Cite as: D. Miyamoto *et al.*, *Science* 10.1126/science.aaf0902 (2016).

Top-down cortical input during NREM sleep consolidates perceptual memory

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During tactile perception, long-range intracortical top-down axonal projections are essential for processing sensory information. Whether these projections regulate sleep-dependent long-term memory consolidation is unknown. We altered top-down inputs from higher order cortex to sensory cortex during sleep and examined the consolidation of memories acquired earlier during waking texture perception. Mice learned novel textures and consolidated them during sleep. Within the first hour of non-rapid eye movement (NREM) sleep, optogenetic inhibition of top-down projecting axons from secondary motor cortex (M2) to primary somatosensory cortex (S1) impaired sleep-dependent reactivation of S1 neurons and memory consolidation. In NREM sleep and sleep-deprived states, closed-loop asynchronous or synchronous M2-S1 co-activation, respectively, reduced or prolonged memory retention. Top-down cortical information flow in NREM sleep is thus required for perceptual memory consolidation.

Non-rapid eye movement (NREM) sleep is essential for memory consolidation of an animal's awake motor (*1*) and sensory (*2*) learning experience. During NREM, synchronous 0.5 to 4 Hz oscillations (slow wave activity, SWA) sweep across cortical areas (*3–5*) suggesting that interregional transfer of internal information in NREM has a role in memory consolidation (*6–8*). We recently identified a reverberating long-range top-down intracortical circuit that underlies somatosensory perception in the mouse hindpaw (*9*) consisting of sensory input from the primary somatosensory cortex (S1) to the secondary motor cortex (M2) and a reciprocal top-down feedback projection from M2 to S1. Optogenetic inhibition of the top-down projection impaired accurate tactile perception. However, whether similar top-down cortical inputs during sleep have a critical role in memory consolidation remains unexamined.

To assess memory consolidation, we developed a floor-texture recognition (FTR) task (Fig. 1A) based on natural novelty preference in mice (*10*). During the sampling period, mice explored objects on the left and right sides of an arena containing smooth floors with no behavioral preference (Fig. 1B). However, during the testing period, mice preferentially explored the object on the novel texture (Fig. 1B). We defined the strength of memory for the familiar texture by the relative amount of time spent exploring an object on the novel texture and for a second texture combination (fig. S2).

The memory retention period lasted for 2 days (fig. S3). Mice do not have an innate preference for groovy or smooth textures (fig. S4). No change in performance was observed with whisker-trimmed mice in a dark room (fig. S5). Optogenetic silencing of S1 sensory cortex hindpaw area impaired task performance (fig. S6). Similar to other perceptual recognition tasks (*11*), behavioral performance declined with sleep deprivation (SD) following the sampling period (fig. S7). In the light phase, SD during the first hour of the resting period, after the sampling period, produced a decline in performance that was not observed with SD in the resting period 6–7 hours later (fig. S7). Even in testing periods starting immediately after SD (i.e., 1 hour interval with SD), mice showed impaired consolidation (fig. S7). Without SD, mice showed impaired consolidation at the start of the dark phase (fig. S7), when mice are active period and in a shorter sleep period (*12*). These results indicate the sleep dependence of the FTR task. Recognition tasks may involve synaptic plasticity (*13*), and *N*-methyl-d-aspartate (NMDA) receptor blockers degraded performance (fig. S8). To test hippocampal dependence, we injected AAV-FLEX-Tetanus Toxin (*14*) into the CA1 region of CaMKIIα-Cre transgenic mice (*15*) and immunohistochemically confirmed the blockade of synaptic transmission (*16*) at the subiculum, the primary site of termination of CA1 axons (fig. S9). Performance in the FTR task did not decline indicating that it is likely independent of the hippocampus (fig. S9).

We examined whether M2 input to S1 affected ongoing perception and consolidation by optogenetically inactivating neural activity during the sampling period, resting period,

or testing period in the FTR task (fig. S10). Inactivation of M2 fibers in S1 during the sampling or testing periods decreased task performance (fig. S10). S1 firing activity in the awake state was significantly reduced with top-down inactivation (86.8 ± 2.8%, n = 24 units, $P < 0.001$, light on vs. off periods, one-sample t-test). Inactivated S1 fibers in M2 resulted in similar data (fig. S10).

We performed optogenetic inactivation of M2 fibers in S1 (Fig. 2A) during the resting period in the first hour after the sampling period that included at least three brain states: 42.9 ± 6.3% (n = 7 mice) in an awake state (normalized to 1 hour) with small high-frequency electroencephalographic (EEG) activity and large electromyographic (EMG) activity; 54.5 ± 5.9% (n = 7) in NREM sleep with slow-wave activity (SWA, 0.5–4 Hz) and a silent EMG; and 2.6 ± 0.96% (n = 7) in rapid eye movement (REM) sleep with EEG activity similar to the awake state and a silent EMG (Fig. 2B). We inactivated M2 fibers or S1 fibers during the resting period in the first hour or 6–7 hours after the sampling period (Fig. 2C) with a closed-loop online photostimulation system (>90% accuracy, see fig. S11). A quiet wakefulness (QW) state was negligible in the first hour (0.4 ± 0.1% in NREM illumination, 3.4 ± 1.2% in awake illumination, fig. S11). Photostimulation in the awake or NREM sleep states did not alter the total duration of the three brain states (fig. S12 and S13). Optogenetic inactivation of M2 fibers during the resting-awake state did not affect task performance in the testing period (Fig. 2D) but significantly decreased task performance during the resting period-NREM sleep immediately after the sampling period (Fig. 2D), while inactivation during resting-NREM sleep 6–7 hours after the sampling period did not (Fig. 2D). Similarly, inactivation of S1 fibers during resting-NREM sleep immediately after (0–1 hour) the sampling period did not alter performance (Fig. 2E). In contrast, visual based task did not require hindpaw M2 to S1 inputs in the three periods (fig. S14). These results indicated that tactile memory consolidation requires M2 input to S1 during NREM sleep shortly after the sampling period.

We asked why M2 to S1 top-down input, and not vice versa, regulates memory consolidation specifically during resting-NREM sleep (Fig. 2D). We hypothesized that the optogenetics disrupts the causal directional regulation by M2 to S1 activity and/or suppresses the prominent emergence of reactivated neurons in S1, as SWA during NREM sleep propagates in an anteroposterior direction (*3*, *4*, *17*). NREM sleep accompanies the reactivation of neurons related to animal's sensory experience before sleep, which is thought to be crucial for memory consolidation (*18*). We performed a Granger causality analysis (*19*, *20*), which can predict directed functional (causal) connections between cortical areas. We recorded and analyzed local field potentials (LFPs) from both M2 and S1 with and without optogenetic inhibition of M2 to S1 projection (Fig. 3, A to C) (note: hereafter, we focused on the resting period immediately after the sampling period). Brain state-dependent photostimulation was performed by visual observation of online EEG and EMG data (fig. S11). Coherence analysis indicated higher synchronized M2–S1 activity in the delta range (0.5–4 Hz) in S1 and M2 LFPs during NREM sleep compared to the awake state (Fig. 3D). A power spectrum in the delta range during NREM sleep was not altered by optogenetic stimulation (Fig. 3E and fig. S15). Without optogenetics, a significant increase in causality in both directions (M2 to S1 and vice versa) during the sampling period and less causality during the resting-awake state (fig. S16) suggesting that ongoing perception requires both pathways (fig. S10) (*9*). The lower causality from M2 to S1 suggests that memory consolidation does not require M2 inputs during the resting-awake state (Fig. 2, D and E). Causality from M2 to S1 during pre-sampling-NREM sleep and resting-NREM sleep were significantly higher than the resting-awake state, indicating brain state dependence (fig. S17). With optogenetic inactivation, we observed a decrease of causality only in the M2→S1 direction during resting-NREM sleep (Fig. 3F).

We tested whether reactivated neurons were suppressed by optogenetic inactivation with tetrode recordings from M2 and S1 (Fig. 3G). Single unit activity was recorded during the sampling period, and NREM sleep before (pre-) and after (post-) the sampling (fig. S18). Reactivated neurons were defined based on total firing activity normalized to pre-sampling NREM sleep (*1*). S1 and M2 neurons that were active in the sampling period were also active during post-sampling NREM sleep. Conversely, neurons that were less active in the sampling period were also less active (Fig. 3, H and I). Optogenetic inactivation of M2 fibers did not suppress this linear correlation in M2 (Fig. 3H), but decreased it in S1 (Fig. 3I). Optogenetic inactivation of M2 axons suppressed M2→S1 causality (Fig. 3F), reactivated S1 neurons (Fig. 3I) and task performance (Fig. 2D).

We tested whether neuronal reactivation is sufficient for memory consolidation by optogenetically activating both areas in a synchronous or anti-synchronous manner (Fig. 4A). We used Thy1-channelrhodopsin-2 (ChR2) transgenic mice in which mostly L5 cortical neurons express ChR2 (*21*). We confirmed that light stimulation to M2 and S1 reliably evokes firing during NREM sleep (Fig. 4, B to E). Under resting-NREM sleep for 30 min in total, M2 and S1 were synchronously activated at 2 Hz with an in-phase (Fig. 4, B and C) or an anti-phase pattern (Fig. 4, D and E). Synchronous activation did not change task performance in the testing period 1 day after the sampling period. In wild-type mice, novelty preference decayed after 2 days (fig. S3), but synchronous stimulation prolonged memory retention to at least 4 days after the sampling period (Fig. 4F). In contrast,

anti-synchronous activation resulted in a decrease in performance in the testing period 1 day after the sampling period (Fig. 4F). Synchronized co-activation of M2 and S1 during SD promoted memory consolidation over 4-day intervals (Fig. 4, G and H).

In this study, we showed that perceptual memory consolidation requires top-down cortico-cortical input during NREM sleep. Memory consolidation was dependent on causal fronto-parietal information flow during ~4% of total sleep time (inactivation during the cumulative 30 min of NREM sleep vs. the 12 hours of total sleep in mice) (*22*). Our findings demonstrate a causal relationship between cortical top-down projections and reactivated neurons for memory consolidation, and suggest a general hierarchical control by presynaptic neurons in higher cortical areas in controlling lower sensory and motor areas to regulate memory consolidation (*23*). Reactivation of cortico-cortical regions underlying a top-down circuit could enhance memory retention periods (Fig. 4F). Further, synchronized co-activation of M2 and S1 could overcome the physiologically adverse effects of SD and retain a long-term memory (Fig. 4H). These results indicate that perceptual memory consolidation may not require sleep per se, but that synchronized co-activation of hierarchical cortical pathways, enabled by slow wave activity in NREM sleep, is necessary and sufficient for sensory experience to be consolidated in memory.

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ACKNOWLEDGMENTS

We thank C. Yokoyama for helpful comments and editing, H. Hirase, T. Toyoizumi, S. Fujisawa, J.P. Johansen and B. Mensh for comments and discussions, S Itohara and K Yasuda for behavioral analysis, T. Tsunematsu for sleep measuring, M. Kobayashi and H. Hioki for anatomical analysis, R. Endo and R. Kato for animal control, K. Tao for a part of firing data analysis, and A. Kamoshida for writing custom-made LabVIEW programs. The archaerhodopsin from the Halorubrum strain TP009 (AAV-CMV-ArchT-EGFP) was kindly gifted by E. Boyden (MIT, Cambridge, MA, USA). The authors acknowledge support from a Grant-in-Aid for Young Scientists (A) from the JSPS to M.M.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Figures S1 to S18
References (*24–40*)

25 December 2015; accepted 12 May 2016
Published online 26 May 2016
10.1126/science.aaf0902

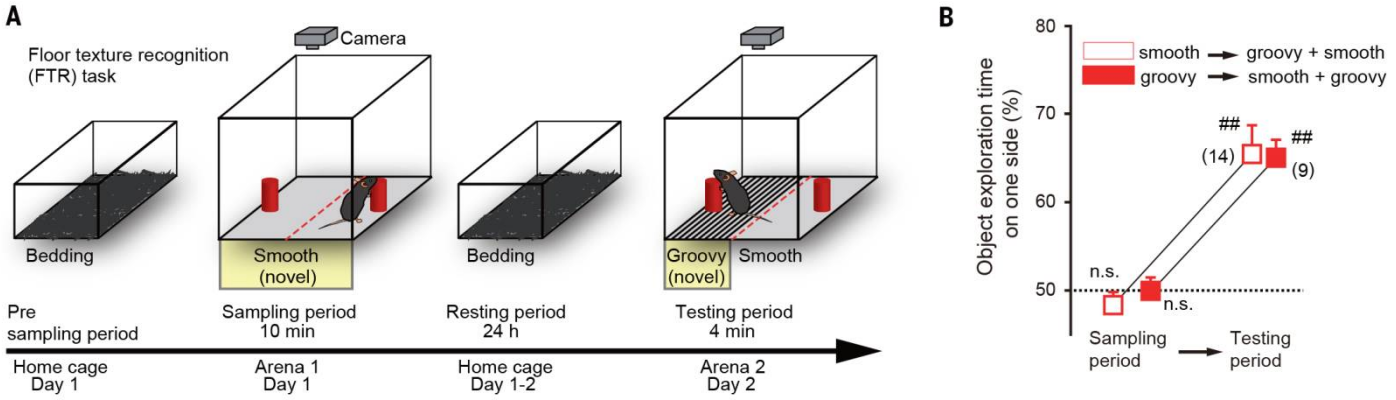

Fig. 1. Floor texture recognition task. (**A**) Behavioral paradigm. (**B**) Object exploration time on a texture in the sampling period and on a novel texture in the testing period. Data are means ± SEM; statistical significance from 50% chance level (##$P < 0.01$) was assessed by one-sample *t*-test.

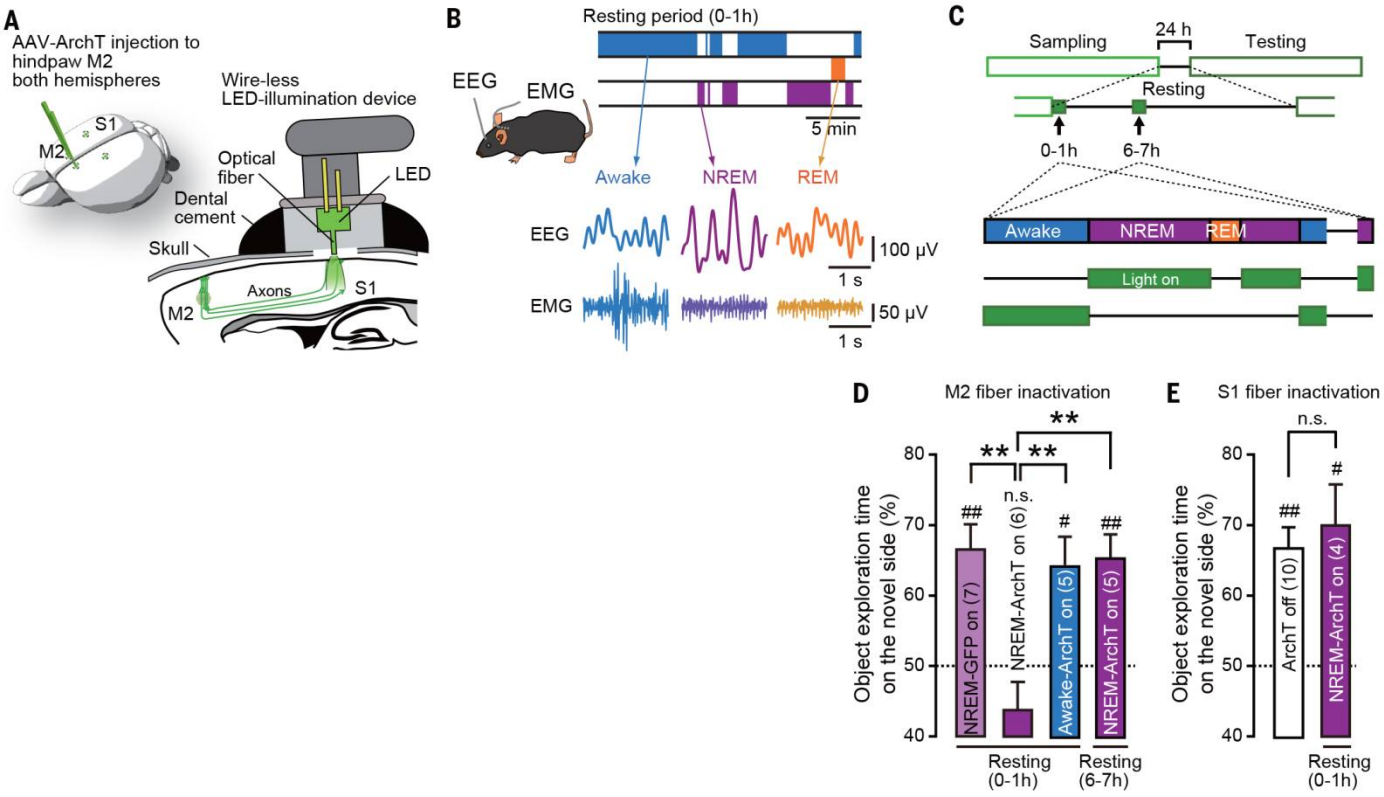

Fig. 2. Optogenetic inactivation of M2 axons impairs memory consolidation. (**A**) Diagram of the miniature wireless LED device that was attached to S1 (or M2) in both hemispheres. AAV-ArchT or AAV-GFP was injected (**Inset**) into M2 (or S1) in both hemispheres. (**B**) Examples of EEG and EMG recordings during the resting period. Brain states were identified with EEG recordings (see Methods). (**C**) Diagram of sleep-state specific optogenetics. (**D**) Summary for the task when M2 fibers were inactivated at S1 during the three periods. (**E**) Summary for the task when S1 fibers were inactivated at M2 during resting-NREM sleep (0–1 hours after sampling period). The cumulative illumination time was 30 min in each state. Statistical significance among more than 2 groups (**$P < 0.01$) was assessed by one-way ANOVA with Tukey's post-hoc test, statistical significance between 2 groups was assessed by Student's *t*-test, statistical significance from 50% chance level (#$P < 0.05$, ##$P < 0.01$) was assessed by one-sample *t*-test.

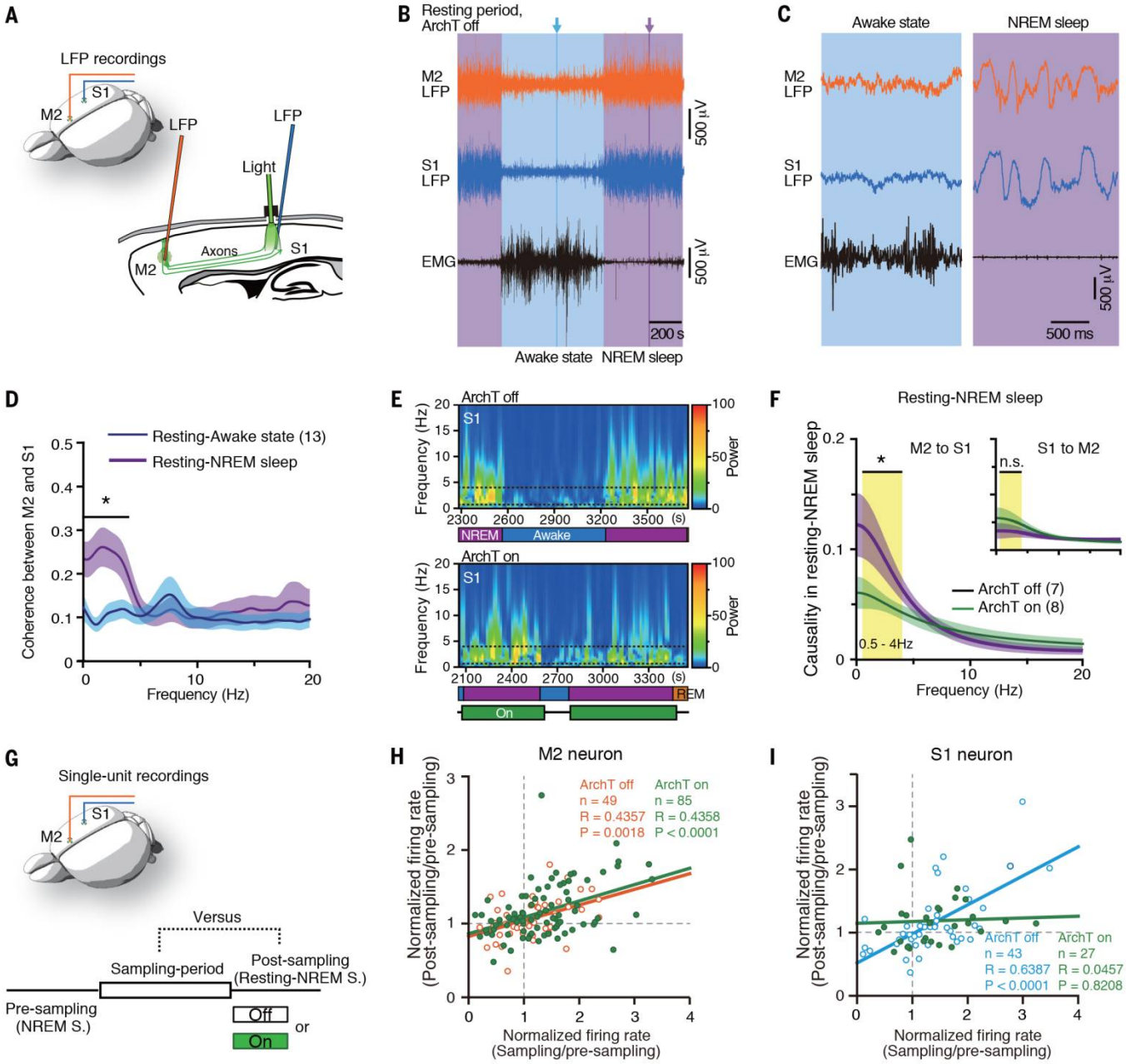

Fig. 3. Optogenetic inactivation of M2 fibers during NREM sleep suppressed M2 causality and S1 reactivated neurons. (**A**) Recording of local field potentials (LFPs) from M2 and S1 with fiberoptic illumination. (**B**,**C**) Representative data of LFPs and EMG (**C**), expanded trace at arrows (**B**) during resting-awake and NREM states without optogenetics. (**D**) Coherence between S1 and M2 during resting-awake and NREM states. (**E**) Representative frequency spectrum of LFPs with and without optogenetic inhibition of M2 fibers at S1. NREM sleep identified by *post hoc* analysis and online visual observation-based photostimulation (green) are shown at the bottom (see fig. S11 for accuracy). (**F**) Granger causality during the resting-NREM periods with and without optogenetics. (**G**) Diagram of tetrode recordings and fiberoptic illumination. Single unit activities were collected during pre-sampling (resting-NREM) for normalization of the sampling and post-sampling (resting-NREM) periods. (**H**, **I**) Normalized firing rate from M2 and S1 individual units (each circle) during the sampling and post-sampling (resting-NREM) periods with (green) and without (black) optogenetic M2 fiber inhibition at S1. Statistical significance among group (*$P < 0.05$) was assessed by Welch's *t*-test.

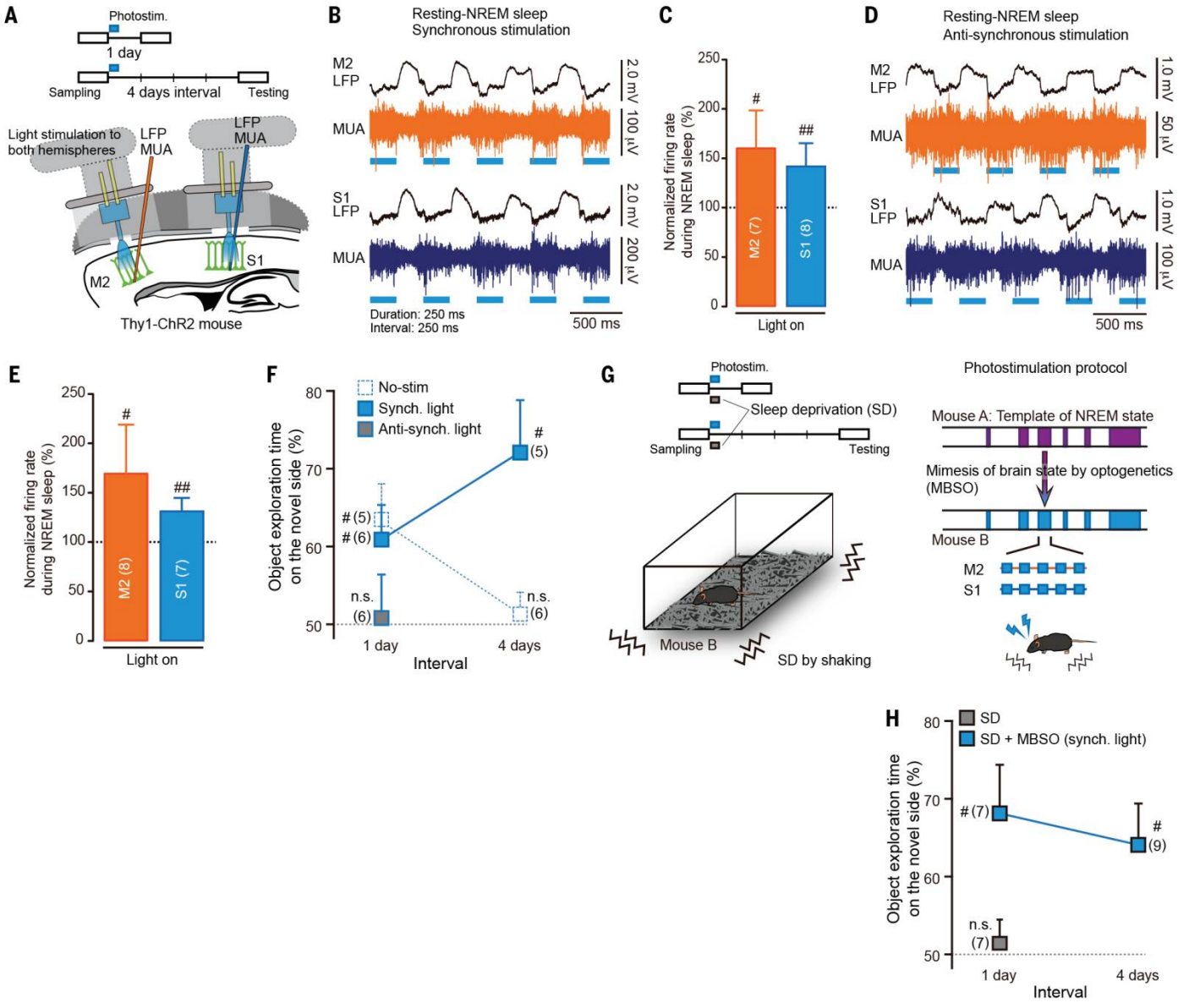

Fig. 4. Memory consolidation depends on the phase synchrony of slow waves. (**A**) Optogenetic activation of M2 and S1 with local field potential (LFP) recordings with single electrodes. (**B**) **Top**: Experimental timeline for synchronous photostimulation. **Middle and Bottom**: LFPs and multi-unit activity (MUA) recordings from M2 (**middle**) and S1 (**bottom**) during resting-NREM sleep. (**C**) Normalized firing activities during light on (normalized to interval off periods). (**D**) LFPs and MUA with anti-synchronous photostimulation. (**E**) Normalized firing activities. (**F**) Task performance after photostimulation during resting-NREM sleep. (**G**) **Left**: behavioral paradigm. Sleep after sampling period was deprived for 1 hour with synchronized co-activation of M2 and S1 in the transgenic mice. **Right**: Photostimulation protocol. The photostimulation (2 Hz) during the SD experiment was applied according to the NREM sleep pattern from Mouse A. (**H**) Summary of task performance after photostimulation. The cumulative illumination time was 30 min. Statistical significance from off period firing rate (#$P < 0.05$, ##$P < 0.01$) or from 50% chance level (#$P < 0.05$) was assessed by one-sample t-test.

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D. Miyamoto, D. Hirai, C. C. A. Fung, A. Inutsuka, M. Odagawa, T. Suzuki, R. Boehringer, C. Adaikkan, C. Matsubara, N. Matsuki, T. Fukai, T. J. McHugh, A. Yamanaka and M. Murayama (May 26, 2016)
published online May 26, 2016

Editor's Summary

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.