

SUPPLEMENTAL MATERIAL

Model of Sleep Deprivation Effects on cAMP Signaling

In our work, we have used electrophysiological, biochemical and pharmacological studies to reveal that sleep deprivation alters cAMP signaling in the hippocampus. In our model, (Supplemental Fig. S1), a sleep deprivation-induced increase in PDE4 activity disrupts subsequent cAMP signaling, possibly by limiting cAMP access to distal PKA targets. This would reduce phosphorylation of PKA targets such as the transcription factor CREB (Supplementary Fig. S7), thus limiting expression of effector and regulator molecules and resulting in impairments in synaptic plasticity and memory consolidation. Treatment with PDE inhibitors would rescue these deficits in sleep-deprived mice (Figures 2, 3, 5, and Supplementary Figure 9) by preventing the breakdown of cAMP, thus allowing it to accumulate to levels that permit activation of effector systems coupled to critical targets. In this regard, it is now well-appreciated that PDE4 isoforms play critical roles in compartmentalizing cAMP signaling, because they are sequestered to distinct signaling scaffolds in cells where they control localized cAMP levels¹. Our data reveal that levels of PDE4A5 are upregulated following sleep deprivation, but it remains unknown what molecular mechanism drives this increase. Interestingly, PDE4A5 is selectively expressed in the brain with highest levels in hippocampal areas CA1 and DG². Prolonged wakefulness during sleep deprivation may initially cause increased neuronal activity^{3,4} and concomitant activity in the cAMP/PKA signaling pathway, and this augmented activity may lead to increased PDE4 expression as a neuroprotective negative feedback mechanism. Indeed, the expression of various PDE isoforms can be induced in a

cAMP-dependent manner¹, and persistent activation of the cAMP pathway often leads to compensatory increases in PDE4 expression and activity⁵⁻⁷.

Brief sleep deprivation does not affect basal synaptic properties in area CA1 of the mouse hippocampus

We report that a brief period of sleep deprivation impairs cAMP/PKA-dependent forms of LTP. To assure that these effects did not simply result from more general effects on hippocampal synaptic function, we assessed two measures of basal synaptic function, slice excitability and paired-pulse facilitation. First, we measured input-output curves in slices from sleep-deprived (SD) and non-sleep-deprived (NSD) mice, comparing the pre-synaptic fiber volley amplitude with the induced fEPSP initial slope. There was no significant difference between the slopes for sleep-deprived and control mice (SD: 3.23 ± 0.27 , $n=20$; NSD: 3.84 ± 0.41 , $n=20$; $p=0.23$), suggesting that slice excitability is unaffected by brief sleep deprivation (Fig. S2a). Consistent with this finding, there was no difference in the maximum fEPSP slope between slices from sleep-deprived and control mice ($p=0.65$, data not shown). We also examined the effects of brief sleep deprivation on paired-pulse facilitation (PPF), a short-term form of plasticity that is sensitive to changes in pre-synaptic transmitter release⁸. There was no overall effect of sleep deprivation on PPF ($F(1, 40)=0.03$, $p=0.85$), and there was no interaction between sleep deprivation and inter-stimulus interval ($F(4,160)=1.01$, $p=0.40$) (Fig. S2b).

Because previous studies have suggested that sleep deprivation can alter NMDA receptor function⁹⁻¹², we performed whole-cell patch clamp recordings to assess the NMDAR I-V curve, NMDAR input-output relationship, and NMDAR/AMPA ratio in the CA1 region of slices from SD and NSD mice (Fig. S3). No differences were found between SD and NSD mice in the I-V

relationship ($F(1, 83)=0.293, p=0.59$) or the input-output relationship ($F(1, 111)=1.428, p=0.235$) of NMDA receptor-mediated currents. The NMDAR/AMPA ratio was also not significantly altered by sleep deprivation (SD: $0.37 \pm 0.03, n=4$; NSD: $0.30 \pm 0.03, n=4, p=0.18$). These findings demonstrate that the brief form of sleep deprivation used in this study does not disrupt basic NMDA receptor function. The absence of any effect of sleep deprivation on basal synaptic properties and short-term plasticity suggests that the disruption of cAMP/PKA-dependent LTP is in fact due to disruption of mechanisms underlying LTP and is not due to non-specific effects on baseline hippocampal function. This contrasts with more extended periods of sleep deprivation, which decrease basal neuronal excitability and generally affect excitatory neurotransmission in the hippocampus^{9,11,13}. The current study is also the first to identify a molecular mechanism induced by sleep deprivation whose manipulation rescues the behavioral and synaptic effects of sleep deprivation.

Controlling for stress effects

When interpreting results of sleep deprivation experiments, there is a concern that the effects of sleep deprivation may be mediated by stress. It is unlikely that stress is responsible for deficits in contextual fear conditioning induced by sleep deprivation, as acute stress is associated with enhanced consolidation of contextual fear conditioning, whereas decreased stress hormone signaling produces memory deficits¹⁴⁻¹⁶. In addition, preventing increases in corticosterone levels using adrenalectomy or metyrapone treatment does not prevent memory deficits produced by sleep deprivation^{17,18}.

However, previous research has shown that increased stress hormone release can produce deficits in LTP¹⁹⁻²¹. Therefore, we measured plasma corticosterone levels from trunk blood

following sleep deprivation by radioimmunoassay, a method that has been used previously to assess stress levels in sleep-deprived mice^{13,22-24}. To determine if stress induction could explain the observed deficits in LTP, we assessed corticosterone levels and spaced 4-train LTP in three groups: mice that were sleep-deprived from ZT 0-5 (Early SD), mice that were sleep-deprived from ZT 5-10 (Normal SD; see Figure 2), and mice that were allowed 2.5 hours of recovery sleep after sleep deprivation from ZT 5-10 (SD + Recovery). We chose our early time point because corticosterone levels in mice are lowest in the early part of the rest period²⁵. Therefore, if LTP deficits due to sleep deprivation are independent of stress hormone effects, we would expect that at our early time point, sleep deprivation would still elicit LTP deficits despite low stress hormone levels. We selected our recovery time point based on other studies showing that LTP deficits due to brief sleep deprivation are reversed by 2-3 hours of undisturbed sleep¹⁰.

We found that Early SD produced significant impairments in spaced 4-train LTP at 130-150 minutes post-induction compared with time-matched control mice (SD: 105.9% \pm 7.7, n=5; NSD: 195.5 \pm 26.4, n=5; F(1,8)=13.3, p=0.007), which were very similar to the deficits produced by Normal SD, suggesting that the time of day when sleep deprivation occurs does not alter the effects on synaptic plasticity (Fig. S4a). The SD + Recovery mice had completely normal spaced 4-train LTP (SD: 198.0% \pm 24.3, n=5; NSD: 183.9% \pm 15.9; n=5, F(1,8)=0.30, p=0.6), demonstrating that 2.5 hours of recovery is capable of rescuing the deficits due to 5 hours of total sleep deprivation (Fig. S4b). Recovery sleep was also capable of rescuing deficits in spaced 4-train LTP following Early SD (data not shown). Interestingly, there was no correlation between LTP stability and levels of the stress hormone corticosterone. Stress hormone levels were lower in the Early SD group (175.8 \pm 30.0 ng/mL) than in the SD + Recovery group (193.4 \pm 49.3 ng/mL), despite the fact that Early SD mice had impaired LTP and the SD + Recovery mice did

not. This is in agreement with findings that a brief period of sleep deprivation can produce significant impairments in LTP unrelated to changes in blood corticosterone levels¹⁰. These results suggest that stress induction cannot explain the deficits in hippocampal function produced by our protocol for sleep deprivation.

mRNA levels of cAMP phosphodiesterase-4 (PDE4) are increased in the hippocampus by brief sleep deprivation and are rescued following 2.5 hours of recovery sleep

Our biochemistry results show that sleep deprivation impairs levels of cAMP induction, and our experiments in which the PDE4-selective inhibitor rolipram¹ rescued deficits in both long-lasting hippocampal LTP and hippocampus-dependent memory consolidation suggested that the effects of sleep deprivation on the cAMP pathway might be due to increased PDE4 activity. Therefore, we used quantitative real-time RT-PCR analysis to assess the expression of mRNA transcripts for three PDE4 genes that are expressed in the hippocampus: *PDE4A*, *PDE4B* and *PDE4D*²⁶. We found that expression of *PDE4B* and *PDE4D* was significantly increased in the hippocampus following sleep deprivation (*PDE4B*, SD: 1.13 +0.05/-0.04, n=9; NSD: 1.00 +0.04/-0.04, n=9; p=0.02, *PDE4D*, SD: 1.12 +0.04/-0.04, n=9; NSD: 1.00 +0.05/-0.04, n=9; p=0.03), and there was a trend towards an increase in *PDE4A* (SD: 1.21 +0.13/-0.11, n=9, NSD: 1.00 +0.08/-0.08, n=9, p=0.08) (Fig. S5a). These findings demonstrate that sleep deprivation selectively upregulates particular PDE4 sub-families. In mice that were sleep-deprived from ZT 5-10 and were then allowed 2.5 hours of recovery sleep (SD + Recovery) before hippocampi were collected, expression levels of all three genes recovered towards baseline (*PDE4B*, SD+Recovery: 1.00 +0.04/-0.04, n=9; NSD: 1.00 +0.04/-0.04, n=9; p=0.97, *PDE4D*, SD+Recovery: 1.08 +0.04/-0.04, n=9; NSD: 1.00 +0.02/-0.02, n=9; p=0.06), *PDE4A*

(SD+Recovery: 1.04 +0.01/-0.01, n=9, NSD: 1.00 +0.02/-0.02, n=9, p=0.09) (Fig. S5b). This demonstrates that a 2.5-hour period of recovery is sufficient to reverse the increases in *PDE4* gene expression produced by 5 hours of sleep deprivation. Therefore, *PDE4* gene expression and LTP (Fig. S4) both return to baseline levels following this period of recovery sleep.

Effects of sleep deprivation on PDE4 protein levels in the hippocampus

Having found that sleep deprivation increased PDE4 activity and mRNA levels of PDE4B and PDE4D in the hippocampus, we were interested to determine if these changes in gene expression were carried over to the protein level. Therefore, we used Western blot analysis to probe for protein levels of several PDE4 isoforms^{27,28}. We found that sleep deprivation upregulated PDE4A5 protein levels (Fig. 3). Using a pan-PDE4A antibody, we saw a non-significant trend towards an increase with sleep deprivation (PDE4A, SD: 1.28 ± 0.22, n=10; NSD: 1.00 ± 0.21, n=10; p=0.18) (Fig. S6). This lack of an effect on PDE4A as a whole is likely because only selected isoforms of PDE4A, such as PDE4A5, are altered by sleep deprivation. This may also explain why we saw a non-significant increase in overall *PDE4A* mRNA levels (Fig. S5). Surprisingly, although sleep deprivation increased *PDE4B* and *PDE4D* gene expression, we did not observe any increase in PDE4B or PDE4D3/5 protein levels (PDE4B, SD: 0.82 ± 0.10, n=9; NSD: 1.00 ± 0.20, n=9; p=0.19), PDE4D3 (SD: 0.82 ± 0.12, n=10, NSD: 1.00 ± 0.22, n=10, p=0.23, PDE4D5 (SD: 0.75 ± 0.13, n=10, NSD: 1.00 ± 0.23, n=10, p=0.16) (Fig. S6). It is possible that translational control mechanisms prevented the effects on *PDE4B* and *PDE4D* mRNA levels from being carried over to the protein level. In contrast, upregulation of *PDE4A* gene expression appears to carry over to the protein level, suggesting that different translation regulation mechanisms are in place for these PDE4 genes. The idea that sleep and

sleep deprivation regulate translation in a complex way is supported by several published studies that have found state-dependent alterations in translation efficacy itself or in levels of genes and proteins that are known to modulate translation²⁹⁻³⁷.

Sleep deprivation decreases CREB phosphorylation in the hippocampus

The transcription factor CREB is one of the downstream targets of cAMP and PKA known to play a key role in hippocampal synaptic plasticity and in memory storage³⁸. Using immunohistochemistry to analyze levels of CREB phosphorylation in the dorsal hippocampus, we found that phosphorylation of CREB at Ser133 was decreased by sleep deprivation in the CA1 region of the hippocampus (NSD: 100% \pm 4.1% (n=13), SD: 84.3% \pm 5.5% (n=14), $p=0.016$) (Fig. S7b), as well as in the dentate gyrus (DG) (NSD: 100% \pm 5.5% (n=13), SD: 87.9% \pm 4.2% (n=14), $p=0.043$) (Fig. S7d). No significant effect of sleep deprivation was observed on CREB phosphorylation in the CA3 region of the hippocampus (NSD: 100% \pm 4.9% (n=13), SD: 93.0% \pm 5.0% (n=14), $p=0.17$) (Fig. S7c). This selective effect on CREB phosphorylation in the dentate gyrus and CA1 hippocampal regions may be explained by the fact that PDE4A5, the PDE4 isoform that we found to be up-regulated in the hippocampus following sleep deprivation (Fig. 3), is expressed highly in the dentate gyrus and CA1, but not in CA3². We also found no effect of sleep deprivation on CREB phosphorylation in the basolateral/lateral amygdala (NSD: 100% \pm 7.9% (n=10), SD: 108.7% \pm 10.9% (n=10), $p=0.53$) (Fig. S7e). These findings demonstrate that deficient cAMP signaling due to sleep deprivation results in impaired activation of at least one major downstream target (CREB) *in vivo*, and that this effect occurs in the hippocampus but not the amygdala.

Sleep deprivation reduces the efficacy of the PDE4-specific inhibitor rolipram to enhance 1-train LTP

In Figure 4, we showed that the PDE4 inhibitor rolipram was capable of rescuing deficits in spaced 4-train LTP caused by sleep deprivation, without any effect on this form of LTP in hippocampal slices from non-sleep-deprived (NSD) mice. We next assessed the effects of rolipram on 1-train LTP. Interestingly, we found that rolipram enhanced 1-train LTP in non-sleep-deprived mice ($p=0.0002$) (Fig. S8a), as has previously been reported³⁹, but had no significant effect on 1-train LTP in sleep-deprived mice ($p=0.56$) (Fig. S8b). This finding suggests that sleep-deprived mice have a reduced sensitivity to rolipram. This is most likely because sleep-deprived mice have higher hippocampal PDE4 activity, consistent with the findings shown in Figure 3. Indeed, treating with a 10-fold higher dose of rolipram modestly enhanced 1-train LTP in sleep-deprived mice, although this was still not a significant effect (data not shown).

Rolipram treatment rescues deficits in hippocampus-dependent memory produced by sleep deprivation

We had previously shown⁴⁰ that a 5-hour period of sleep deprivation immediately following fear conditioning impairs memory for the trained context 24 hours later, with no effect on cued memory. This resulted in a significant impairment in context-specific memory, as measured by the difference in freezing between the trained and an altered context⁴⁰. In this study, our training procedure consisted of a 3-minute exposure to a novel environment, with a 2-second, 1.5 mA footshock delivered at 2.5 minutes. Mice were either deprived of sleep by gentle handling for 5

hours immediately following training (SD), or were left undisturbed in their cages (NSD). Mice were injected i.p. immediately after training and 2.5 hours later with either vehicle (VEH) or 1.0 mg/kg rolipram (ROL). Testing was performed in the trained context 1 day after training, and in an altered context 2 days after training. We found that injections at the 2.5 hour time point only kept the NSD mice awake for about 15 minutes.

There was an overall significant interaction between sleep deprivation and rolipram treatment with testing context as a within-subject repeated measure ($F(1,76)=7.0$, $p=0.01$). Rolipram treatment improved memory for the trained context in sleep-deprived mice (SD+ROL: $31.8 \pm 2.7\%$, $n=20$; SD+VEH: $19.5 \pm 1.9\%$, $n=20$; $p=0.001$), with no effect on non-sleep-deprived mice (NSD+ROL: $27.8 \pm 2.7\%$, $n=20$; NSD+VEH: $26.5 \pm 3.2\%$, $n=20$; $p=0.99$) (Fig. S9). No significant effects of either sleep deprivation or rolipram treatment were found in the altered context (SD+ROL: $12.5 \pm 1.2\%$, $n=20$; SD+VEH: $14.3 \pm 1.8\%$, $n=20$; NSD+ROL: $11.4 \pm 1.5\%$, $n=20$; NSD+VEH: $11.1 \pm 1.9\%$, $n=20$). This result shows that neither brief sleep deprivation nor rolipram treatment alters freezing behavior non-selectively, but instead both specifically affect the consolidation of hippocampus-dependent contextual memory. All groups except for the SD+VEH group froze significantly more in the trained context than in the altered context (SD+VEH: $p=0.43$, SD+ROL: $p=0.0001$, NSD+VEH: $p=0.0001$, NSD+ROL: $p=0.0001$). Therefore, sleep deprivation caused deficits in the specificity of contextual memory, a measure that has been shown to be particularly sensitive to disruptions of hippocampal function⁴¹, and rolipram reversed this effect.

Our data suggest that rolipram rescues memory deficits induced by sleep deprivation because the drug counteracts the increased PDE4 activity produced in the hippocampus by sleep deprivation, allowing for efficient cAMP signaling involved in memory consolidation. One

alternative explanation for how rolipram rescues memory deficits in sleep-deprived mice is that treatment *in vivo* increases time spent asleep. However, we (data not shown) and others⁴² have found that rolipram treatment actually *reduces* time spent asleep. Using beam break-based activity monitoring, we found that injections of rolipram performed as in our rescue experiments reduced the time spent asleep by ~20% over the 1.5 hours following each injection, compared with vehicle injections (data not shown).

Additionally, it has been suggested that sleep deprivation might cause memory impairments by inducing a shift in circadian rhythms⁴³⁻⁴⁶. Based on the following findings, we do not believe that shifted circadian rhythms underlie the effects of sleep deprivation on LTP or memory. First, sleep deprivation causes similar impairments in 4-train LTP regardless of whether sleep deprivation is carried out during the first or second half of the sleep period (see Fig. 1 and Supplementary Figure S3). A lack of dependence on circadian time has also been shown previously for the effects of sleep deprivation on memory consolidation⁴⁰. Second, using EEG/EMG recordings we have seen that sleep deprivation does not cause a shift in daily rhythm in light/dark conditions. Following sleep deprivation, mice anticipate the dark phase, waking up at the same time of day as non-sleep-deprived mice, despite having lost several hours of sleep earlier in the day (data not shown). Because we did not look for shifts in behavioral rhythms following sleep deprivation in dark/dark conditions, we may not have been able to detect shifts in rhythms due to masking effects of light. In addition, we have not explicitly tested whether sleep deprivation shifts the *in vivo* cycling of cAMP that has been reported recently to occur in the hippocampus⁴⁶. Therefore, it remains possible that a shift in molecular rhythms within the hippocampus occurs during sleep deprivation to impair cAMP signaling, synaptic plasticity, and memory consolidation.

Because the present study utilized systemic IP injections of rolipram, our data do not demonstrate conclusively that the site of rolipram action is the hippocampus. However, our slice studies confirm that rolipram is capable of reversing the effects of sleep deprivation on synaptic plasticity in the isolated hippocampus (Fig. 4). In addition, it has been shown previously that sleep deprivation selectively disrupts hippocampus-dependent tasks, such as contextual fear conditioning or spatial water maze learning, without altering related but hippocampus-independent tasks^{13,40,47,48}. Therefore, it seems likely that sleep deprivation impairs memory by acting on the hippocampus, and that rolipram treatment rescues hippocampus-dependent memory deficits by blocking the effects of sleep deprivation on cAMP signaling within the hippocampus.

Supplemental Figures

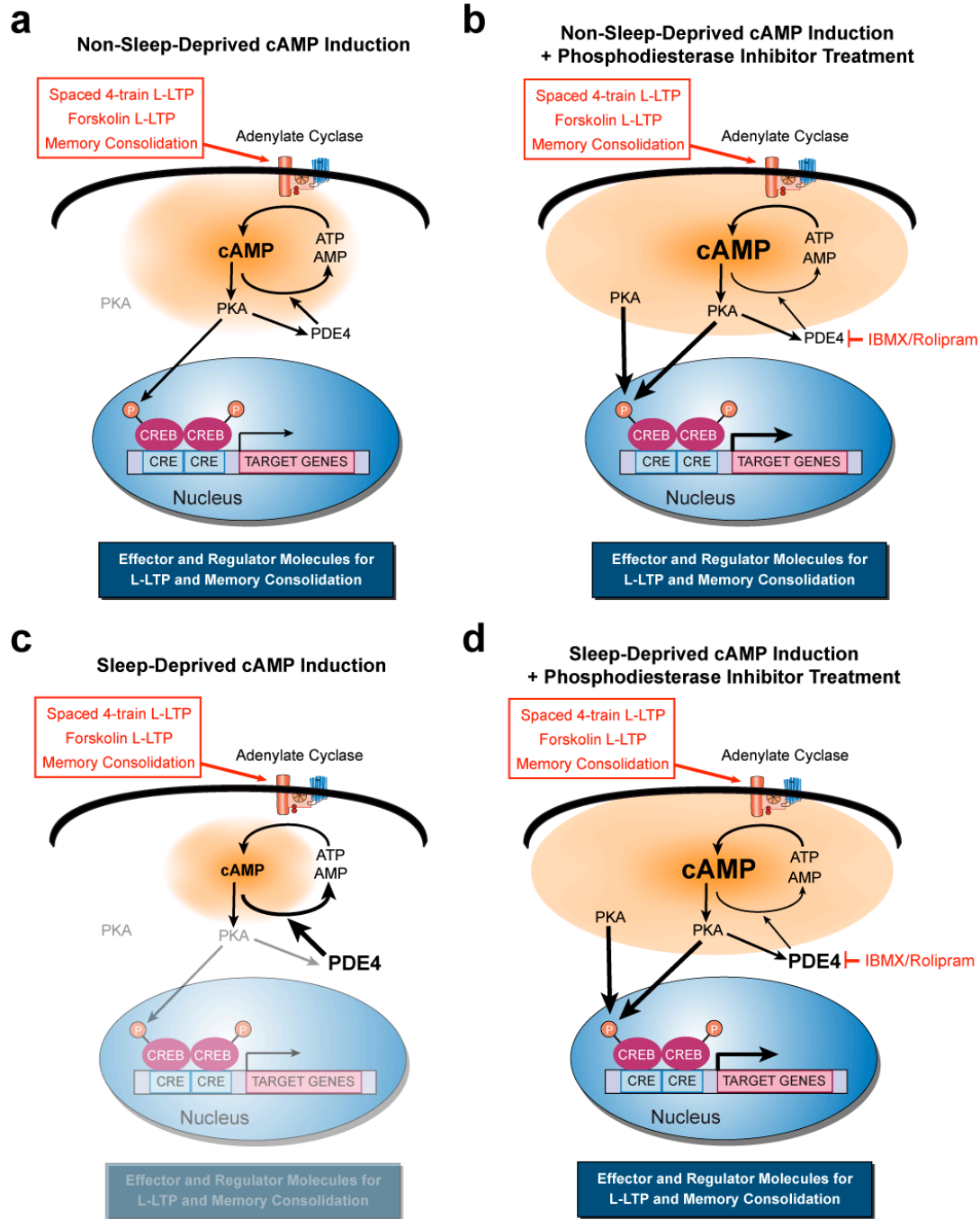


Figure S1

Figure S1. Brief sleep deprivation alters cAMP signaling in the hippocampus. Shown is a model of how sleep deprivation may impact cAMP function in the hippocampus. (a) In control

(NSD) mice, cAMP induction due to forskolin treatment, spaced 4-train L-LTP induction, or memory consolidation, is sufficient to signal to PKA, which then signals to the nucleus to generate plasticity-related proteins that support long-term synaptic changes. As a negative feedback mechanism, cAMP/PKA signaling may activate PDE4 through a variety of mechanisms, including phosphorylation and regulation of transcription and translation. (b) In NSD mice, cAMP induction in conjunction with treatment with phosphodiesterase (PDE) inhibitors IBMX or rolipram results in an even greater level and spread of cAMP, which activates PKA more strongly, and may also signal via additional pools of PKA, increasing signaling to the nucleus. (c) In sleep-deprived (SD) mice, increased PDE4 activity limits the induction of cAMP signaling, resulting in incomplete PKA activation and deficits in hippocampal synaptic plasticity. (d) Treatment with PDE inhibitors negates the effect of additional PDE4 activity in SD mice, thus rescuing the induction of cAMP and PKA-mediated signaling to the nucleus. As we have shown, PDE4 inhibition rescues both long-term synaptic plasticity and memory consolidation.

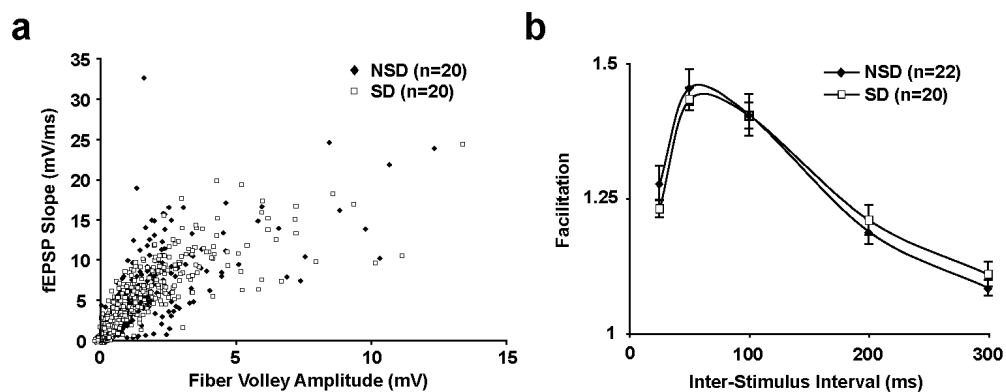


Figure S2

Supplemental Figure S2. Sleep deprivation does not alter basal synaptic properties in area CA1 of the mouse hippocampus. C57BL/6J mice were deprived of sleep for 5 hours by gentle

handling (SD) or were left undisturbed in their home cages (NSD). *In vitro* field recordings were made from the Schaffer collateral pathway in hippocampal slices taken immediately following the deprivation period. (a) The strength of the synaptic input, as measured by the pre-synaptic fiber volley amplitude, was gradually increased, and the resulting post-synaptic output was plotted. The resulting input-output curve showed no difference in basal excitability following sleep deprivation. (b) Paired-pulse facilitation (PPF), a short-term form of pre-synaptic plasticity, was unaltered in sleep-deprived mice at any of the intervals examined. Representative sample sweeps are shown for each LTP experiment. In each case, the gray sweep represents the average of the first 5 baseline sweeps, and the black sweep represents the average of the last 5 sweeps in the recording. Scale bars are 5 mV/5 ms. Error bars indicate +/- SEM.

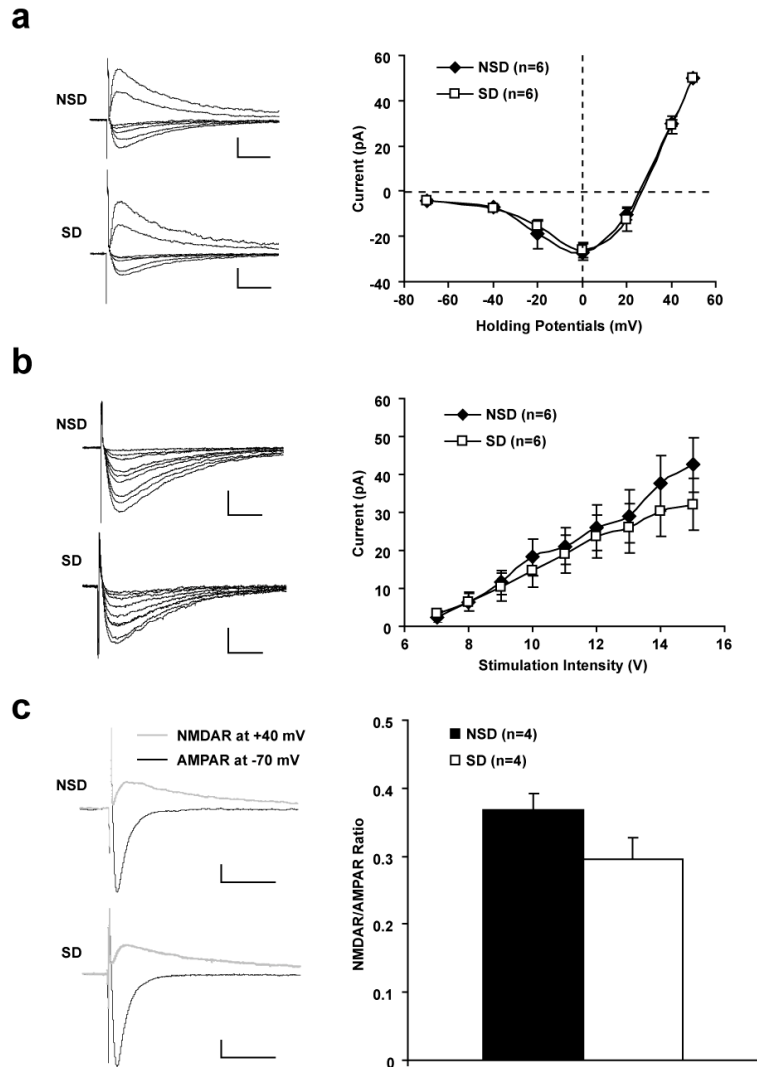


Figure S3

Figure S3. Sleep deprivation does not alter NMDA receptor characteristics or the NMDAR/AMPA ratio. (a) To examine the voltage-dependence of NMDAR-mediated EPSCs in slices from SD and NSD mice, currents were recorded in the presence of CNQX at different holding potentials (-70mV, -40mV, -20 mV, 0mV, +20mV, +40mV, and +50mV). (b) To examine the NMDAR input-output relationship, the strength of stimulation at 0mV was plotted against the elicited current in slices from SD and NSD mice. (c) Average NMDAR and AMPAR currents were recorded for each animal at +40mV and -70mV, respectively. The ratio of the size

of these currents was compared between SD and NSD mice. No effect of sleep deprivation was observed for any of these measures of NMDAR function.

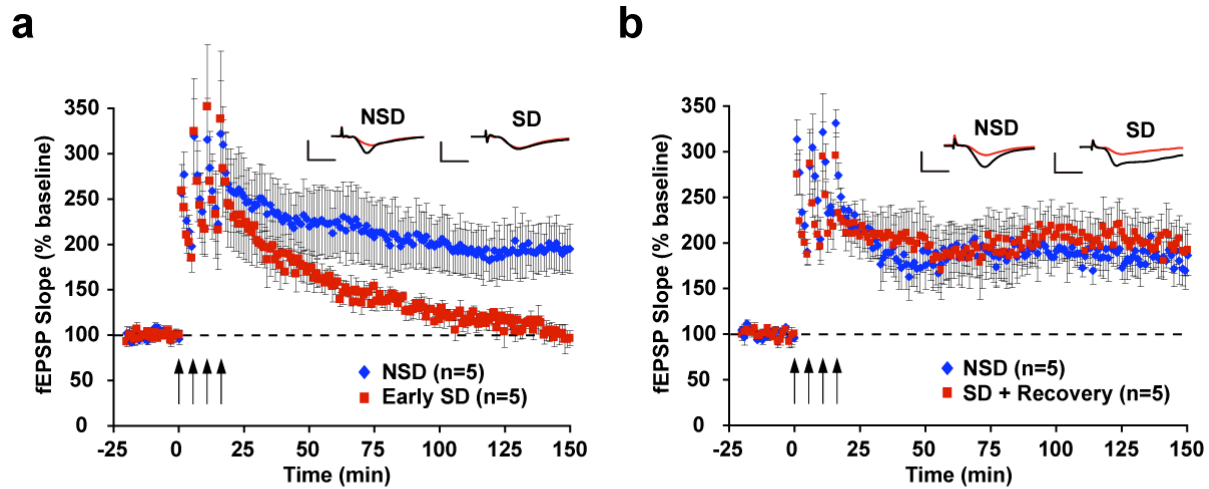


Figure S4

Supplemental Figure S4. LTP maintenance is impaired in mice receiving an early window of sleep deprivation and is rescued by 2.5 hours of recovery sleep. (a) The maintenance of spaced 4-train LTP was impaired in mice that were sleep-deprived from ZT 0-5 (Early SD) relative to time-matched non-sleep-deprived mice (NSD), demonstrating that the time of day during which deprivation occurs does not affect the impairments in LTP induced by sleep deprivation. (b) Spaced 4-train LTP was completely recovered in mice that were sleep-deprived from ZT 5-10 and were then allowed 2.5 hours of recovery sleep (SD + Recovery) before slices were taken, relative to time-matched non-sleep-deprived mice (NSD), demonstrating that a 2.5 hour period of recovery is sufficient to reverse LTP deficits due to 5 hours of sleep deprivation. Representative sample sweeps are shown for each LTP experiment. In each case, the red sweep represents the average of the first 5 baseline sweeps, and the black sweep represents the average of the last 5 sweeps in the recording. Scale bars are 5 mV/5 ms. Error bars represent +/- SEM.

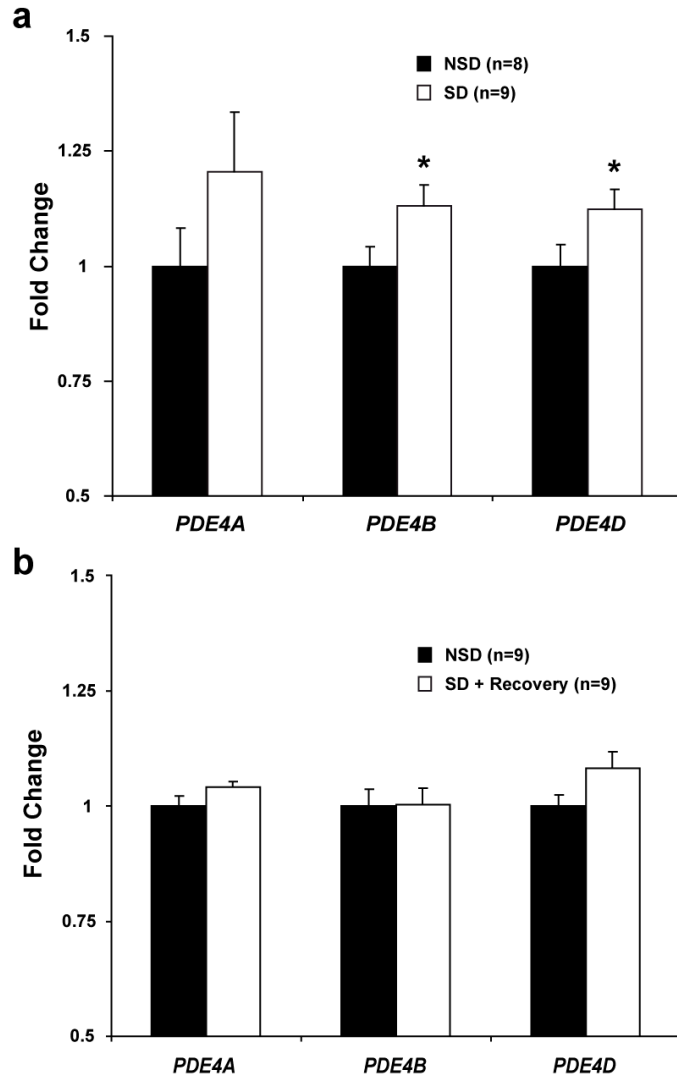


Figure S5

Supplemental Figure S5. PDE4 mRNA expression is upregulated by sleep deprivation and recovers following 2.5 hours of recovery sleep. Quantitative RT-PCR was used to determine the level of mRNA transcripts for three PDE4 isoforms (*PDE4A*, *PDE4B*, and *PDE4D*) in hippocampal tissue. Expression levels of all three genes were increased following sleep deprivation from ZT 5-10, significantly so for *PDE4B* and *PDE4D*. In mice that were sleep-deprived from ZT 5-10 and were then allowed 2.5 hours of recovery sleep (SD + Recovery) before hippocampi were collected, expression levels of all three genes recovered towards

baseline. This demonstrates that a 2.5 hour period of recovery is sufficient to reverse the increases in PDE4 gene expression produced by 5 hours of sleep deprivation. For each gene, expression is represented as the fold change relative to control mice, normalized relative to the expression of housekeeping genes *Actg*, *Hprt*, and *Tuba4a*. * indicates a comparison with $p < 0.05$. Error bars indicate \pm SEM.

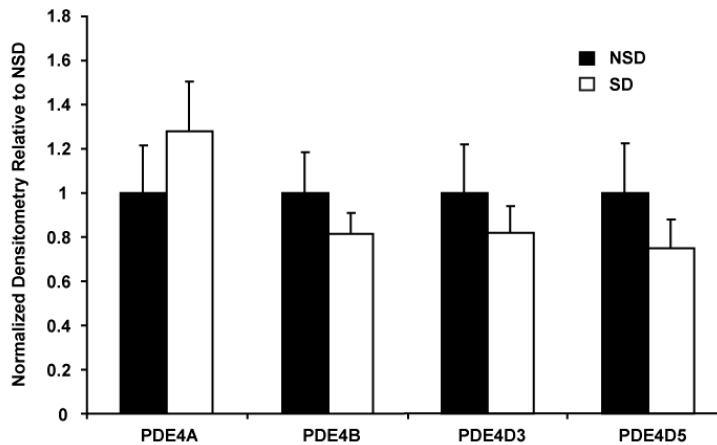


Figure S6

Supplemental Figure S6. Effects of sleep deprivation on PDE4 protein levels in the hippocampus. Western blot analysis was used to determine the levels of several PDE4 proteins (PDE4A, PDE4B, PDE4D3, and PDE4D5) in hippocampal tissue taken from sleep-deprived (SD) or non-sleep-deprived (NSD) mice. Only PDE4A protein levels showed a trend towards an increase in SD mice. For each protein, data is represented as the densitometry for the band of interest, normalized to densitometry measurements for α -tubulin. The NSD average for each protein was set to 1. Error bars indicate \pm SEM.

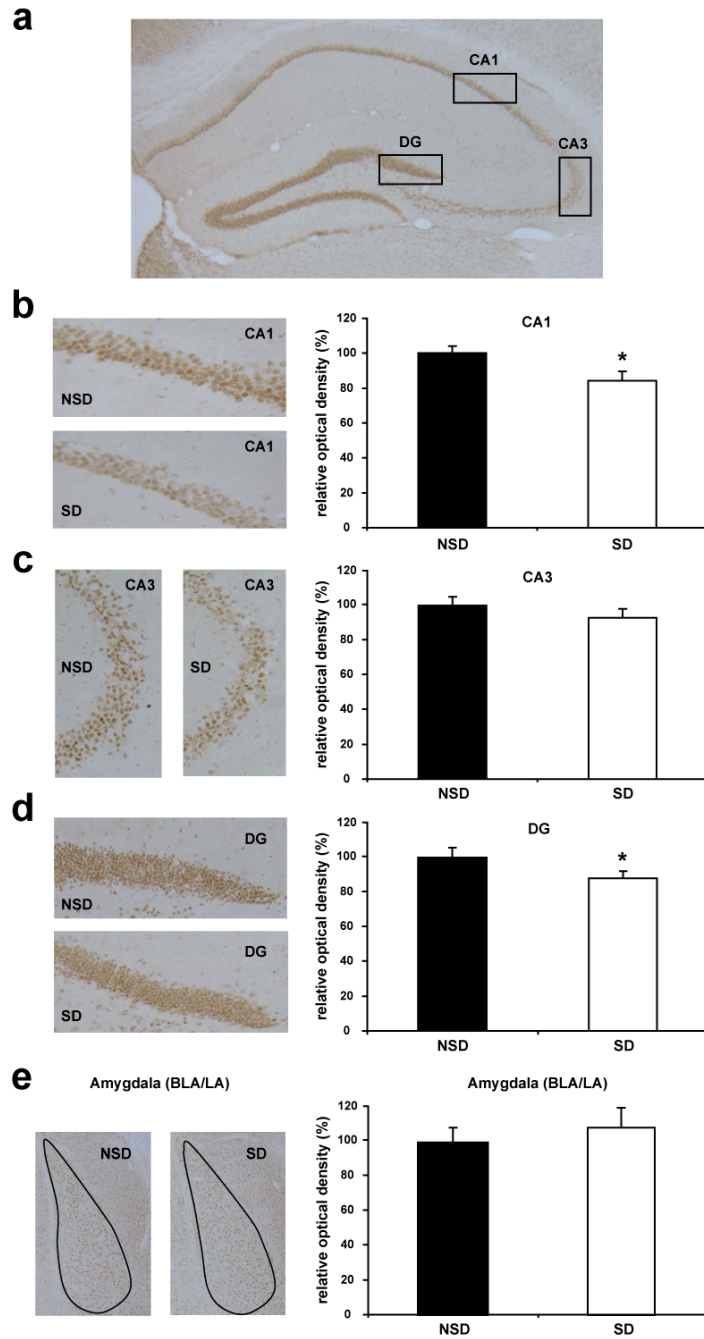


Figure S7

Supplemental Figure S7. Sleep deprivation decreases phospho-CREB immunoreactivity in the CA1 and DG regions of the hippocampus. We used immunohistochemistry to measure the levels of phosphorylated CREB in subregions of the dorsal hippocampus and in the basolateral/lateral amygdala in sections taken from sleep-deprived (SD) or non-sleep-deprived

(NSD) mice. (a) An example section showing the hippocampal formation, with boxes illustrating where measurements were made for CA1, CA3, and DG. Insets show higher magnification views of representative measured regions from SD and NSD mice. SD mice had significantly lower phospho-CREB immunoreactivity specifically in area CA1 (b) and in the DG (d), but not in CA3 (c) or in the basolateral/lateral amygdala (e).

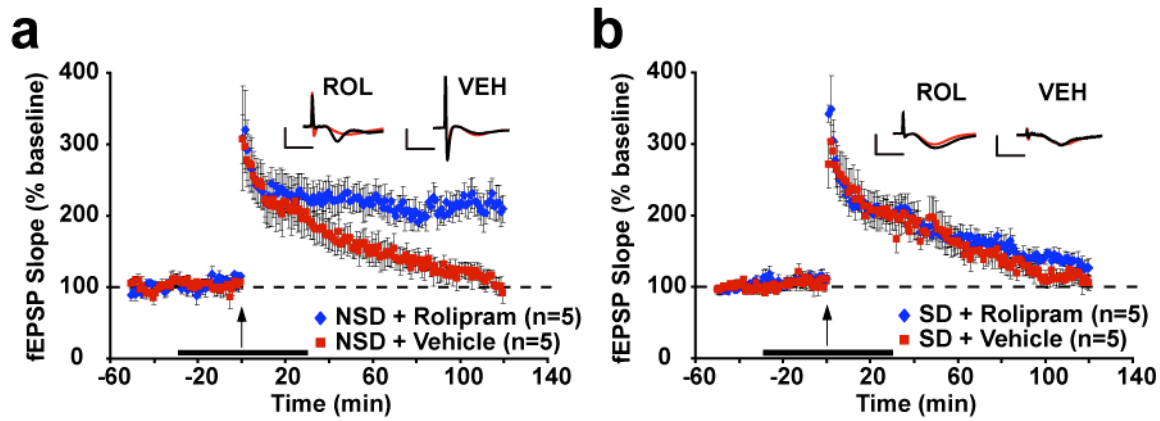


Figure S8

Supplemental Figure S8. Sleep deprivation reduces the sensitivity of hippocampal slices to the enhancing effects of rolipram. (a) Rolipram enhanced 1-train LTP in non-sleep-deprived mice, but (b) had no significant effect on 1-train LTP in sleep-deprived mice. Representative sample sweeps are shown for each LTP experiment. In each case, the red sweep represents the average of the first 5 baseline sweeps, and the black sweep represents the average of the last 5 sweeps in the recording. Scale bars are 5 mV/5 ms. Error bars indicate +/- SEM.

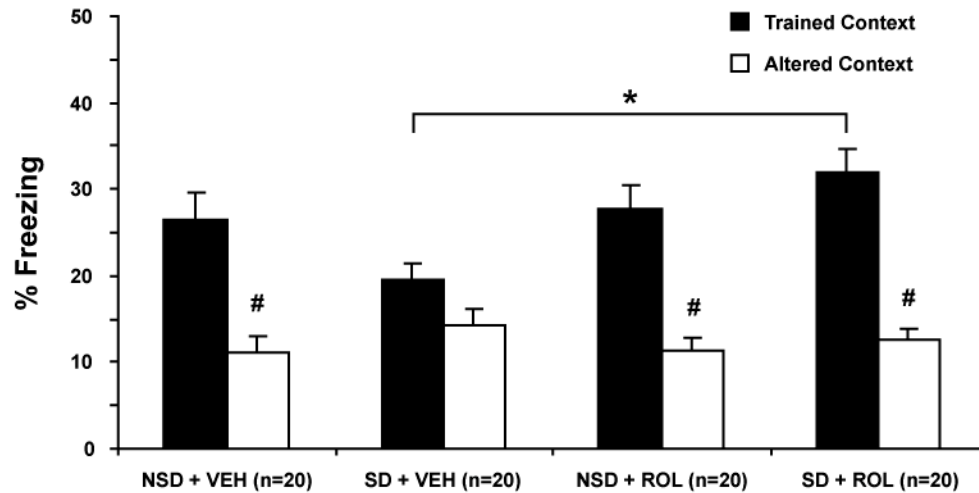


Figure S9

Supplemental Figure S9. Rolipram treatment rescues deficits produced by sleep deprivation in hippocampus-dependent memory consolidation. C57BL/6J mice received single-trial foreground contextual fear conditioning (1.5 mA footshock) followed by 5 hours of total sleep deprivation by gentle handling (SD), or 5 hours undisturbed in their home cages (NSD). Mice were injected i.p. immediately after training and 2.5 hours later with either vehicle (VEH) or 1.0 mg/kg rolipram (ROL). Testing was performed in the trained context 1 day after training, and in an altered context 2 days after training. Memory was assessed by % freezing during the 5-minute test. Rolipram treatment enhanced freezing in response to the trained context in sleep-deprived mice (SD + ROL vs. SD + VEH), without any effect on non-sleep-deprived mice (NSD + ROL vs. NSD+VEH). Rolipram treatment had no effect on freezing in response to the altered context in either SD or NSD mice. All groups had significantly higher freezing in the trained context than in the altered context except for vehicle-treated SD mice. * indicates a comparison with $p < 0.05$. # indicates a comparison between the trained and altered context for a given group with $p < 0.05$. Error bars represent +/- SEM.

Supplemental References

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