optogenetic circuit probing. To map the synaptic inputs to dPFC PV and VIP neurons, we injected AAV2/1-CMV-Flex-TVA-mCherry-2A-oG and RV-CMV-ENVA-EGFP (EGFP, enhanced green fluorescent protein) (29) in PV- and VIP-IRES-Cre mice, respectively.
Fig. 2. Shift in inhibitory balance during REM sleep in dPFC circuits.
(A) Expression profile of GCaMP6 interneurons in the dPFC. Scale bars, 1 mm (top) and 100 μm (bottom). (B) (Left) Schematic of the experimental procedure. (Right) GCaMP6 PV neurons. Scale bar, 30 μm. (C) Hypnogram and color-coded and raw ΔF/F0 traces of PV neurons. Red arrowheads indicate motion artifacts. (D) Mean ± SEM (red line) ΔF/F0 of PV neurons (n = 137 neurons; m = 7 mice) during wake, NREM, and REM sleep. **p < 0.01; ***p < 0.001; one-way RM ANOVA with Tukey post hoc test. (E) Percentages of PV neurons with maximal values of ΔF/F0 in wake (light blue), NREM (gray), and REM (fuchsia) sleep. (F) Line (top) and scatter (bottom) plots showing the distribution of PV neurons ΔF/F0 values during wake, NREM, and REM sleep. (G) (Left) Schematic of the experimental procedure. (Right) GCaMP6 VIP neurons. Scale bar, 30 μm. (H) Hypnogram and color-coded and raw ΔF/F0 traces of VIP neurons. Red arrowheads indicate motion artifacts. (I) Mean ± SEM (green line) ΔF/F0 of VIP neurons (n = 230 neurons; m = 5 mice) during wake, NREM, and REM sleep. **p < 0.01; ***p < 0.001; one-way RM ANOVA with Tukey post hoc test. (J) Percentages of PV neurons with maximal values of ΔF/F0 in wake (light blue), NREM (gray), and REM (fuchsia) sleep. (K) Line (top) and scatter (bottom) plots showing the distribution of VIP neurons ΔF/F0 values during wake, NREM, and REM sleep. (L) (Left) Schematic of the experimental procedure. (Right) GCaMP6 SST neurons. Scale bar, 30 μm. (M) Hypnogram and color-coded and raw ΔF/F0 traces of SST neurons. Red arrowheads indicate motion artifacts. (N) Mean ± SEM (dark blue line) ΔF/F0 of SST neurons (n = 64 neurons; m = 3 mice) during wake, NREM, and REM sleep. ***p < 0.001; one-way RM ANOVA with Tukey post hoc test. (O) Percentages...
of SST neurons with maximal values of ΔF/F₀ in wake (light blue), NREM (gray), and REM (fuschia) sleep. (P) Line (top) and scatter (bottom) plots showing the distribution of SST neurons ΔF/F₀ values during wake, NREM, and REM sleep. (Q) (Left) Mean ± SEM activity profiles of dPFC neuronal populations during NREM-to-REM sleep transitions (black = PYR, red = PV, green = VIP, dark blue = SST). (Right) ΔF/F₀ change ± SEM across transitions. ***P < 0.001; one-way ANOVA with Tukey post hoc test. (R) (Left) Mean ± SEM activity profiles of dPFC neuronal populations during the transition from REM sleep to wake. (Right) ΔF/F₀ change ± SEM across transitions. ***P < 0.001; one-way ANOVA with Tukey post hoc test. (S) Correlation analysis between peak REM sleep theta frequency ± SEM and the respective ΔF/F₀ mean ± SEM of all recorded neurons per session.

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Fig. 3. Thalamic control of REM sleep–specific PV-mediated inhibition of PYR somas. (A) Schematic of the experimental procedure. (B) Viral injection site of flex-mCherry and RV-GFP in the dPFC. Scale bars, 500 μm (leftmost panel) and 50 μm (right three panels). (C) Expression profiles of the major brain regions projecting to dPFC PV neurons (CM, RSA and RSG, and S1BF). Scale bars, 1 mm. (D) Total distribution of identified neurons ± SEM (n = 3 mice) in the CM, RS, and SL. (E) Density of identified neurons ± SEM in the CM, RS, and SL. (F) Schematic of the experimental strategy. (G) Example trace of light-evoked EPSC in a PV neuron. Black traces indicate single evoked sweeps, the red trace indicates the sweeps’ mean, and the blue vertical line indicates optical stimulation. (H) (Left) EPSC mean ± SEM latency from light stimulation (n = 7 neurons; m = 3 mice). (Right) Mean ± SEM amplitude of evoked EPSCs. (I) Percentages of cells responsive to light-evoked stimulation. (J) Schematic of the experimental procedure. (K) (Left) Expression profile of GCaMP6 in the CM. Scale bar, 1 mm. (Right) GCaMP6 CM axons. Scale bar, 30 μm. (Bottom) Time-lapse activity profile of a CM. Scale bar, 10 μm. (L) Hypnogram and color-coded ΔF/F₀ activity profiles of CM axons. (M) Mean ΔF/F₀ ± SEM (black line) of CM axons (n = 112 axons; m = 5 mice) during wake, NREM, and REM sleep. ***P < 0.001; one-way RM ANOVA with Tukey post hoc test. (N) Line (top) and scatter (bottom) plots showing the distribution of CM axons ΔF/F₀ values during wake, NREM, and REM sleep. (O) Percentages of CM axons with maximal values of ΔF/F₀ in wake (light blue), NREM (gray), and REM (fuchsia) sleep. (P) Schematic of the experimental procedure. (Q) GCaMP6 PYR neurons and RCaMP PV neurons. Scale bar, 30 μm. (R) Hypnogram and color-coded ΔF/F₀ activity profiles of dPFC PYR and PV somas. Blue and red vertical lines indicate 473-nm and 638-nm optical stimulations, respectively. (S) REM sleep ΔF/F₀ ± SEM of a group of PYR somas (n = 475 neurons; m = 8 mice) upon REM sleep–specific CM silencing (opto) versus a control group (no opto) (n = 238 neurons; m = 6 mice). ***P < 0.001; Student’s t test. (T) PYR ΔF/F₀ change ± SEM (difference between REM sleep and wake) in opto versus no opto. ***P < 0.001; Student’s t test. (U) REM sleep ΔF/F₀ ± SEM of PV neurons (n = 92 neurons; m = 4 mice) upon REM sleep–specific CM silencing (opto) versus control (no opto) (n = 137 neurons; m = 7 mice). ***P < 0.001; Student’s t test. (V) PV ΔF/F₀ change ± SEM (difference between REM sleep and wake) in opto versus no opto. ***P < 0.001; Student’s t test.
**Fig. 4.** VIP-mediated disinhibition of PYR dendrites. (A) Schematic of the experimental procedure. (B) GCaMP6 PYR dendrites (right) and SwitchRCA VIP neurons (left). Scale bar, 30 μm. (C) Hypnogram and ΔF/ΔF₀ traces of ROIs obtained from the segmentation of a single dendrite (represented on the left). Scale bar, 10 μm. (D) Dendritic confined event frequency change ± SEM (difference between REM sleep and wake) in no opto (n = 308 dendrites; m = 5 mice) versus opto (n = 51 dendrites; m = 5 mice). **P < 0.01; Student’s t test. Raw frequency values during REM sleep: no opto = 0.0066 ± 0.004 Hz; opto = 0.0211 ± 0.009 Hz. (E) Dendritic spreading event frequency change ± SEM (difference between REM sleep and wake) in no opto versus opto. ***P < 0.001; Student’s t test. Raw frequency values during REM sleep: no opto = 0.2012 ± 0.0326 Hz; opto = 0.0066 ± 0.004 Hz.

**Fig. 5.** VIP- and PV neuron–dependent tuning of REM sleep–specific memory consolidation. (A) Schematic of the experimental procedure. (B) Experimental timeline. Electrical stimulation is indicated in the diagram. (C) Sleep-wake state total duration during optogenetic manipulation in PV YFP (m = 11 mice), PV Arch (m = 11 mice), VIP YFP (m = 7 mice), and VIP Arch (m = 10 mice). (D) Freezing percentage (normalized to YFP) ± SEM during CS− and CS+ in VIP YFP (green) versus VIP Arch (orange). ***P < 0.001; ns, not significant; one-way ANOVA with Tukey post hoc test. (E) Freezing percentage (normalized to YFP) ± SEM during CS− and CS+ in PV YFP (green) versus PV Arch (orange). ***P < 0.001; ns, not significant; one-way ANOVA with Tukey post hoc test. (F) Discrimination score (normalized to YFP) ± SEM in VIP (left) and PV (right) mice (orange = Arch; green = YFP). ***P < 0.001; Student’s t test. (G) AMPA/NMDA ratio traces. (H) Mean AMPA/NMDA ratios ± SEM (normalized to YFP) of VIP YFP (green) (n = 22 cells; m = 5 mice), VIP Arch (orange) (n = 23 cells; m = 5 mice), and naive (gray, no behavior) (n = 14 cells; m = 3 mice). *P < 0.05; ns, not significant; one-way ANOVA with Tukey post hoc test. (I) Mean AMPA/NMDA ratios ± SEM (normalized to YFP) of PV YFP (green) (n = 19 cells; m = 5 mice), PV Arch (orange) (n = 21 cells; m = 4 mice), and naive (gray, normalized to YFP, raw value, 1.283 ± 0.22). **P < 0.01; ***P < 0.001; one-way ANOVA with Tukey post hoc test. (J) Correlation between discrimination score and AMPA/NMDA ratios. Estimations of 95% confidence ellipses are represented.
somatic inhibition may preclude a top-down reinforcement of physiological and behavioral responses to fear in downstream pathways. A lack of somatic inhibition may interfere with local dendritic computation through back-propagating activity (33, 36) and facilitate non-linear integration of synaptic inputs during REM sleep. This may result in overconsolidation of emotional memories observed in post-traumatic stress disorders and other affective psychiatric and mood disorders often associated with REM sleep disturbances (37, 38).

These findings are consistent with a simultaneous upscaling and downscaling of synaptic plasticity at the PYR dendrites and cell bodies, respectively. Yet, whether similar NREM sleep-dependent mechanisms support memory consolidation and synaptic plasticity remains to be investigated (39, 40). Somatodendritic decoupling during REM sleep provides a mechanism that allows flexibility or adaptation to environmental changes and optimizes the discrimination of danger versus safety and physiological responses to behavioral stressors.

**REFERENCES AND NOTES**


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**Competing interests:** The authors declare no competing interests.

**Data and materials availability:** All presented data and analysis scripts, including mat-files and Matlab scripts and functions, are available on https://github.com/2ENLabCode.

**SUPPLEMENTARY MATERIALS**

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**Materials and Methods**

Figs. S1 to S13

References (41–43)

MDAR Reproducibility Checklist

Movie S1
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Emotional memory encoding in REM sleep
During rapid eye movement (REM) sleep, emotional memories are consolidated in the prefrontal cortex. However, we are still far from a mechanistic understanding of the processes involved. Aime et al. investigated the impact of REM sleep on cellular and subcellular activities, microcircuit connectivity, plasticity, and behavior. The authors quantified how somatic and dendritic activity differ in mice during wakefulness, REM, and non-REM sleep and probed how interneurons cause these differences. They further identified a specific area in the thalamus that activates a subpopulation of interneurons that decouple the dendrite from the somatic output. Finally, by optogenetic intervention during REM sleep, learning of discrimination between danger and safety could be enhanced or diminished. These findings provide a better understanding of the processing of emotions during sleep. —PRS

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