Sleep loss drives acetylcholine- and somatostatin interneuron–mediated gating of hippocampal activity to inhibit memory consolidation

James Delorme[a], Lijing Wang[a], Femke Roig Kuhn[b], Varna Kodoth[a], Jingqun Ma[c], Jessy D. Martinez[a], Frank Raven[a], Brandon A. Toth[a], Vinodh Balendran[a], Alexis Vega Medina[a], Sha Jiang[a], and Sara J. Aton[a,1]

[a]Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48019; [b]Program in Behavioural and Cognitive Neurosciences, University of Groningen, 9700 AB Groningen, The Netherlands; and [c]Bioinformatics Core, Biomedical Research Core Facilities, University of Michigan, Ann Arbor, MI 48019

Sleep loss disrupts consolidation of hippocampus-dependent memory. To characterize effects of learning and sleep loss, we quantified activity-dependent phosphorylation of ribosomal protein S6 (pS6) across the dorsal hippocampus of mice. We find that pS6 is enhanced in dentate gyrus (DG) following single-trial contextual fear conditioning (CFC) but is reduced throughout the hippocampus after brief sleep deprivation (SD; which disrupts contextual fear memory [CFM] consolidation). To characterize neuronal populations affected by SD, we used translating ribosome affinity purification sequencing to identify cell type–specific transcripts on pS6 ribosomes (pS6-TRAP). Cell type–specific enrichment analysis revealed that SD selectively activated hippocampal somatostatin-expressing (Sst+) interneurons and cholinergic and orexinergic hippocampal inputs. To understand the functional consequences of SD-elevated Sst+ interneuron activity, we used pharmacogenetics to activate or inhibit hippocampal Sst+ interneurons or cholinergic input from the medial septum. The activation of either cell population was sufficient to disrupt sleep-dependent CFM consolidation by gating activity in granule cells. The inhibition of either cell population during sleep promoted CFM consolidation and increased S6 phosphorylation among DG granule cells, suggesting their disinhibition by these manipulations. The inhibition of either population across post-CFC SD was insufficient to fully rescue CFM deficits, suggesting that additional features of sleeping brain activity are required for consolidation. Together, our data suggest that state-dependent gating of DG activity may be mediated by cholinergic input and local Sst+ interneurons. This mechanism could act as a sleep loss–driven inhibitory gate on hippocampal information processing.

consolidation | sleep deprivation | interneurons | acetylcholine | bioinformatics

Hippocampal plasticity and memory storage are gated by vigilance states. In both human subjects and animal models, sleep loss disrupts consolidation of multiple types of hippocampus-dependent memories (1, 2). This effect has been extensively studied in mice in which as little as a few hours of experimental sleep deprivation (SD) can disrupt hippocampally mediated consolidation of object–place memory (3–5) and contextual fear memory (CFM) (6, 7). Recent work has characterized biochemical pathways involved in memory consolidation which are disrupted in the hippocampus by SD (4, 5, 8, 9). However, much less is known about how SD affects hippocampal microcircuit function.

SD disrupts patterns of hippocampal network activity which are associated with memory consolidation. For example, SD interferes with network activity changes induced in hippocampal area CA1 by prior learning (contextual fear conditioning, or CFC); these post-CFC changes predict successful CFM consolidation, and their loss predicts consolidation disruption (7, 10). The reason for this SD-mediated disruption is unknown. Recently, activity-dependent regulation of protein translation machinery within the dorsal hippocampus was found to be essential for sleep-dependent memory consolidation (9). SD interferes with biochemical pathways which drive increased protein synthesis following learning (9, 11). This suggests a link between state-dependent changes in network activity and biosynthetic events occurring in the first few hours following learning (12–15), which are necessary for appropriate CFM consolidation.

To better characterize the link between neuronal activity and protein synthesis in the hippocampus during CFM consolidation, we characterized effects of learning and subsequent sleep or SD on activity-dependent phosphorylation of ribosomal protein S6 (pS6). S6 is phosphorylated in an activity-dependent manner by ERK, PKA, and mTOR kinase pathways in neurons and thus is a cellular marker of activated neurons (16). We find that CFC increases S6 phosphorylation at a terminal serine residue (pS6 Ser244-247) and that SD reduces pS6 Ser244-247 throughout the dorsal hippocampus. To identify cell populations differentially expressing pS6 after sleep versus SD, we used a pSer244-247 as an affinity tag for translating ribosome affinity purification (pS6-TRAP). We then identified modules of cell type–specific transcripts with expression correlated to wake time in sleeping and SD mice and verified these

Significance

Sleep loss disrupts long-term storage of episodic and spatial memories that require activity in the hippocampus. We find that sleep deprivation in mice leads to selective disruption of activity-dependent phosphorylation of ribosomal protein S6 in the hippocampus, which is normally increased in the hippocampus after learning. We used an unbiased approach to profile messenger RNAs associated with phosphorylated ribosomes and found evidence that cholinergic hippocampal inputs and somatostatin-containing hippocampal neurons are selectively activated with sleep deprivation. We find functional evidence supporting the idea that during sleep loss, activity in these two cell populations may act as an inhibitory gate, reducing the activity of dentate gyrus neurons. We find that this inhibitory gating mechanism is capable of disrupting memory consolidation.

findings with qPCR. These analyses indicate that SD selectively activates (i.e., leads to increased S6 phosphorylation in) hippocampal somatostatin-expressing (Sst+) interneurons and orexinergic (lateral hypothalamic) and cholinergic (MS) neurons, which send input to the hippocampus. We used TRAP in Sst+ interneurons (Sst-TRAP) to verify that activity-dependent transcripts are increased in these neurons with SD. To assess how increased activity in the hippocampus Sst+ interneuron population during SD affects memory consolidation, we used pharmacogenetics to selectively activate these neurons in the hours following CFC. We find that mimicking the effects of SD on Sst+ interneuron activity is sufficient for disruption of CFM consolidation in freely sleeping mice and that inactivation of Sst+ interneurons during post-CFC sleep augments CFM consolidation. Lastly, we tested the hypothesis that state-dependent regulation of the dorsal hippocampal network is mediated by changes in activity of MS cholinergic neurons. We find that pharmacogenetic activation of MS cholinergic inputs to the hippocampus following CFC impairs sleep-dependent CFM consolidation. In contrast, pharmacogenetic inhibition of these cholinergic inputs during sleep promotes CFM consolidation and increases S6 phosphorylation in the dorsal hippocampus. Together, these data provide evidence for a state-dependent gate on network activity in the hippocampus, regulated by Sst+ interneurons and MS cholinergic input, which likely contributes to SD-induced disruption of memory consolidation.

Results

Learning Increases and Sleep Loss Decreases Phosphorylation of S6 in the Hippocampus. Brief SD of only a few hours is sufficient to disrupt many forms of hippocampus-dependent memory consolidation in mice (3–7). We first confirmed disruptive effects of post-CFC SD on CFM consolidation in wild-type mice. Beginning at lights on (Zeitgeber time [ZT]0), mice underwent single-trial CFC followed by either 24-h ad libitum sleep (Sleep) or 6 h of SD by gentle handling (followed by recovery sleep). SD mice showed significant reductions in context-specific freezing during CFM testing (Fig. 1A).

We next characterized the effects of CFC and subsequent sleep or SD on neuronal activity-driven S6 phosphorylation in the dorsal hippocampus, a region critical for CFM consolidation (7, 12, 17). Mice either underwent CFC or remained in their home cage (HC) at ZT0 and then had either SD or Sleep until euthanasia at ZT3 (Fig. 1B). The behavioral observation of mice in the HC + Sleep and CFC + Sleep groups indicated a high proportion of the 3-h ad libitum sleep window (72 ± 3% and 71 ± 3%, respectively [mean ± SEM]) was spent asleep (quiescent, in stereotyped sleep postures, and within the nest). Because S6 is sequentially phosphorylated at five serine residues, we first quantified phosphorylation using an antibody recognizing the initial Ser235-236 phosphorylation sites. Consistent with previous reports (9), 3-h SD did not alter either the number of pS6(Ser235-236)+ neurons in the dentate gyrus (DG) or the intensity of phosphorylation at Ser235-236 in the pyramidal cell body layers of CA1 or CA3 (Fig. 1D). We then quantified phosphorylation at the terminal S6 phosphorylation sites (Ser244-247). We observed significant SD-driven decreases in the number of pS6(Ser244-247)+ neurons in DG and reduction in pS6(Ser244-247)+ staining in CA1 and CA3 (Fig. 1 C and D). While CFC increased S6 phosphorylation at Ser244-247 throughout the hippocampus (relative to HC controls; Fig. 1 B and C), post-CFC SD decreased pS6(Ser244-247)+ staining in DG and CA1 (Fig. 1 C and D). In contrast to the effects of SD in the dorsal hippocampus, adjacent neocortical regions (i.e., primary somatosensory cortex) showed increased numbers of pS6+ neurons at both sites after 3-h SD (SI Appendix, Fig. S2).

We verified that S6(Ser244-247) phosphorylation is neuronal activity driven, consistent with previous reports (18), by quantifying coexpression of the activity-regulated protein Arc in pS6+ neurons. Consistent with our previous findings (19), 3-h SD reduced
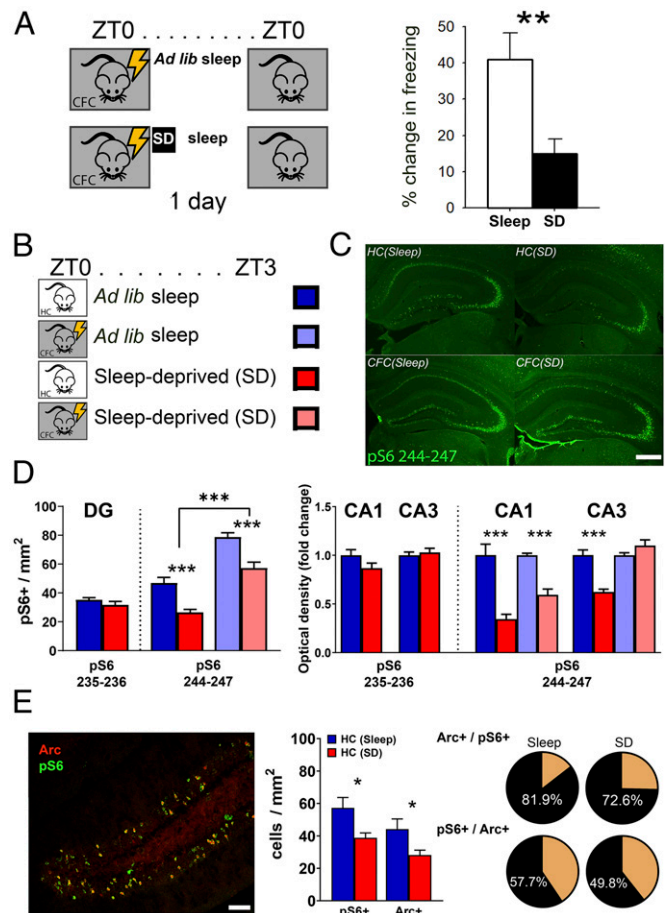

Fig. 1. Hippocampal S6 phosphorylation increases after learning and is reduced by SD. (*A, Left*) Experimental paradigm. Mice underwent single-trial CFC at ZT0. One group of mice was allowed ad libitum sleep over the next 24 h prior to testing at ZT0 (Sleep, n = 9). A second group was SD (n = 10) by gentle handling for the first 6 h after CFC and was allowed recovery sleep until testing the following day. (*Right*) CFM consolidation (measured as percent changes in context-dependent freezing compared with pre-CFC baseline) was significantly reduced after SD. **P < 0.01, Student's t test. (*B*) Experimental paradigm. Mice underwent single-trial CFC at ZT0 or were left in their HC. Over the next 3 h, mice in CFC and HC groups were then permitted ad libitum sleep (Sleep) or were SD by gentle handling. (*C*) Fluorescent images of pS6 (S244-247) staining in the dorsal hippocampus of representative mice in the four treatment groups (Scale bar, 500 μm). (*D, Left*) pS6+ neurons in DG were counted using antibodies detecting S6 phosphorylation at either S235-236 or S244-247 sites. SD selectively reduced S244-247 pS6+ neurons in both HC (n = 5/group) and CFC (n = 5/group) mice (***P < 0.001, Tukey test). CFC increased the number of pS6+ neurons (two-way ANOVA: main effect of CFC, F = 87.09, P < 0.001; main effect of SD, F = 38.94, P < 0.001; CFC × SD interaction, not significant [N.S.]). (*Right*) pS6 in pyramidal cell layers CA1/CA3 was quantified as background subtracted optical density. SD values were calculated as the fold change relative to the Sleep condition HC + Sleep or CFC + Sleep, respectively. pS6 (S244-247) OD was reduced in both CA1 and CA3 (***P < 0.001, Tukey test) after SD in HC mice. After CFC, SD reduced pS6 in CA1 neurons (***P < 0.001, Tukey test) following SD. (*E, Left*) Representative image of pS6 and Arc colocalization in DG. (Scale bar, 100 μm.) (*Middle*) Quantification of pS6 and Arc expression in HC + Sleep and HC + SD mice (n = 5/group). A 3-h SD reduced both Arc+ and pS6+ (*P < 0.05, Student's t test) neurons in DG. (*Right*) ~77% of Arc+ DG neurons expressed pS6+; ~54% of pS6+ neurons expressed Arc.

numbers of both Arc+ and pS6+ neurons in the DG. Arc and pS6 were colocalized to a similar extent in DG of both Sleep and SD mice (Fig. 1E), with 77 ± 3.2% of Arc+ DG neurons also being pS6(Ser244-247)+ and 54 ± 2.8% of pS6(Ser244-247)+ neurons also being Arc+ (mean ± SEM from n = 10 mice).

We next tested whether hippocampal S6(Ser244-247) phosphorylation (hereafter referred to simply as S6 phosphorylation, or pS6) was affected by learning a hippocampus-dependent memory task. Mice underwent single-trial CFC (in which exploration of a novel chamber is paired with a foot shock) or, for comparison, were left in their HC at lights on. After this, both CFC and HC mice were either allowed ad libitum sleep or had SD by gentle handling in their HC. In freely sleeping mice, CFC increased the number of pS6+ DG neurons at both 30 min and 3 h post-CFC, relative to HC controls (two-way ANOVA: main effect of time, $F = 50.63$, $P < 0.001$; main effect of learning, $F = 33.59$, $P < 0.001$; time × learning interaction, $F = 2.22$, $P = 0.16$) (*SI Appendix*, Fig. S1). In contrast, CFC did not alter S6 phosphorylation in CA1 or CA3 pyramidal cell layers of freely sleeping mice relative to HC controls. Consistent with greater S6 phosphorylation in the hippocampus after periods rich in sleep, DG pS6+ neurons increased between the two timepoints (ZT0 versus ZT3) in both naïve (HC + Sleep) and CFC-trained (CFC + Sleep) mice (*SI Appendix*, Fig. S1). Conversely, 3-h SD disrupted S6 phosphorylation in the hippocampus following CFC, with fewer pS6+ neurons in the DG and reduced pS6+ expression in CA1 (Fig. 1D). Taken together, these data suggest that learning increases and SD reduces S6 phosphorylation in the hippocampus.

Identification of Hippocampal Cell Types with Altered S6 Phosphorylation during SD. We next used an unbiased RNA sequencing (RNA-seq) approach to identify cells in which S6 phosphorylation differs between Sleep and SD. As previously described (16), we used pS6 as an affinity tag to isolate ribosomes and associated transcripts in active cells, and we performed pS6 translating ribosome affinity purification (pS6-TRAP) (16). Hippocampi were collected from CFC and HC mice after 3-h ad libitum sleep or SD. Ribosome-associated transcripts were then isolated by pS6-TRAP for RNA-seq. To identify clusters of coregulated transcripts in our RNA-seq data (such as might be expected for genetically defined cell types), we used weighted gene correlation network analysis (WGCNA) (20) on transcripts with a variance greater than 0.03 ($n = 1662$ transcripts; *Materials and Methods*). WCGNA yielded 10 clusters (modules) of highly correlated transcripts in our data, and a separate (Gray) cluster representing unassigned (uncorrelated) transcripts (Fig. 2A and Dataset S1). To determine which modules' expression varied as a function of sleep versus SD, we correlated the level of expression of module eigengene with the percent time mice spent sleeping over the 3-h period prior to sacrifice (Sleep groups = 74.4 ± 2.2%, SD groups = 0.0 ± 0.0% [mean ± SEM]). The results from the analysis revealed two significantly correlated eigengene clusters (Brown, Magenta) whose expression negatively correlated with sleep time (Fig. 2A and *SI Appendix*, Fig. S3). Since these represented subclusters of the same module, we combined them for further analysis (Brown/Magenta cluster).

Since our data suggested that the population of pS6+ neurons in the hippocampus may differ in freely sleeping and SD mice (Fig. 1), we used cell type–specific expression analysis (CSEA) (21, 22) to quantify cell type–specifying transcripts represented in the Brown/Magenta cluster, which were significantly affected by SD (*Materials and Methods*). CSEA was used to generate a p_{adj} value for overlap between transcripts in the Brown/Magenta cluster and known cell type–specific enriched transcripts of a particular specificity index P value (pSI) (based on a multiple comparisons–corrected Fisher's exact test). Using the most stringent CSEA (pSI < 0.0001), the analysis identified Brown/Magenta cluster transcripts to be most enriched in cholinergic (Chat+) neurons ($p_{adj} = 0.036$) and orexinergic (Hcrt+) neurons ($p_{adj} = 0.047$) as well as Pnoc+ and Cort+ interneurons (both $p_{adj} = 0.045$) (Fig. 2 B and C and *SI Appendix*, Fig. S3 and Datasets S1 and S2). This suggests that during extended SD, S6 phosphorylation increases in orexinergic/cholinergic neurons and interneurons despite the fact that overall S6 phosphorylation in pyramidal and granule cell layers is reduced after SD. The former likely reflects transcripts present in orexinergic inputs to the hippocampus from lateral hypothalamus and cholinergic input from the medial septum, respectively—both of which are more active during active wake versus sleep (23, 24). With respect to the latter finding, overlap between the Brown/Magenta cluster and transcripts expressed selectively in Cort+ and Pnoc+ interneurons (25, 26) included transcripts encoding interneuron-specific transcription factors (Dlx1) and secreted neuropeptides somatostatin (Sst), neuropeptide Y (Npy), and corticotrophin-releasing hormone (Crh).
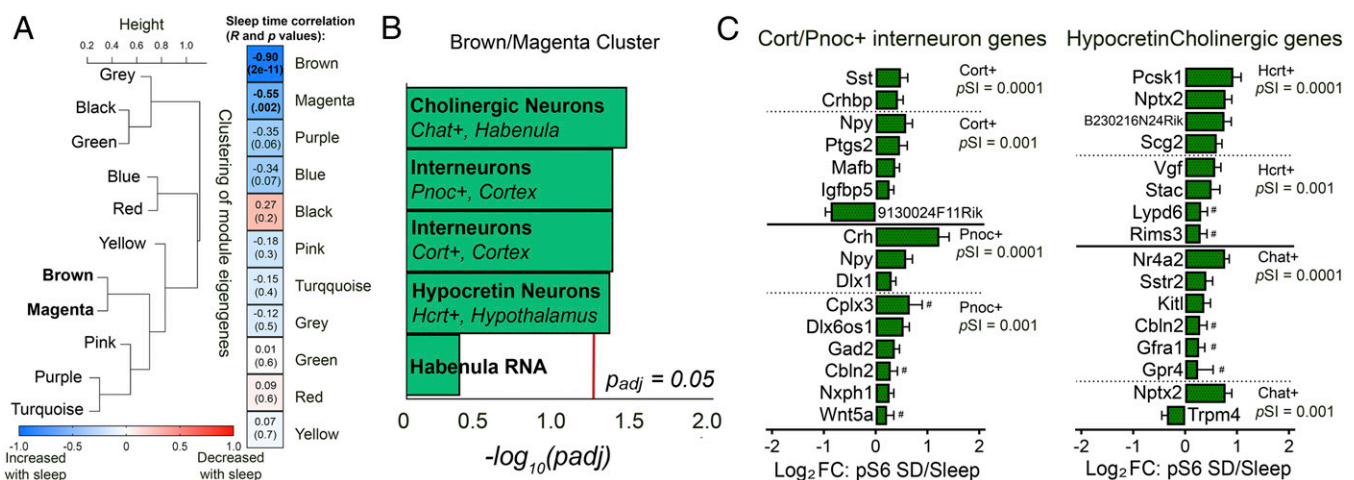

Fig. 2. Phosphorylated ribosome capture following SD selectively enriches transcripts specific to GABAergic, cholinergic, and orexinergic neurons. (*A*, *Left*) WGCNA identified modules of similarly correlated pS6 transcripts. Each module is identified with a color name, and Gray represents transcripts not assigned to a coexpression module. The color scale at bottom indicates the R values for the correlations at *Right*. (*Right*) Module-trait correlation between eigengene expression in each module and total sleep time prior to sacrifice (R and P values for Pearson correlation in parentheses for each module). $n = 16$ and 14 mice for sleep and SD conditions, respectively, with CFC and HC mice combined for module correlations with sleep amount. (*B*) Transcripts in the Brown/Magenta module with expression correlated to sleep time were used for CSEA. The Brown/Magenta transcripts showed significant overlap with mRNAs enriched most selectively (pSI < 0.0001) in cholinergic (Epi.ChAT, $p_{adj} = 0.036$), orexinergic (Hyp.Hcrt, $p_{adj} = 0.047$), and GABAergic (Ctx.Pnoc, $p_{adj} = 0.045$; Ctx.Cort, $p_{adj} = 0.045$) neuron populations. (*C*) Deseq2 Log2FC (SD/Sleep) values from Brown/Magenta transcripts identified by CSEA. All genes are statistically significant ($p_{adj} < 0.1$) unless otherwise indicated (# indicates $p_{adj} > 0.1$).

Delorme et al.
Sleep loss drives acetylcholine- and somatostatin interneuron–mediated gating of hippocampal activity to inhibit memory consolidation

PNAS | 3 of 10
https://doi.org/10.1073/pnas.2019318118

Hippocampal Sst+ Interneurons and Cholinergic Inputs Show Increased Activity during Brief SD. Sst and Npy neuropeptides are coexpressed in dendritic-targeting interneurons in DG, CA3, and CA1 and play a role in gating neighboring neuronal activity (27–29). To confirm enrichment of *Sst*, *Npy*, and other CSEA-identified transcripts in the pS6+ cell population after a longer period of SD [sufficient to disrupt CFM consolidation (6)], we carried out a second experiment in which CFC and HC mice either were allowed 5 h of ad libitum sleep or underwent 5-h SD. For mice in the Sleep group, total sleep time constituted 85.0 ± 1.7% (mean ± SEM) of the 5-h period prior to euthanasia. pS6-TRAP was followed by qPCR to measure cell type–specific transcripts from the hippocampus. We found that independent of prior training (CFC or HC), SD caused similar enrichment for transcripts present in GABAergic neurons in pS6-TRAP. While *Gad67* and *Pvalb* transcripts were only moderately increased (by roughly 30%) following 5-h SD, *Sst* and *Npy* showed larger (two- to fourfold) increases (Fig. 3A and Dataset S3). SD also increased *Cht* expression in both CFC and HC mice. These data support our unbiased CSEA-based finding of increased abundance of Sst+ interneuron and cholinergic neuron markers in the SD pS6+ population.

These data suggest that despite reduced total activation of hippocampal neurons during SD (Fig. 1), Sst+ interneurons in the hippocampus are selectively more activated in SD versus sleep. To confirm SD-driven activation, we next quantified the expression of activity markers in Sst+ interneurons directly. We used TRAP to isolate messenger RNAs (mRNAs) associated with translating ribosomes in this cell population using *SST-IRES-CRE* transgenic mice expressing hemagglutinin (HA)-tagged Rpl-22 (RiboTag) in a Cre-dependent manner (30). Ribosome-associated transcripts from the hippocampus of these mice, isolated following a 3-h period of sleep or SD, were quantified with qPCR. Mice in the

Sleep group (*n* = 5) spent 70.8 ± 4.6% (mean ± SEM) of the 3-h period prior to euthanasia in sleep. We first verified the enrichment of cell type–specific (i.e., Sst+ interneuron–specific) mRNAs by comparing transcript levels from TRAP versus Input (whole hippocampus) mRNA. Sst-TRAP significantly de-enriched glial (*Gfap*, *Mbp*) and excitatory neuron (*Vlugt1*, *Vglut2*) cell markers and significantly enriched for Sst+ interneuron–expressed transcripts *Gad1*, *Vgat*, *Sst*, *Npy*, and *Crhbp* (Fig. 3B). We then tested whether 3-h SD increased the expression of activity-regulated transcripts in Sst+ interneurons and found that *Cfos* (but not *Npas4* or *Arc*) was significantly elevated at Sst+ interneurons' ribosomes after SD (Fig. 3C and Dataset S3). We also tested whether SD-driven increases in *Sst*, *Npy*, and *Crhbp* in pS6-TRAP were due to increased expression levels within Sst+ interneurons. Using qPCR for these neuropeptide transcripts in mRNA isolated using Sst-TRAP pulldown, we found that following 3-h SD, *Sst*, *Npy*, and *Crhbp* transcripts were all less abundant rather than more abundant (Fig. 3C). The same change was not present in the Input fraction (whole hippocampus) mRNA, in which expression of *Sst*, *Npy*, and *Crhbp* mRNAs were all unchanged by SD (*SI Appendix*, Fig. S4 and Dataset S3). We also used qPCR to quantify expression of other cell type–specific transcripts identified in pS6-TRAP by WCGNA/CSEA. Of the transcripts tested, we found that SD increased expression of *Kcnf1* (encoding the voltage-gated potassium channel subunit Kv5.1) in Sst+ interneurons (Fig. 3C). Kv5.1 acts as a physiological modifier of Kv2 family channel subunits (31, 32). While little is known about its function in neurons, available patch-seq data suggest that greater expression of *Kcnf1* correlates with a reduced action potential threshold and increased neuronal firing rate (33). Taken together, these data support the conclusion that SD-induced increases in *Sst*, *Npy*, and *Crhbp* transcripts in pS6-TRAP
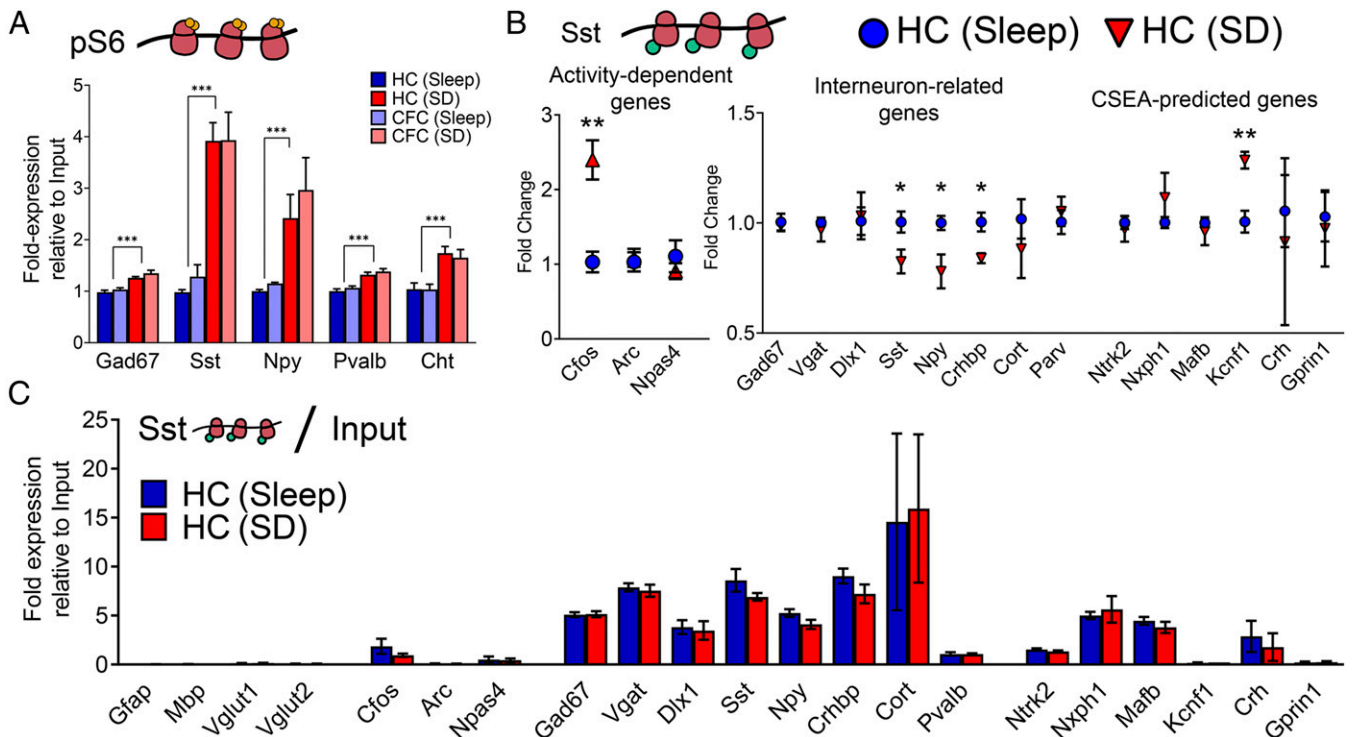

Fig. 3. SD increases activity in Sst+ interneurons. (*A*) qPCR data for pS6-associated transcripts from CFC (*n* = 6/group) or HC (*n* = 5/group) mice with 5 h subsequent ad libitum sleep or SD (two-way ANOVA: main effect of SD, *P* < 0.001; main effect of CFC, *N.S.*; CFC × SD interaction, *N.S.*). (*B*) Expression of cell type–specific markers in mRNA from Sst-TRAP versus Input. Sst-TRAP de-enriched transcripts expressed in glial cells and preferentially enriched transcripts expressed in Sst+ (GABAergic) interneurons. These enrichment values did not differ between HC + Sleep (*n* = 5) and HC + SD (*n* = 4) mice. (*C*) Changes in expression of activity-regulated, interneuron-specific, and CSEA-predicted transcripts associated with Sst+ interneuron ribosomes following 3 h of ad libitum sleep or SD. Sleep versus SD, ***P < 0.001, **P < 0.01, and *P < 0.05, Student's *t* test.

reflect increases in the activity, and thus activity-driven S6 phosphorylation, within Sst+ interneurons.

To further validate increases in Sst+ interneuron activity after SD, we examined SD-driven changes in S6 phosphorylation in Sst+ and parvalbumin-expressing (Pvalb+) interneurons in the dorsal hippocampus using immunohistochemistry (Fig. 4A). As observed previously (Fig. 1), 3-h SD reduced the total number of pS6+ neurons in the DG (Fig. 4B). However, at the same time, 3-h SD increased S6 phosphorylation in Sst+ interneurons in the DG and showed a strong trend for increased expression in CA3 Sst+ interneurons (Fig. 4C). Overall numbers of Sst+ interneurons were similar between Sleep and SD mice, and, consistent with qPCR results from Sst-TRAP, the intensity of Sst staining among Sst+ interneurons was decreased after SD (*SI Appendix,* Fig. S5).

Mimicking SD-Driven Increases in Sst+ Interneuron Activity in the Hippocampus Suppresses Granule Cell Activity and Disrupts Sleep-Dependent Memory Consolidation. Because the SD-associated increase in Sst+ interneuron activity has the potential to profoundly suppress surrounding hippocampal network activity (27, 34), we next tested how this process affects sleep-dependent memory consolidation. To test this, we transduced the dorsal hippocampus of *SST-IRES-CRE* mice with an AAV vector to express either the activating DREADD hM3Dq-mCherry or mCherry alone in a Cre-dependent manner (Fig. 5A and *SI Appendix,* Fig. S6). To confirm the effects of pharmacogenetic manipulation on Sst+ interneuron and surrounding DG granule cells' neuronal activity, hM3Dq- and mCherry-expressing (n = 5 and 4, respectively) mice were injected with clozapine-N-oxide (CNO; 3 mg/kg) at lights on and allowed 3-h ad libitum sleep in their HC prior to euthanasia. hM3Dq-mCherry–transduced DG neurons showed significantly higher levels of cFos expression at this time point compared to mCherry-expressing control mice (hM3Dq: 68.0 ± 15.9% versus mCherry: 2.0 ± 1.4%; *P* < 0.01 Student's *t* test) (Fig. 5 *A* and *B*). To assess the effects of CNO on Sst+ interneuron–mediated inhibition in the surrounding DG, we quantified cFos expression in nontransduced neurons in the DG granule cell layer. hM3Dq expression significantly reduced the number of cFos+ neurons in the surrounding DG relative to mCherry-expressing control mice (*P* < 0.05, Student's *t* test) (Fig. 5C). To test how the activation of Sst+ interneurons affected memory consolidation, hM3Dq-mCherry– (n = 6) and mCherry-expressing (n = 5) mice underwent single-trial CFC at lights on, after which they were immediately injected with CNO and returned to their HC for ad libitum sleep. After 24 h, at lights on, mice were returned to the CFC

context to assess CFM. Mice expressing hM3Dq showed significant decreases in context-specific freezing compared to mCherry-expressing control mice (*P* < 0.001, Student's *t* test) (Fig. 5D). While these mice did not undergo polysomnographic recording, there was no observed change in locomotor activity or apparent sleep behavior between mCherry and hM3Dq-expressing mice in the first hour following CNO administration. Together, these data show that sleep-dependent consolidation of CFM can be disrupted via activation of Sst+ interneurons, which provide strong inhibition to the surrounding hippocampal network.

Suppressing Hippocampal Sst+ Interneuron Activity Augments Post-CFC Granule Cell Activity and Sleep-Dependent Memory Consolidation. Since SD was associated with elevated Sst+ interneuron activity, we next tested whether reducing activity in Sst+ interneurons was sufficient to augment consolidation of CFM. We transduced the hippocampus of *SST-IRES-CRE* mice with an AAV vector to express either the inhibitory DREADD hM4Di-mCitrine or YFP in a Cre-dependent manner. To confirm the effects of pharmacogenetic manipulation on the DG network, hM4Di- and YFP-expressing mice (n = 5/group) underwent single-trial CFC at lights on, followed by injection with CNO, and were allowed 3 h ad libitum sleep in their HC prior to euthanasia (Fig. 6A). cFos expression in the DG granule cell layer was again quantified as a measure of network activation. Pharmacogenetic inhibition of Sst+ interneurons after CFC led to increased expression of cFos in DG granule cells (*P* < 0.05, Mann–Whitney *U* rank sum test), consistent with a role for this interneuron population in gating DG network activity (27).

To test how the suppression of Sst+ interneuron activity affects memory consolidation during sleep, hM4Di-mCitrine– and YFP-expressing mice underwent single-trial CFC at lights on, after which they were immediately injected with CNO and returned to their HC for ad libitum sleep. After 24 h, at lights on, mice were returned to the CFC context to assess CFM. Mice expressing hM4Di showed significant enhancement in context-specific freezing compared to YFP-expressing control mice (*P* < 0.001, Student's *t* test) (Fig. 6B). This memory-enhancing effect appeared to be specific to freely sleeping mice. Pharmacogenetic suppression of Sst+ interneuron activity in the context of post-CFC SD led to a modest, but not statistically significant, increase in context-specific freezing 24 h after CFC (*P* = 0.27, Student's *t* test) (*SI Appendix,* Fig. S7A). While the inactivation of Sst+ interneurons was insufficient to rescue CFM from SD-induced disruption, our data show that sleep-dependent consolidation of CFM can be augmented by inactivating
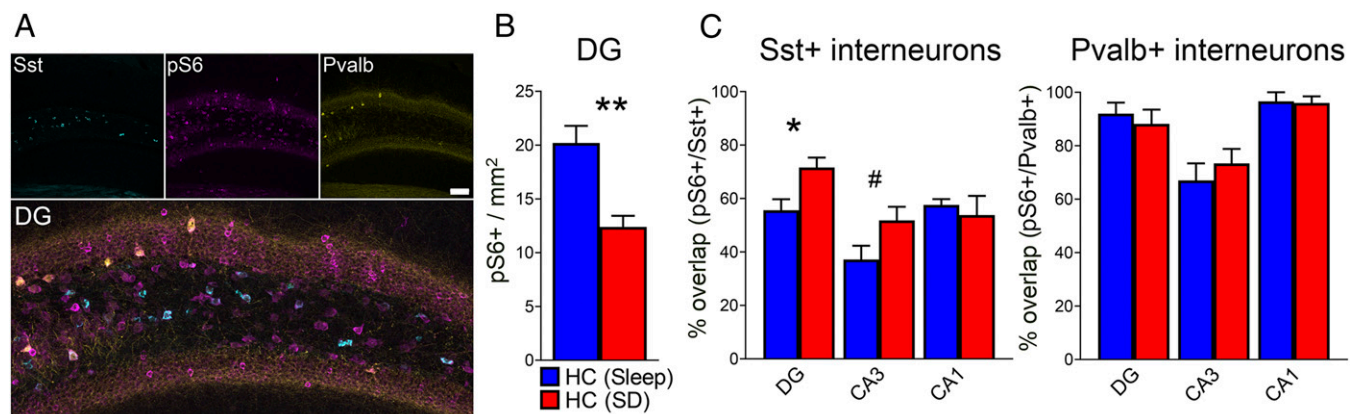
Fig. 4. DG Sst+ interneurons show increased S6 phosphorylation following SD. (A) Representative images showing expression of Sst, Pvalb, and pS6 in DG. (Scale bar, 100 μm.) (B) DG pS6+ neurons decreased following 3-h SD (*P* < 0.01, Student's *t* test, n = 5 mice/group). (C) pS6 colocalization in Sst+ and Pvalb+ DG interneurons was compared for HC mice after 3-h SD or ad libitum sleep. SD-elevated S6 phosphorylation in Sst+ interneurons in the DG hilus (*P* < 0.05, Student's *t* test) and trended in CA3 (*P* < 0.1). S6 phosphorylation in Pvalb+ interneurons was unaffected by SD. Sleep versus SD, **P* < 0.01, **P* < 0.05, and #*P* < 0.1, Student's *t* test.

Delorme et al.
Sleep loss drives acetylcholine- and somatostatin interneuron–mediated gating of hippocampal activity to inhibit memory consolidation

PNAS | 5 of 10
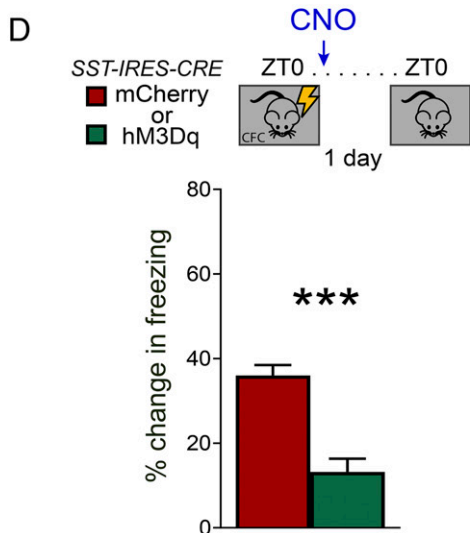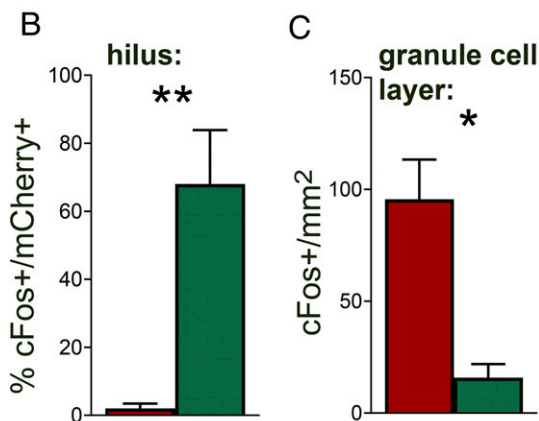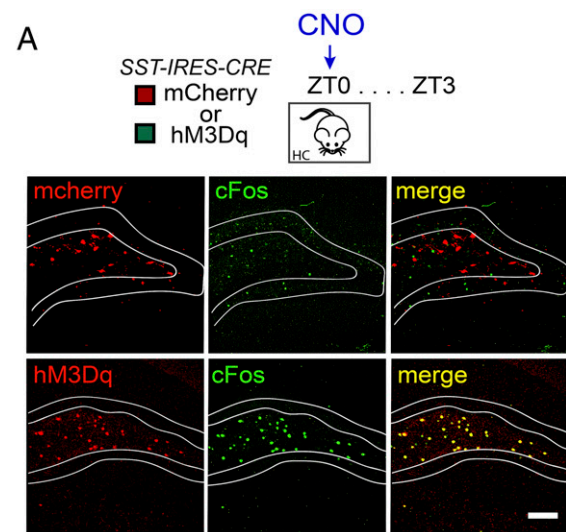https://doi.org/10.1073/pnas.2019318118

Fig. 5. Mimicking SD effects on activity in Sst+ interneurons reduces DG granule cell activity and disrupts sleep-dependent memory consolidation. (*A, Top*) Experimental design: *SST-IRES-CRE* mice expressing either mCherry (*n* = 4) or hM3Dq-mCherry (*n* = 5) in the dorsal hippocampus were injected with CNO (3 mg/kg, i.p.) at lights on and allowed 3 h ad libitum sleep prior to sacrifice for immunohistochemistry. (*Bottom*) Confocal images of mCherry and cFos expression in the DG granule cell layer (white outlines) and hilar Sst+ interneurons. (*B*) Expression of cFos in virally transduced Sst+ interneurons was significantly higher in the hilus of hM3Dq-expressing mice than in mCherry control mice (**P* < 0.01, Student's *t* test). (*C*) Expression of cFos

Sst+ interneurons, releasing inhibition to the surrounding hippocampal network.

Increasing Cholinergic Input to Hippocampus Reduces S6 Phosphorylation in Granule Cells and Disrupts Sleep-Dependent Memory Consolidation. Cholinergic input from MS selectively increases activity, functional plasticity, and structural plasticity in Sst+ interneurons; these effects have been attributed to both nicotinic and muscarinic receptors expressed by Sst+ interneurons (34–41). Acetylcholine release in the hippocampus is significantly higher during active wake versus non-rapid eye movement (NREM) sleep (23, 42), which is the predominant state in the hours following CFC (7, 10, 17). Thus, one possibility is that cholinergic input drives higher Sst+ interneuron activity during SD. To test whether increased MS cholinergic input to the hippocampus is sufficient to disrupt the sleep-dependent consolidation of CFM, we transduced the MS of *Chat-CRE* mice with an AAV vector to express either hM3Dq or mCherry in a Cre-dependent manner (Fig. 7). To confirm the effects of pharmacogenetic manipulation on the DG network, hM3Dq- and mCherry-expressing mice (*n* = 5/group) underwent single-trial CFC at lights on, followed by injection with CNO, and were allowed 3 h ad libitum sleep in their HC prior to euthanasia (Fig. 7*A*). S6 phosphorylation in the DG granule cell layer was quantified as a measure of network activation. Pharmacogenetic activation of MS cholinergic neurons after CFC led to reduced numbers of pS6+ DG granule cells (*P* < 0.001, Student's *t* test), consistent with a role for cholinergic input in gating DG network activity. To determine how this manipulation affected sleep-dependent memory consolidation, a second cohort of hM3Dq- or mCherry-expressing *Chat-CRE* mice (*n* = 9/group) underwent single-trial CFC followed by CNO administration and ad libitum sleep. CFM (assessed 24 h later) was reduced in hM3Dq-expressing mice relative to mCherry-transduced controls (*P* < 0.05, Student's *t* test) (Fig. 7*B*).

Reducing Cholinergic Input to Hippocampus Improves Sleep-Dependent Memory Consolidation and Increases Hippocampal S6 Phosphorylation. We next tested whether reducing MS cholinergic input to the hippocampus following CFC enhances CFM consolidation. To do this, we transduced the MS of *Chat-CRE* mice with an AAV vector to express the inhibitory DREADD hM4Di in a Cre-dependent manner. To characterize the effects of reduced MS cholinergic input on network activity in the hippocampus, hM4Di-expressing mice were treated with CNO or vehicle (VEH) at lights on and allowed 3 h ad libitum sleep. The inhibition of cholinergic MS neurons in CNO-treated mice increased numbers of pS6+ neurons in DG relative to VEH-injected mice (*n* = 5 mice/group, *P* < 0.05, Student's *t* test) (Fig. 8*A*). Transduced mice underwent single-trial CFC at lights on, after which they were immediately injected with either CNO or VEH (*n* = 10 mice/group) and were returned to their HCs for ad libitum sleep. After 24 h, at lights on, mice were returned to the CFC context to assess CFM. Mice administered CNO showed significant increases in context-specific freezing compared to VEH-treated mice (*P* < 0.05, Student's *t* test) (Fig. 8*B*). However, as was true for Sst+ interneuron inhibition, the memory-enhancing effect of pharmacogenetic inhibition was specific to post-CFC sleep. Pharmacogenetic suppression

in surrounding DG neurons (in the DG granule cell layer) was reduced in hM3Dq-expressing mice (**P* < 0.05, Student's *t* test). (*D, Top*) Experimental design: *SST-IRES-CRE* mice expressing mCherry (*n* = 5) or hM3Dq-mCherry (*n* = 6) in the dorsal hippocampus underwent single-trial CFC at lights on and then were immediately administered CNO (3 mg/kg, i.p.) and allowed ad libitum sleep in their HC. After 24 h, all mice were returned to the CFC context for assessment of CFM. (*Bottom*) hM3Dq-expressing mice showed significantly reduced CFM consolidation after 24 h with ad libitum sleep compared with mCherry control mice. ****P* < 0.001, Student's *t* test.
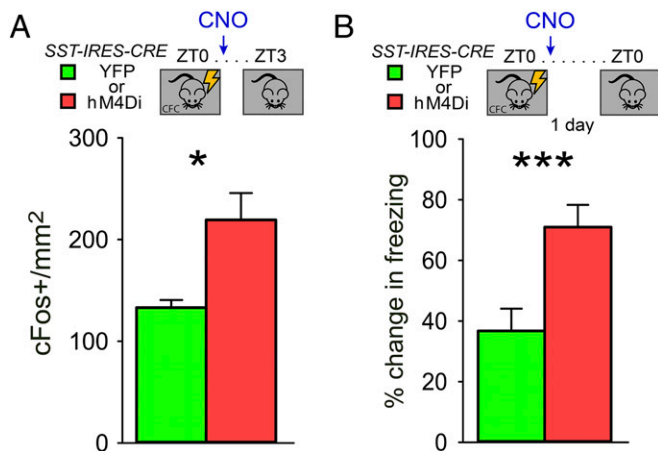
Fig. 6. Suppression of Sst+ interneuron activity increases DG granule cell activity and improves sleep-dependent memory consolidation. (*A*, *Top*) Experimental design: *SST-IRES-CRE* mice expressing either YFP or hM4Di-mCitrine (n = 5/group) in the dorsal hippocampus underwent single-trial CFC at lights on, were injected with CNO (3 mg/kg, i.p.), and allowed 3 h ad libitum sleep prior to sacrifice for immunohistochemistry. (*Bottom*) Expression of cFos in surrounding DG neurons (in the DG granule cell layer) was reduced in hM4Di-expressing mice (*P < 0.05, Mann–Whitney *U* rank sum test). (*B*, *Top*) Experimental design: *SST-IRES-CRE* mice expressing YFP (n = 6) or hM4Di-mCitrine (n = 8) in the dorsal hippocampus underwent single-trial CFC at lights on and then were immediately administered CNO (3 mg/kg, i.p.) and allowed ad libitum sleep in their HC. After 24 h, all mice were returned to the CFC context for assessment of CFM. (*Bottom*) hM4Di-expressing mice showed significantly greater CFM consolidation (measured as percent time freezing) after 24 h with ad libitum sleep compared with YFP control mice. ***P < 0.001, Student's *t* test.

of MS cholinergic neurons' activity during post-CFC SD failed to fully rescue the consolidation of CFM (P = 0.21, Student's *t* test) (*SI Appendix*, Fig. S7B). Together, these data suggest that MS cholinergic input to the hippocampus mediates gating of hippocampal network activity in the same way that Sst+ interneuron activity does, by acting a brake on memory consolidation. They also suggest that lifting this inhibitory gate on the hippocampal network (which is activated during SD) can promote memory consolidation but is insufficient to rescue consolidation from SD-driven disruption.

Discussion

Here, we present converging lines of evidence that indicate SD disrupts activity in the dorsal hippocampus and disrupts memory consolidation via a Sst+ interneuron–mediated inhibitory gate (summarized in Fig. 9). First, we find that, similar to *Arc* mRNA and Arc protein (19), activity-dependent expression of pS6 in dorsal hippocampus increases across a brief period of sleep (*SI Appendix*, Fig. S1) but is reduced by a period of SD (Fig. 1). This effect of SD is enhanced by prior learning (Fig. 1 and *SI Appendix*, Fig. S1) and seems to occur selectively in the hippocampus—it is not seen in the neocortex (*SI Appendix*, Fig. S2). We find that under SD conditions, S6 phosphorylation in Sst+ interneurons (but not other cell types) is increased rather than decreased (Fig. 4). Using pS6 itself as an affinity tag, we took an unbiased TRAP-seq approach to characterize cell types active in the hippocampus during sleep versus SD. We found that transcripts up-regulated on pS6+ ribosomes after SD included those with expression unique to specific interneuron subtypes (e.g., *Sst*, *Npy*, and *Crhbp*) and markers of cholinergic and orexinergic neurons (e.g., *Cht*) (Figs. 2 and 3). Hippocampal Sst+ interneurons are enriched in the same interneuron-specific markers identified as increasing in abundance after SD with pS6 TRAP-seq (Fig. 3B). SD-driven changes in their expression

appear to be caused by greater activity in these neurons as a function of SD (Fig. 3 and *SI Appendix*, Fig. S4).

Previous reports have shown that Sst+ interneurons gate DG network activity during memory acquisition and that their activation likewise gates initial learning (27, 34). Considering we observe fewer activated DG neurons expressing either Arc or pS6 after SD (Fig. 1) (19), one possibility is that by driving higher firing activity in Sst+ interneurons, SD may disrupt memory consolidation by acting as an inhibitory gate (i.e., limiting activity in the surrounding network). Within the hippocampus, Sst+ interneurons target both neighboring pyramidal cells and other interneuron types (such as Pvalb+ interneurons) for inhibition (28, 43–47). Our present data demonstrate that activation of Sst+ interneurons is sufficient to disrupt activity-regulated gene expression in neighboring neurons and CFM consolidation (Fig. 5). Because selective activation of Sst+ interneurons in the hippocampus appears to be characteristic of SD, we conclude that this mechanism may explain the sleep dependence of CFM consolidation (4, 6, 7). It may also contribute to other effects of SD on the dorsal hippocampal network, including disruption of long-term potentiation (4, 5), reduction of plasticity-associated gene expression (19), and decreases in dendritic spine density on pyramidal neurons (5, 48). CFM consolidation itself relies on intact network activity in the dorsal hippocampus (12) and is associated with increased network activity and amplified sleep-associated oscillations in structures such as CA1 (7, 10, 17, 49) and regularization of spike-timing relationships during post-CFC sleep (7, 10, 17). For example, in the hours following CFC, both fast-spiking (putative Pvalb+) interneurons and pyramidal neurons in CA1 show increases in firing rate (10), NREM sharp wave–ripple oscillations are more frequent and higher in amplitude, and delta and theta oscillations are enhanced during NREM and rapid eye movement (REM) sleep (7, 17). CA1 circuit manipulations that interfere with these events also disrupt CFM consolidation (7, 17). Thus, the disruption of network activity patterns via activation of an
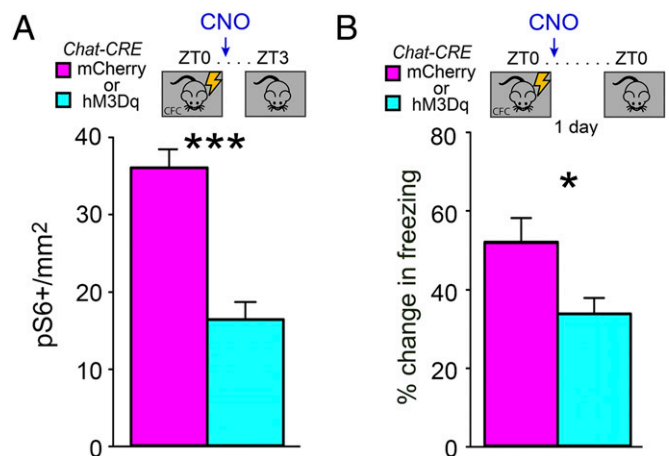

Fig. 7. Activation of MS cholinergic input to the hippocampus decreases DG network activity and disrupts sleep-dependent memory consolidation. (*A*, *Top*) Experimental design: *Chat-CRE* mice expressing either hM3Dq-mCherry or mCherry alone in MS (n = 5/group) underwent single-trial CFC at lights on, after which they were immediately administered CNO (3 mg/kg, i.p.) and allowed 3 h ad libitum sleep prior to sacrifice for immunohistochemistry. (*Bottom*) The number of pS6+ neurons in the DG granule cell layer was reduced in hM3Dq-expressing mice (***P < 0.001, Student's *t* test). (*B*, *Top*) Experimental design: *Chat-CRE* mice expressing hM3Dq or mCherry in MS (n = 9/group) underwent single-trial CFC at lights on, after which they were immediately administered CNO (3 mg/kg, i.p.) and allowed ad libitum sleep in their HC. After 24 h, mice were returned to the CFC context for a CFM test. (*Bottom*) CFM 24 h post-CFC was reduced in hM3Dq-expressing mice relative to mCherry controls (*P < 0.05, Student's *t* test).

Delorme et al.
Sleep loss drives acetylcholine- and somatostatin interneuron–mediated gating of hippocampal activity to inhibit memory consolidation

PNAS | 7 of 10
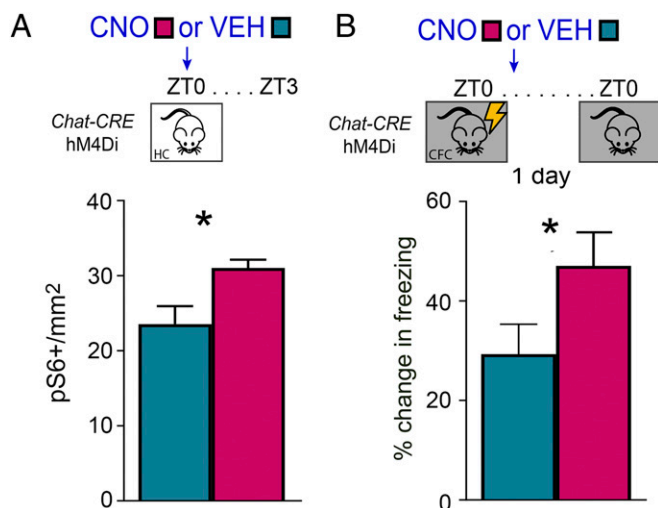https://doi.org/10.1073/pnas.2019318118

Fig. 8. Reduced MS cholinergic input to the hippocampus increases DG network activity and improves sleep-associated memory consolidation. (*A, Top*) Experimental design: *Chat-CRE* mice expressing hM4Di in the medial septum were administered CNO (3 mg/kg, i.p.) or VEH (*n* = 5/group) at ZT0 and were allowed 3 h ad libitum sleep prior to euthanasia. (*Bottom*) Inhibition of cholinergic inputs to hippocampus increased numbers of pS6+ neurons in the DG granule cell layer after 3 h of ad libitum sleep (*P* < 0.05, Student's *t* test). (*B, Top*) Experimental design: *Chat-CRE* mice expressing hM4Di in the medial septum underwent single-trial CFC at lights on, after which they were immediately administered CNO (3 mg/kg, i.p.) or VEH (*n* = 10/group) and allowed ad libitum sleep in their HC. After 24 h, mice were returned to the CFC context for a CFM test. (*Bottom*) CFM performance 24 h post-CFC was improved in CNO-treated mice relative to VEH-treated controls (**P* < 0.05, Student's *t* test).

inhibitory circuit element during SD is likely to interfere with consolidation mechanisms. Critically, Sst+ interneurons may act as a gate on the hippocampal network, inhibiting sharp wave–ripple oscillations (46, 50) and hippocampal–cortical communication

(51, 52)—features which correlate with the sleep-dependent consolidation of CFM (17, 49). Thus, activation of Sst+ interneurons may interfere with consolidation by either disrupting post-learning "reactivation" of memory-encoding neurons (27, 53), by disrupting sleep-associated hippocampal oscillations (54), or both. The latter mechanism, in which sleep-specific oscillations are a necessary element of consolidation, may explain why pharmacogenetic inhibition of Sst+ interneurons augments CFM consolidation in freely sleeping mice but does not fully rescue CFM consolidation in SD mice.

Increased representation of cholinergic and orexinergic cell type–specific transcripts with SD using pS6-TRAP (Fig. 2) is consistent with increased activity in lateral hypothalamic (orexinergic) and MS (cholinergic) inputs to the dorsal hippocampus during wake (23, 24) (Fig. 9). Does activation of cholinergic or orexinergic inputs to the hippocampus likewise contribute to disruption of CFM consolidation? And do these modulators drive selective activation of hippocampal Sst+ interneurons during SD?

Behavioral data from both human subjects (38, 39) and animal models (55) demonstrate that reduced acetylcholine signaling is essential for the benefits of sleep for memory consolidation (37–39). MS cholinergic neurons send the majority of their output to the hippocampus, although a smaller subset provide cholinergic input to neocortical sites, including entorhinal and primary sensory cortices (56, 57). Our present data (Fig. 7) are consistent with reductions in MS cholinergic input to the hippocampus being vital for sleep-dependent CFM consolidation, although modulation of neocortical areas may also play an important role. While cholinergic input to the hippocampus is relatively high during both REM sleep and wake (58), MS cholinergic neurons release significantly less acetylcholine during NREM sleep (42). Like Sst+ interneurons, MS cholinergic inputs suppress sharp wave–ripple oscillations (58), which are prominent in the dorsal hippocampus during post-CFC NREM sleep (17, 49). The relative contributions of REM (with higher acetylcholine release) versus NREM (with lower acetylcholine release) to specific aspects of memory storage are still poorly defined. NREM sleep is the predominant state in the hours following CFC (comprising 60 to 70% of recording time over the first 6 h compared with ~5% of time spent
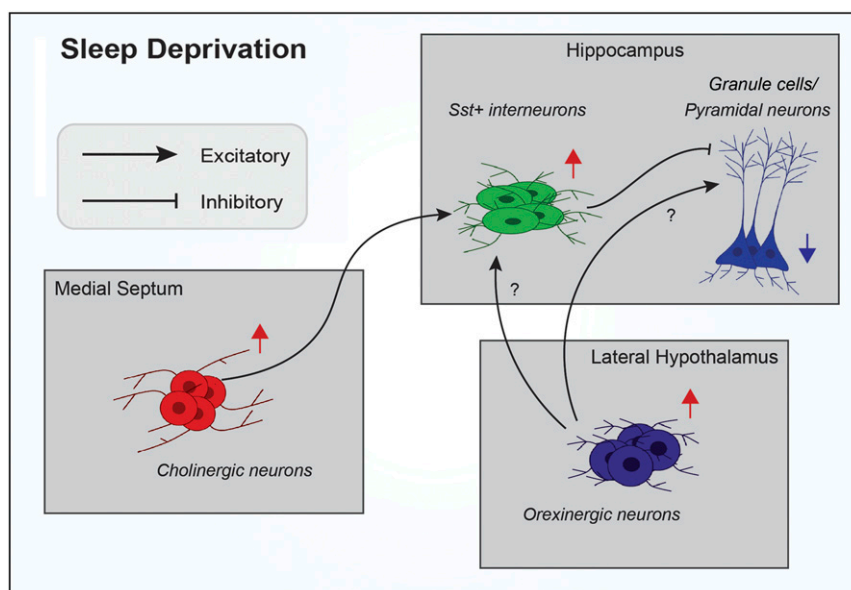

Fig. 9. Schematic model summarizing SD effects on the hippocampal network, which lead to memory consolidation deficits. SD leads to increased activity among cholinergic and orexinergic inputs to hippocampus, which in turn activate Sst+ interneurons (direction of neuronal activity change indicated by red arrows). GABAergic signaling from Sst+ interneurons reduces activity in surrounding glutamatergic granule cells/pyramidal neurons (blue arrow), gating hippocampal network activity.

in REM), and features of NREM sleep appear to be essential for CFM consolidation (7, 10). However, REM sleep, and the network oscillations prominent in the hippocampus during REM, also appear to play a vital role in memory storage (7, 17, 59). It remains to be determined how state-specific changes in acetylcholine release promote memory storage, but our present data suggest that these effects could be mediated, at least in part, by cholinergic regulation of Sst+ interneurons' activity. For example, we find that that S6 phosphorylation in the hippocampus is augmented when MS cholinergic input is reduced (Fig. 7), consistent with disinhibition. Others have found that stimulation of septohippocampal cholinergic neurons causes GABAergic inhibition of DG granule cells, mediated by cholinergic receptors on hilar (Sst- and Npy-expressing) interneurons (60). In contrast, while less is known about the role of orexinergic signaling in memory consolidation, available data suggests that orexin can promote, rather than inhibit, consolidation (61). Moreover, orexinergic input to the hippocampus appears to activate glutamatergic neurons to a greater extent than GABAergic neurons (62). Thus, while we cannot rule out a role for orexinergic input in SD-driven activation of Sst+ interneurons (Fig. 9), based on our present data, it is plausible that cholinergic input is both necessary and sufficient for interneuron activation.

An outstanding question is whether SD-associated, selective activation of Sst+ interneurons is driven mainly by network interactions (e.g., input from MS cholinergic interneurons) or cellautonomous mechanisms (e.g., cell type–specific changes in theexpression of proteins that alter intrinsic excitability and neuronal firing). Our present data do not discriminate between these two mechanisms but provide some circumstantial evidence of both. For example, data from pharmacogenetic manipulations (Figs. 7 and 8) suggest that alterations in MS cholinergic neuron activity affect activity in the granule cell layer of DG. At the same time, our TRAP data (Fig. 3) suggest that Sst+ interneurons undergo SD-driven increases in expression of *Kcnf1*, which may lead to increased spontaneous firing rates (33), regardless of neuromodulatory input.

A second outstanding question is why post-CFC suppression of Sst+ interneurons or cholinergic input to the hippocampus is insufficient to fully rescue CFM consolidation in the context of SD. As described above, CFM consolidation is associated with post-learning changes in a number of sleep-associated oscillations during both NREM and REM sleep (7, 10, 17, 49). One likely possibility is that during SD, these oscillations are suppressed despite the pharmacogenetic inhibition of these cell populations. In other words, it is plausible that hippocampal network activity remains "wake-like" to some extent. In this scenario, sleep-associated decreases in the activity of these populations would be a permissive precondition that is, on its own, insufficient for memory consolidation. Future studies will be needed to determine how the suppression of Sst+ interneuron and MS cholinergic neuron activities affects neuronal and network activity (during post-CFC sleep and SD), in order to address this possibility. Another plausible explanation is that (particularly in the case of Sst+ interneurons in the hippocampal formation), transduction of the cell population with inhibitory DREADDs is incomplete. Thus, it is another possibility that incomplete rescue is related to incomplete manipulation of the population.

A third, and final, outstanding question is whether these mechanisms are unique to the hippocampus or whether similar selective activation of Sst+ interneurons occurs during SD in other structures, such as the neocortex. Our present data suggest that neuronal activity-driven S6 phosphorylation is higher after SD in at least some neocortical regions (*SI Appendix*, Fig. S2). This is in contrast to what is seen in the dorsal hippocampus, where S6 phosphorylation is markedly lower after SD. This finding is consistent with our

recent work, showing that expression of the immediate early gene *Arc* is increased after SD in the somatosensory cortex while simultaneously being suppressed in hippocampal DG (19). More recently, we have found that while SD drives higher expression of a number of activity-regulated genes in both pyramidal cells and PV+ interneurons in the neocortex, expression of the same genes in the hippocampus is refractory to SD (63). Together, these findings might suggest that SD-driven activation of Sst+ interneurons, leading to general network inhibition, could be unique to the hippocampal network. On the other hand, recent calcium imaging studies of the mouse neocortex have demonstrated higher activity of Sst+ interneurons in superficial cortical layers during wake versus NREM sleep (64, 65). This effect (which would lead to reductions in dendrite-targeted inhibition during sleep) may be related to the recent finding of dendritic calcium spikes in these cortical layers during NREM oscillations (66). Critically, the activation of neocortical Sst+ interneurons during active wakefulness is driven by cholinergic signaling (67). While this mechanism is necessary for effective circuit-level information processing during brief periods of arousal, one possibility is that extended wake may act as an inhibitory gate, preventing information processing altogether—as we see evidence for in the hippocampus. Indeed, recent data suggest that overactivation of Sst+ neocortical neurons disrupts both appropriately timed activation of neighboring pyramidal neurons in the context of learning and storage of newly learned information (68). Together, these findings suggest that a similar mechanism could plausibly underlie some SD-induced disruption of memory consolidation mechanisms outside of the hippocampus (69) as well.

Prior work has identified a number of intracellular pathways as being critical targets for SD-mediated disruption of memory in the hippocampus (4, 5, 9) and neocortex (70–72). Our present data indicate that microcircuit-level regulation of brain activity is another SD-driven mechanism underlying the disruption of memory consolidation by sleep loss.

Materials and Methods

Mouse Husbandry, Handling, and Behavioral Procedures. All animal husbandry and experimental procedures were approved by the University of Michigan Institutional Animal Care and Use Committee. For behavioral experiments, 3- to 4-mo-old C57Bl6/J mice (Jackson) or transgenic mice on a C57Bl6 background were habituated to experimenter handling (5 min/day) for 5 d prior to experimental procedures. At lights on (ZT0), animals were either left in their HC or underwent single-trial CFC. Mice were then either were permitted Sleep or were SD by gentle handling (7, 73) over the next 3 to 5 h for immunohistochemical or biochemical studies. For behavioral analysis of CFM (with or without pharmacogenetic manipulations), mice of the appropriate genotypes from the same litter were randomly assigned to experimental and control groups. All mice underwent single-trial CFC as described above followed by either ad libitum sleep or 6-h SD [a length of time sufficient for the disruption of CFM (4, 6, 7)] and subsequent recovery sleep. CFM was assessed behaviorally 24 h after CFC. Complete materials and methods are in *SI Appendix, Materials and Methods*.

Data Availability. All study data are included in the article and/or supporting information.

1. T. Abel, R. Havekes, J. M. Saletin, M. P. Walker, Sleep, plasticity and memory from molecules to whole-brain networks. *Curr. Biol.* **23**, R774–R788 (2013).
2. R. Havekes, T. Abel, The tired hippocampus: The molecular impact of sleep deprivation on hippocampal function. *Curr. Opin. Neurobiol.* **44**, 13–19 (2017).

3. T. M. Prince et al., Sleep deprivation during a specific 3-hour time window post-training impairs hippocampal synaptic plasticity and memory. *Neurobiol. Learn. Mem.* **109**, 122–130 (2014).
4. C. G. Vecsey et al., Sleep deprivation impairs cAMP signalling in the hippocampus. *Nature* **461**, 1122–1125 (2009).

Delorme et al.
Sleep loss drives acetylcholine- and somatostatin interneuron–mediated gating of hippocampal activity to inhibit memory consolidation

PNAS | 9 of 10
https://doi.org/10.1073/pnas.2019318118

5. R. Havekes et al., Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area CA1. *eLife* **5**, e13424 (2016).

6. L. A. Graves, E. A. Heller, A. I. Pack, T. Abel, Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learn. Mem.* **10**, 168–176 (2003).

7. N. Ognjanovski, C. Broussard, M. Zochowski, S. J. Aton, Hippocampal network oscillations rescue memory consolidation deficits caused by sleep loss. *Cereb. Cortex* **28**, 3711–3723 (2018).

8. S. J. Aton, J. Seibt, M. G. Frank, "Sleep and memory" in *Encyclopedia of Life Sciences* (John Wiley and Sons, Ltd., Chichester, 2009), https://doi.org/10.1002/9780470015902.a0021395.

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

10. N. Ognjanovski, D. Maruyama, N. Lashner, M. Zochowski, S. J. Aton, CA1 hippocampal network activity changes during sleep-dependent memory consolidation. *Front. Syst. Neurosci.* **8**, 61 (2014).

11. C. G. Vecsey et al., Genomic analysis of sleep deprivation reveals translational regulation in the hippocampus. *Physiol. Genomics* **44**, 981–991 (2012).

12. S. Daumas, H. Halley, B. Francés, J. M. Lassalle, Encoding, consolidation, and retrieval of contextual memory: Differential involvement of dorsal CA3 and CA1 hippocampal subregions. *Learn. Mem.* **12**, 375–382 (2005).

13. G. V. Wallenstein, D. R. Vago, A. M. Walberer, Time-dependent involvement of PKA/PKC in contextual memory consolidation. *Behav. Brain Res.* **133**, 159–164 (2002).

14. J. Cho et al., Fear memory consolidation in sleep requires protein kinase A. *Learn. Mem.* **25**, 241–246 (2018).

15. R. Bourtchouladze et al., Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. *Learn. Mem.* **5**, 365–374 (1998).

16. Z. A. Knight et al., Molecular profiling of activated neurons by phosphorylated ribosome capture. *Cell* **151**, 1126–1137 (2012).

17. N. Ognjanovski et al., Parvalbumin-expressing interneurons coordinate hippocampal network dynamics required for memory consolidation. *Nat. Commun.* **8**, 15039 (2017).

18. P. S. Pirbhoy, S. Farris, O. Steward, Synaptic activation of ribosomal protein S6 phosphorylation occurs locally in activated dendritic domains. *Learn. Mem.* **23**, 255–269 (2016).

19. J. E. Delorme, V. Kodoth, S. J. Aton, Sleep loss disrupts Arc expression in dentate gyrus neurons. *Neurobiol. Learn. Mem.* **160**, 73–82 (2019).

20. P. Langfelder, S. Horvath, WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics* **9**, 559 (2008).

21. X. Xu, A. B. Wells, D. R. O'Brien, A. Nehorai, J. D. Dougherty, Cell type-specific expression analysis to identify putative cellular mechanisms for neurogenetic disorders. *J. Neurosci.* **34**, 1420–1431 (2014).

22. B. P. Coe et al., Neurodevelopmental disease genes implicated by de novo mutation and copy number variation morbidity. *Nat. Genet.* **51**, 106–116 (2019).

23. L. M. Teles-Grilo Ruivo et al., Coordinated acetylcholine release in prefrontal cortex and hippocampus is associated with arousal and reward on distinct timescales. *Cell Rep.* **18**, 905–917 (2017).

24. L. I. Kiyashchenko et al., Release of hypocretin (orexin) during waking and sleep states. *J. Neurosci.* **22**, 5282–5286 (2002).

25. H. Taniguchi et al., A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. *Neuron* **71**, 995–1013 (2011).

26. J. P. Doyle et al., Application of a translational profiling approach for the comparative analysis of CNS cell types. *Cell* **135**, 749–762 (2008).

27. T. Stefanelli, C. Bertollini, C. Lüscher, D. Muller, P. Mendez, Hippocampal somatostatin interneurons control the size of neuronal memory ensembles. *Neuron* **89**, 1074–1085 (2016).

28. K. A. Pelkey et al., Hippocampal GABAergic inhibitory interneurons. *Physiol. Rev.* **97**, 1619–1747 (2017).

29. T. Kosaka, J.-Y. Wu, R. Benoit, GABAergic neurons containing somatostatin-like immunoreactivity in the rat hippocampus and dentate gyrus. *Exp. Brain Res.* **71**, 388–398 (1988).

30. E. Sanz, J. C. Bean, D. P. Carey, A. Quintana, G. S. McKnight, RiboTag: Ribosomal tagging strategy to analyze cell-type-specific mRNA expression in vivo. *Curr. Protoc. Neurosci.* **88**, e77 (2019).

31. J. W. Kramer, M. A. Post, A. M. Brown, G. E. Kirsch, Modulation of potassium channel gating by coexpression of Kv2.1 with regulatory Kv5.1 or Kv6.1 alpha-subunits. *Am. J. Physiol.* **274**, C1501–C1510 (1998).

32. W. R. Kobertz, Oddballs in the Shaker family: Kv2-related subunits. *J. Gen. Physiol.* **150**, 1599–1601 (2018).

33. C. Bomkamp et al., Transcriptomic correlates of electrophysiological and morphological diversity within and across excitatory and inhibitory neuron classes. *PLOS Comput. Biol.* **15**, e1007113 (2019).

34. S. A. Raza et al., HIPP neurons in the dentate gyrus mediate the cholinergic modulation of background context memory salience. *Nat. Commun.* **8**, 189 (2017).

35. M. Lovett-Barron et al., Dendritic inhibition in the hippocampus supports fear learning. *Science* **343**, 857–863 (2014).

36. N. Hájos, E. C. Papp, L. Acsády, A. I. Levey, T. F. Freund, Distinct interneuron types express m2 muscarinic receptor immunoreactivity on their dendrites or axon terminals in the hippocampus. *Neuroscience* **82**, 355–376 (1998).

37. L. C. Schmid et al., Dysfunction of somatostatin-positive interneurons associated with memory deficits in an Alzheimer's disease model. *Neuron* **92**, 114–125 (2016).

38. S. Gais, J. Born, Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 2140–2144 (2004).

39. B. H. Rasch, J. Born, S. Gais, Combined blockade of cholinergic receptors shifts the brain from stimulus encoding to memory consolidation. *J. Cogn. Neurosci.* **18**, 793–802 (2006).

40. H. Nichol, B. Amilhon, F. Manseau, S. Badrinarayanan, S. Williams, Electrophysiological and morphological characterization of Chrna2 cells in the subiculum and CA1 of the hippocampus: An optogenetic investigation. *Front. Cell. Neurosci.* **12**, 32 (2018).

41. Y. Jia, Y. Yamazaki, S. Nakauchi, K.-I. Ito, K. Sumikawa, Nicotine facilitates LTP induction in oriens-lacunosum molecular cells via Ca2+ entry through non-α7 nicotinic acetylcholine receptors. *Eur. J. Neurosci.* **31**, 463–476 (2010).

42. H. Kametani, H. Kawamura, Alterations in acetylcholine release in the rat hippocampus during sleep-wakefulness detected by intracerebral dialysis. *Life Sci.* **47**, 421–426 (1990).

43. I. Katona, L. Acsády, T. F. Freund, Postsynaptic targets of somatostatin-immunoreactive interneurons in the rat hippocampus. *Neuroscience* **88**, 37–55 (1999).

44. E. B. Bloss et al., Structured dendritic inhibition supports branch-selective integration in CA1 pyramidal cells. *Neuron* **89**, 1016–1030 (2016).

45. K. D. Harris et al., Classes and continua of hippocampal CA1 inhibitory neurons revealed by single-cell transcriptomics. *PLoS Biol.* **16**, e2006387 (2018).

46. L. Katona et al., Sleep and movement differentiates actions of two types of somatostatin-expressing GABAergic interneuron in rat hippocampus. *Neuron* **82**, 872–886 (2014).

47. P. Somogyi, L. Katona, T. Klausberger, B. Lasztóczi, T. J. Viney, Temporal redistribution of inhibition over neuronal subcellular domains underlies state-dependent rhythmic change of excitability in the hippocampus. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **369**, 20120518 (2013).

48. F. Raven, P. Meerlo, E. A. Van der Zee, T. Abel, R. Havekes, A brief period of sleep deprivation causes spine loss in the dentate gyrus of mice. *Neurobiol. Learn. Mem.* **160**, 83–90 (2019).

49. F. Xia et al., Parvalbumin-positive interneurons mediate neocortical-hippocampal interactions that are necessary for memory consolidation. *eLife* **6**, e27868 (2017).

50. T. Klausberger, P. Somogyi, Neuronal diversity and temporal dynamics: The unity of hippocampal circuit operations. *Science* **321**, 53–57 (2008).

51. A. I. Abbas et al., Somatostatin interneurons facilitate hippocampal-prefrontal synchrony and prefrontal spatial encoding. *Neuron* **100**, 926–939.e3 (2018).

52. J. Haam, J. Zhou, G. Cui, J. L. Yakel, Septal cholinergic neurons gate hippocampal output to entorhinal cortex via oriens lacunosum molecular interneurons. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E1886–E1895 (2018).

53. B. C. Clawson et al., Causal role for sleep-dependent reactivation of learning-activated sensory ensembles for fear memory consolidation. *Nat. Commun.* **12**, 1200 (2021).

54. C. Puentes-Mestril, J. Roach, N. Niethard, M. Zochowski, S. J. Aton, How rhythms of the sleeping brain tune memory and synaptic plasticity. *Sleep (Basel)* **42**, zsz095 (2019).

55. S. Inayat et al., Low acetylcholine during early sleep is important for motor memory consolidation. *Sleep (Basel)* **43**, zsz297 (2020).

56. S. Desikan, D. E. Koser, A. Neitz, H. Monyer, Target selectivity of septal cholinergic neurons in the medial and lateral entorhinal cortex. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E2644–E2652 (2018).

57. X. Li et al., Generation of a whole-brain atlas for the cholinergic system and mesoscopic projectome analysis of basal forebrain cholinergic neurons. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 415–420 (2018).

58. Y. Zhang et al., Cholinergic suppression of hippocampal sharp-wave ripples impairs working memory. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2016432118 (2021).

59. R. Boyce, S. D. Glasgow, S. Williams, A. Adamantidis, Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. *Science* **352**, 812–816 (2016).

60. M. Pabst et al., Astrocyte intermediaries of septal cholinergic modulation in the hippocampus. *Neuron* **90**, 853–865 (2016).

61. V. Mavanji et al., Orexin/hypocretin treatment restores hippocampal-dependent memory in orexin-deficient mice. *Neurobiol. Learn. Mem.* **146**, 21–30 (2017).

62. E. M. Stanley, J. R. Fadel, Aging-related alterations in orexin/hypocretin modulation of septo-hippocampal amino acid neurotransmission. *Neuroscience* **195**, 70–79 (2011).

63. C. Puentes-Mestril et al., Sleep loss drives brain region- and cell type-specific alterations in ribosome-associated transcripts involved in synaptic plasticity and cellular timekeeping. *J. Neurosci.* **41**, 5386–5398 (2021).

64. N. Niethard et al., Sleep-stage-specific regulation of cortical excitation and inhibition. *Curr. Biol.* **26**, 2739–2749 (2016).

65. N. Niethard, A. Burgalossi, J. Born, Plasticity during sleep is linked to specific regulation of cortical circuit activity. *Front. Neural Circuits* **11**, 65 (2017).

66. J. Seibt et al., Publisher correction: Cortical dendritic activity correlates with spindle-rich oscillations during sleep in rodents. *Nat. Commun.* **8**, 1838 (2017).

67. W. Muñoz, R. Tremblay, D. Levenstein, B. Rudy, Layer-specific modulation of neocortical dendritic inhibition during active wakefulness. *Science* **355**, 954–959 (2017).

68. A. Adler, R. Zhao, M. E. Shin, R. Yasuda, W.-B. Gan, Somatostatin-expressing interneurons enable and maintain learning-dependent sequential activation of pyramidal neurons. *Neuron* **102**, 202–216.e7 (2019).

69. C. Puentes-Mestril, S. J. Aton, Linking network activity to synaptic plasticity during sleep: Hypotheses and recent data. *Front. Neural Circuits* **11**, 61. (2017).

70. S. J. Aton et al., Mechanisms of sleep-dependent consolidation of cortical plasticity. *Neuron* **61**, 454–466 (2009).

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

72. M. C. Dumoulin et al., Extracellular signal-regulated kinase (ERK) activity during sleep consolidates cortical plasticity in vivo. *Cereb. Cortex* **25**, 507–515 (2015).

73. J. Durkin et al., Cortically coordinated NREM thalamocortical oscillations play an essential, instructive role in visual system plasticity. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 10485–10490 (2017).