Article

Current Biology

Recovering object-location memories after sleep deprivation-induced amnesia

Highlights

- Sleep deprivation-induced amnesia is a problem of memory retrievability
- Memory engram activation after sleep deprivation restores memory retrievability
- Amnesia due to sleep loss can be permanently reversed multiple days after learning
- Roflumilast and optogenetics can be used to restore memory access

Authors

Youri G. Bolsius, Pim R.A. Heckman, Camilla Paraciani, ..., Steve Ramirez, Peter Meerlo, Robbert Havekes

Correspondence

r.havekes@rug.nl

In brief

Sleep deprivation after learning leads to hippocampal memory deficits. Bolsius et al. show that this amnesia is not caused by information loss but is rather a problem of retrievability. Memory access can be restored by optogenetic engram activation or the drug roflumilast.

Bolsius et al., 2023, Current Biology *33*, 1–11
January 23, 2023
https://doi.org/10.1016/j.cub.2022.12.006

CellPress

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

Current Biology

CellPress
OPEN ACCESS

Article

Recovering object-location memories after sleep deprivation-induced amnesia

Youri G. Bolsius,[1,4,5] Pim R.A. Heckman,[1,2,4] Camilla Paraciani,[1] Sophia Wilhelm,[1] Frank Raven,[1,6] Elroy L. Meijer,[1] Martien J.H. Kas,[1] Steve Ramirez,[3] Peter Meerlo,[1] and Robbert Havekes[1,7,*]

[1]Neurobiology Expertise Group, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG Groningen, the Netherlands

[2]Department of Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, Maastricht University, Universiteitssingel 40, 6229 ER Maastricht, the Netherlands

[3]Department of Psychological and Brain Sciences, The Center for Systems Neuroscience, Boston University, Boston, MA 02215, USA

[4]These authors contributed equally

[5]Present address: Department of Brain Sciences, Weizmann Institute of Science, Rehovot 7610001, Israel

[6]Present address: Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA

[7]Lead contact

*Correspondence: r.havekes@rug.nl

https://doi.org/10.1016/j.cub.2022.12.006

SUMMARY

It is well established that sleep deprivation after learning impairs hippocampal memory processes and can cause amnesia. It is unknown, however, whether sleep deprivation leads to the loss of information or merely the suboptimal storage of information that is difficult to retrieve. Here, we show that hippocampal object-location memories formed under sleep deprivation conditions can be successfully retrieved multiple days following training, using optogenetic dentate gyrus (DG) memory engram activation or treatment with the clinically approved phosphodiesterase 4 (PDE4) inhibitor roflumilast. Moreover, the combination of optogenetic DG memory engram activation and roflumilast treatment, 2 days following training and sleep deprivation, made the memory more persistently accessible for retrieval even several days later (i.e., without further optogenetic or pharmacological manipulation). Altogether, our studies in mice demonstrate that sleep deprivation does not necessarily cause memory loss but instead leads to the suboptimal storage of information that cannot be retrieved without drug treatment or optogenetic stimulation. Furthermore, our findings suggest that object-location memories, consolidated under sleep deprivation conditions and thought to be lost, can be made accessible again several days after the learning and sleep deprivation episode, using the clinically approved PDE4 inhibitor roflumilast.

INTRODUCTION

Sleep loss is a common hallmark of modern society, which affects people of all ages[1,2] and has a severe impact on body and brain (e.g., Abel et al.,[3] Areal et al.,[4] Havekes and Aton,[5] Holmer et al.,[6] Liew and Aung,[7] Meerlo et al.,[8] and Raven et al.[9]). Even a brief period of sleep deprivation, following learning and processing of new information, can result in cognitive deficits, particularly in the case of hippocampus-dependent memories.[3,10–13] Several misregulated signaling mechanisms have been identified that may contribute to these cognitive impairments.[5,9–11,14–16] Specifically, previous work by a number of different laboratories demonstrated that sleep deprivation leads to molecular as well as cellular perturbations in the hippocampus.[5,17] For instance, a brief period of sleep deprivation decreases the levels of cAMP and PKA, hampers subsequent cAMP/PKA-dependent forms of LTP (e.g., Vecsey et al.[18]), and leads to structural alterations, resulting in an overall net reduction in the number of dendritic spines in the CA1 and dentate gyrus (DG).[19–23] Furthermore, sleep deprivation attenuates mTORC1-dependent protein synthesis in the hippocampus.[24] Altogether, these molecular and cellular alterations impacting synaptic plasticity are suggested to be (at least partly) responsible for the cognitive deficits observed after a brief period of sleep deprivation.

Importantly, a crucial question that has not been addressed is whether amnesia induced by sleep loss after learning reflects a loss of information in the brain or, alternatively, reflects a suboptimal storage of information resulting in a memory trace that is difficult to access. The development of novel approaches to label and activate neural ensembles forming a memory engram has greatly advanced our understanding of the molecular basis of memory.[25–27] This allowed researchers to identify cognitive problems arising from suboptimally stored memories, which can be retrieved by means of optogenetic engram activation. For example, studies in retrograde amnesia models such as Alzheimer's disease and infantile amnesia showed that the memory can still be successfully recalled upon optogenetic stimulation of the memory engram.[26,28,29] Moreover, optogenetic engram

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

stimulation also led to successful memory recall when the memory consolidation process was disrupted by the systemic treatment with a protein synthesis inhibitor.[27] These findings are reshaping the ideas about how brain disorders, as well as societal challenges, affect brain plasticity critical for cognitive processes. In fact, these new insights led us to hypothesize that sleep deprivation during the consolidation of a memory does not lead to a loss of information but instead leads to suboptimal storage of information difficult to retrieve. To test this hypothesis, we applied optogenetic approaches and pharmacological strategies aimed at reactivating spatial memory engrams to recover the information that was stored under sleep deprivation conditions.

RESULTS

Optogenetic reactivation of object-location memories consolidated under sleep deprivation conditions

We examined whether sleep deprivation following learning leads to a loss of information or merely hampers the access/retrieval in adult male mice exposed to an object-location memory (OLM) task. This behavioral paradigm is based on the innate preference for spatial novelty (i.e., the spatial relocation of an object), it relies on the hippocampus,[30] and it is susceptible to sleep deprivation after the training trial.[19,24,31–34] DG engram cells were successfully and specifically labeled when animals were taken off doxycycline (dox)-containing food from 24 h before OLM training until immediately after training (which corresponds with the start of the 6-h sleep deprivation period) (Figures 1A–1C). Labeling did not occur when animals were kept on dox (Figure 1C). We then performed a control experiment to examine (1) whether mice instrumented for optogenetic manipulation would still have the expected preference for relocated objects in the OLM task and (2) whether sleep deprivation would still lead to an impairment of OLM. Mice were maintained on dox throughout the experiment to prevent DG engram labeling. Shortly before the retrieval test, the mice were moved to the empty arena and exposed to laser stimulation (15 ms pulses of 20 Hz [473 nm] for 3 min at the level of the DG) (Figure 1D). The non-sleep-deprived animals indeed showed a strong preference for the relocated object (Figure 1E). In line with our previous work,[19,30–34] mice subjected to 6 h of sleep deprivation directly following training explored both objects to a similar extent, indicating that they failed to detect the spatial novelty (i.e., relocation of one object) (Figures 1E; exploration times, Figures S1A and S1B). It is important to note that the 5-min window provides a brief interval between optogenetic stimulation and the testing trial, as laser stimulation directly during the test session (instead of 5 min preceding the testing session) resulted in a failure to detect spatial novelty regardless of sleep deprivation (Figures S1G and S1H). For this reason, with all subsequent studies, laser stimulation was applied 5 min prior to the test session, which did result in successful detection of spatial novelty (see below).

We subsequently replicated the control experiment, but now took animals off dox 24 h preceding the training to allow for DG engram labeling (Figure 1F). Optogenetic reactivation of this engram directly preceding the retention test successfully prevented the sleep deprivation-induced failure to detect spatial novelty (Figures 1G; for exploration times, Figures S1C and S1D). Next, we investigated whether the context pairing (i.e., training context/empty arena) during optogenetic engram stimulation was essential for the successful detection of spatial novelty after sleep deprivation. Hence, we repeated the experiment but now optogenetically stimulated the memory engram in the home cage instead of the training context (Figure 1H). We found that engram stimulation in the home cage, preceding the testing session, also successfully prevented the sleep deprivation-induced failure to detect spatial novelty (Figures 1I; for exploration times, Figures S1E and S1F). Together, these findings suggest that sleep deprivation during the consolidation window does not lead to a loss of information, but it rather makes the OLM inaccessible without optogenetic stimulation.

Based on these observations after 24 h, we wondered whether engram reactivation several days following training with sleep deprivation would still be able to restore memory retrieval and subsequent detection of spatial novelty in the OLM task. We therefore re-exposed the mice (of Figures 1D–1G) to two additional test sessions with and without laser stimulation at 5 and 8 days following training (Figure 2A). Mice once again successfully detected the relocated object only if the retention test was preceded by laser stimulation (Figures 2B; for exploration times, Figure S2A). We also determined whether the context pairing during optogenetic stimulation was necessary for the successful detection of the spatial novelty, 5 and 8 days after the learning episode and sleep deprivation. The mice tested at 24 h (Figures 1H and 1I) were therefore re-exposed to two additional testing sessions in which the engram was again optogenetically stimulated in the home cage (i.e., 5 and 8 days after the training session) (Figure 2C). In line with the previous findings, we found that optogenetic stimulation of the memory engram in the home cage led to successful detection of the spatial novelty (Figures 2D; for exploration times, Figure S2B). Lastly, to exclude the possibility of carry-over effects due to the used within-subject design, we used a new batch of mice that was exposed to a training trial followed by sleep deprivation and subsequently tested 5 days after training only (Figure 2E). Engram reactivation in the home cage, 5 days after training with sleep deprivation, again led to the successful detection of spatial novelty (Figures 2F; for exploration times, Figures S2C and S2D), confirming our previous results. The latter observation is in line with previous work showing that optogenetic engram reactivation in itself is insufficient to permanently restore the retrievability of memories in the absence of optogenetic activation (e.g., Ramirez et al.,[25] Roy et al.,[26] and Liu et al.[35]).

As a next step, we determined whether the successful optogenetic retrieval was mediated specifically by reactivation of the OLM engram or whether activation of any sparse population of neurons in the DG would lead to the same result. To label a subset of granular cells in the DG unrelated to the OLM task, we subjected a new batch of mice to an alternative context prior to training in the OLM task (Figure 3A). Half of this group was taken off dox during exposure to the alternative context, whereas the other half was taken off dox during OLM training (Figure 3A). Off-dox exposure to the alternative context and OLM training resulted in a similar number of neurons labeled in the DG (Figure 3C). Following training and sleep deprivation, mice were subjected to the retention test 5 and 8 days thereafter with or without laser stimulation (Figure 3A). Optogenetic activation of the neuronal population encoding the OLM resulted in a

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

CellPress
OPEN ACCESS

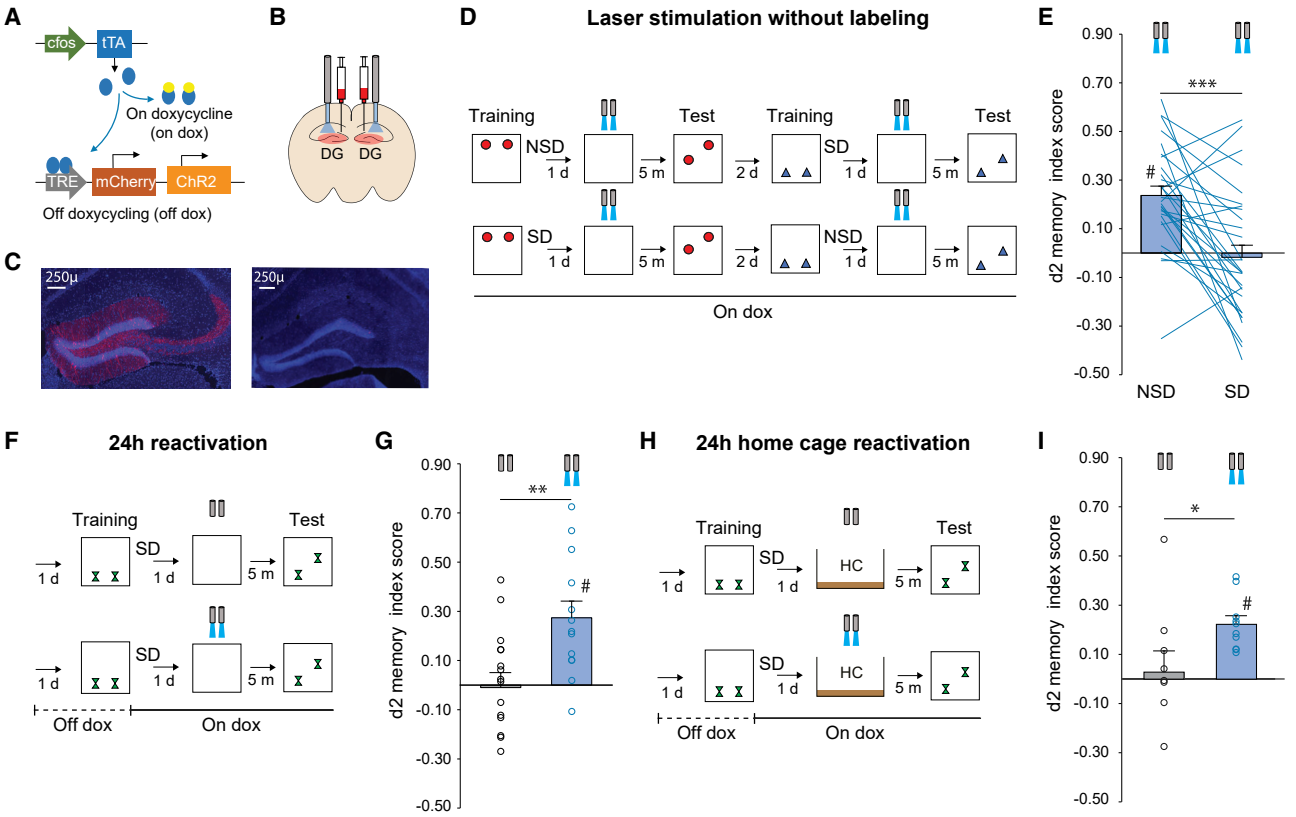

Figure 1. Object-location memories consolidated under sleep deprivation can still be retrieved by optogenetically reactivating the memory engram after 24 h

(A–C) Mice expressing c-*fos*-tTA were virally injected with AAV9-TRE-ChR2-mCherry and implanted with an optical fiber targeting the dentate gyrus (DG). Expression of FOS drives the synthesis of ChR2 and mCherry only in the absence of doxycycline (dox).

(D) Mice were trained in the object-location memory (OLM) task and subjected to 6 h of sleep deprivation (SD) directly following training or left undisturbed (NSD). All mice underwent laser treatment in the training context/empty arena, 5 min preceding the retention test. The experiment was replicated 2 days later with new objects and new object locations in a cross-design fashion. SD, sleep deprivation group; NSD, non-sleep-deprived controls.

(E) Mice subjected to sleep deprivation failed to detect spatial novelty (i.e., showed no preference for the relocated object) 24 h after training, regardless of laser stimulation (both groups, n = 29; paired-sample t test, $t_{28} = 4.545$; $p < 0.001$).

(F) Mice were taken off dox 1 day before OLM training to enable engram labeling, immediately put back on dox thereafter, subjected to 6 h of sleep deprivation, and tested 24 h after training.

(G) Optogenetic engram reactivation in the empty arena 24 h after the training with sleep deprivation resulted in the successful detection of spatial novelty (laser off, n = 16; laser on, n = 13; independent-sample t test, $t_{27} = -3.096$; $p = 0.005$).

(H) Similar procedure as in (F), but optogenetic reactivation of the engram was conducted in the home cage (HC) instead of the empty arena.

(I) Engram reactivation in the home cage instead of in the empty arena yielded similar results, i.e., the successful detection of spatial novelty (laser off, n = 9; laser on, n = 10; independent-sample t test, $t_{17} = -2.156$; $p = 0.046$).

All data are mean ± SEM. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$; # indicates significantly different from zero (i.e., successful detection of spatial novelty).

See also Figure S1 for more information.

successful detection of spatial novelty (Figures 3B; for exploration times, Figures S3A and S3B). By contrast, activation of the neural ensemble encoding the alternative context resulted in a failure to discriminate between the relocated and non-relocated object. These data indicate that activation of the neural ensemble encoding the OLM, rather than a non-specific subpopulation of DG cells, is required to overcome the sleep deprivation-induced failure to detect spatial novelty. Altogether, our findings suggest that the behavioral deficit, as a result of sleep deprivation during the consolidation window, is not caused by a loss of object-location information but is rather due to the inaccessibility of this information in the absence of optogenetic engram stimulation.

Treatment with a PDE4 inhibitor is sufficient to overcome the inability to retrieve the memory consolidated under sleep deprivation conditions

To increase the translational potential of our findings, we turned toward a non-invasive approach to overcome the inability to retrieve suboptimally stored memories as a consequence of sleep deprivation. Previous work indicated that boosting cAMP signaling either systemically or locally in hippocampal excitatory neurons during sleep deprivation was sufficient to make memory processes resilient to the negative impact of sleep loss.[18,31,36] Therefore, in the current study, we determined whether boosting cAMP during the retention test, instead of during the sleep deprivation procedure as we did in our earlier studies,[31,36] would lead to

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

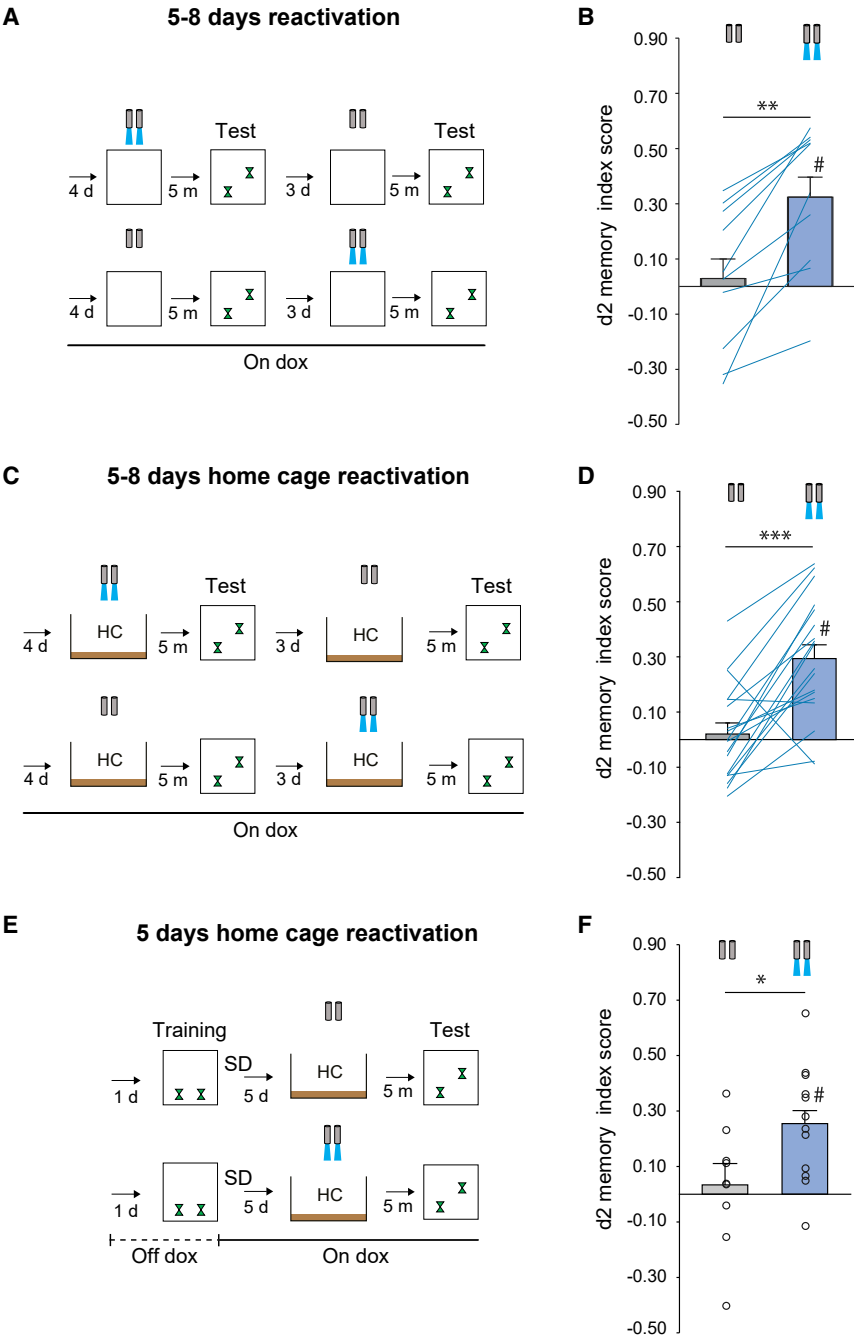

Figure 2. Object-location memories consolidated under sleep deprivation can still be retrieved by optogenetically reactivating the memory engram after 5 and 8 days

(A) After the 24-h test session (Figures 1F and 1G), mice received a second and third retention test at 5 and 8 days, respectively, after training with sleep deprivation. Of note, during the consecutive test trials at 5 and 8 days, the objects remained at the same locations as during the test trial given at 24 h (Figures 1F–1I).

(B) Engram reactivation in the empty arena 5 and 8 days after training with sleep deprivation led to the successful detection of spatial novelty. n = 10; repeated-measures ANOVA using "laser" (on versus off) as within-subject factor and "order" (day 5 versus day 8) as between-subject factor. No significant "laser*order" effect was found ($F_{1,8}$ = 0.779; NS). Subsequent analysis showed a main effect for laser ($F_{1,8}$ = 25.250; p = 0.001).

(C) Next, we repeated the experiment, but this time optogenetic reactivation of the engram was conducted in the home cage (HC) rather than the empty test arena.

(D) Optogenetic engram reactivation in the home cage instead of the empty arena 5 and 8 days after training with sleep deprivation also led to the successful detection of spatial novelty. n = 18; repeated-measures ANOVA using laser (on versus off) as within-subject factor and order (day 5 versus day 8) as between-subject factor. No significant laser*order effect was found ($F_{1,16}$ = 0.829; NS). Subsequent analysis showed a main effect for laser ($F_{1,16}$ = 29.966; p = 0.000051).

(E) To exclude the possibility of carry-over effects due to the within-subject design being responsible for these outcomes, we used optogenetic reactivation of OLM engram in the home cage after 5 days, without a prior testing session at 24 h.

(F) Similar to previous experiments, engram reactivation in the home cage 5 days after training with sleep deprivation (SD) led to the successful detection of spatial novelty (laser off, n = 9; laser on, n = 12; independent-sample t test, t_{19} = 2.331; p = 0.031).

All data are mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001; # indicates significantly different from zero (i.e., successful detection of spatial novelty). See also Figure S2 for more information.

proper retrieval of OLM. To increase cAMP levels, we applied the clinically approved phosphodiesterase 4 (PDE4) inhibitor roflumilast.[36–39] To this end, 24 h after OLM training and sleep deprivation, mice were injected intraperitoneally (i.p.) with roflumilast (0.03 mg.kg^{-1}) or vehicle solution 30 min preceding the test (Figure 4A). Roflumilast treatment resulted in a proper detection of spatial novelty, not different from that of non-sleep-deprived mice (Figures 4B; for exploration times, Figures S4A and S4B). Subsequently, we showed in a new batch of mice that we could successfully overcome the sleep deprivation-induced retrieval deficit with roflumilast even 5 days after training (Figures 4C and 4D; for exploration times, Figures S4C and S4D). The roflumilast treatment also resulted in a proper detection of spatial novelty 24 h and 5 days after the training trial followed by sleep deprivation when mice were not exposed to the training context 5 min preceding the testing session (Figures 4E–4H; for exploration times, Figures S4E–S4H). Together, these data show that the clinically approved drug roflumilast can successfully be used to retrieve information previously stored under sleep deprivation conditions.

Restoring the access of memories consolidated under sleep deprivation conditions

Optogenetic activation of hippocampal DG memory engrams only provides temporary access to information stored under

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

CellPress
OPEN ACCESS

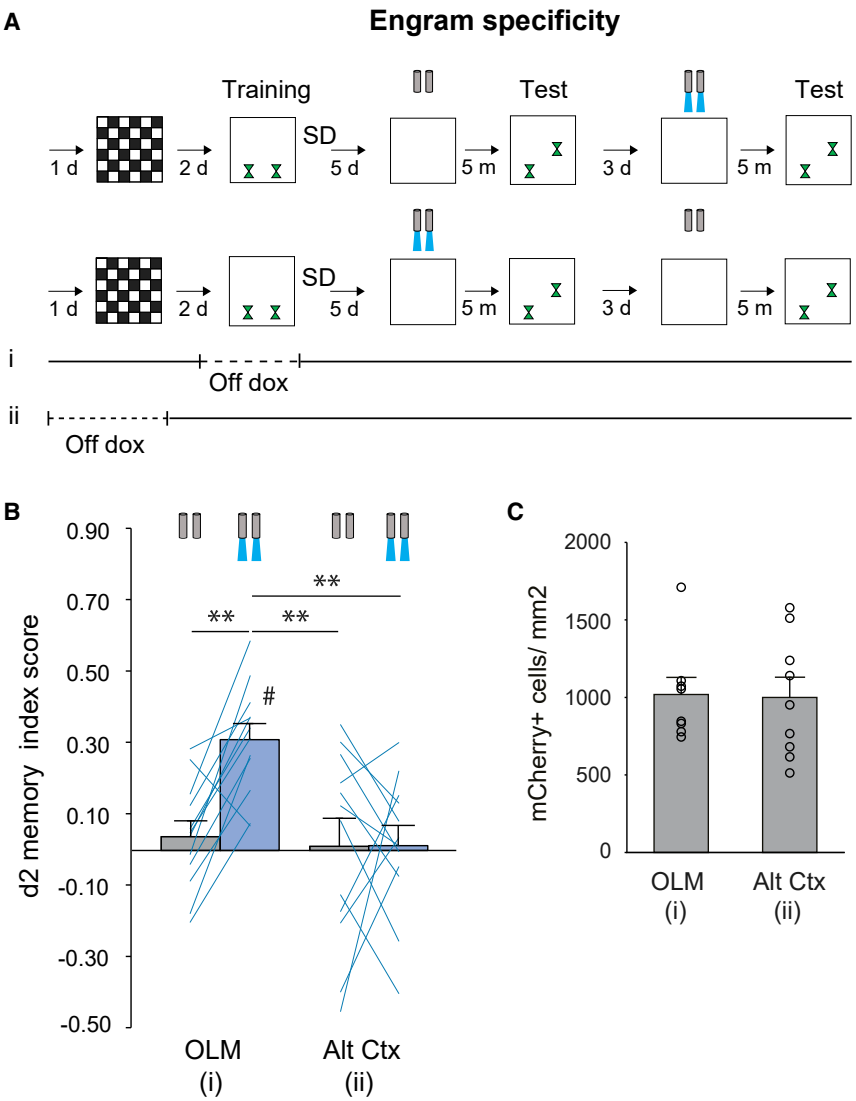

Figure 3. Reactivation of the OLM engram, but not the alternative context engram, resulted in the successful detection of spatial novelty

(A) To induce engram labeling, animals were taken of dox either during training in the OLM task or exposure to the alternative context (Alt Ctx). Engram reactivation was elicited 5 and 8 days thereafter.

(B) Reactivation of the OLM engram, but not Alt Ctx engram, resulted in the successful detection of spatial novelty. All groups, n = 12; repeated-measures ANOVA using laser (on versus off) as within-subject factor and context (OLM versus Alt Ctx) and order (laser "on-then-off" versus "off-then-on") as between-subject factors. No three-way interaction ($F_{1,20}$ = 0.256; p = 0.618) or a laser*order interaction ($F_{1,20}$ = 0.000; p = 0.992) was found, indicating that the order of laser stimulation had no effect. A significant "laser*context" interaction was detected ($F_{1,20}$ = 5.733; p = 0.027). Pairwise comparisons using Dunnett's test with "OLM laser ON" as a reference against (1) OLM laser OFF, p = 0.005; (2) Alt Ctx laser ON, p = 0.002; and (3) Alt Ctx laser off, p = 0.002.

(C) Quantification of the ChR2-mCherry-positive cells in the dentate gyrus neuronal ensemble of the object-location engram and alternative context engram (3–4 hippocampal slices per animal) showed a similar number of tagged cells (OLM, n = 8; Alt Ctx, n = 9; independent-sample t test, t_{15} = 0.108; p = 0.915).

All data are mean ± SEM. **p < 0.01; # indicates significantly different from zero (i.e., successful detection of spatial novelty).

See also Figure S3 for more information.

suboptimal conditions and by itself does not lead to lasting memory restoration (e.g., Roy et al.,[26] Ryan et al.,[27] and Pignatelli et al.[40]). For example, the day after the optogenetic stimulation, mice failed to express the proper behavioral response (e.g., a freezing response in the training context in the case of fear memories), suggesting that they cannot access/retrieve information stored under suboptimal conditions despite the optogenetic stimulation the day before. Transient short-term memories in rodents can be converted into more robust memories through systemic treatment with PDE4 inhibitors 3–5.5 h following training[37,41,42] (i.e., during the consolidation window). We therefore combined optogenetic stimulation of the OLM engram formed under sleep deprivation conditions with application of the PDE4 inhibitor roflumilast to test if we could more permanently restore access to the OLMs, resulting in successful natural retrieval even days later (i.e., without the need for further optogenetic or pharmacological stimulation). Specifically, we trained a new batch of mice in the OLM task (and labeled DG neurons activated by learning as described; Figures 1A–1C) and subjected them to sleep deprivation for 6 h (Figure 5A). Also, 3 days later, animals were re-exposed to the empty arena for 3 min and subjected to (1) laser stimulation to activate the engram, (2) roflumilast treatment (0.03 mg.kg^{-1} i.p.; 3 h following arena exposure), or (3) a combination of laser stimulation and roflumilast treatment (3 h after optogenetic engram reactivation). The 3-h time point was specifically chosen as previous work indicated that boosting cAMP signaling (by means of PDE4 inhibition) facilitates memory consolidation when administered 3–5.5 h after learning.[42] In our studies, the "learning episode" is replaced by optogenetic reactivation of the engram, which is then "reconsolidated" in the presence of boosted cAMP signaling. Mice subjected to engram activation or drug treatment alone failed to detect the spatial novelty during the retrieval test 2 days later, i.e., 5 days after the original training (Figures 5A and 5B; for exploration times, Figures S5A and S5B). However, when optogenetic engram reactivation was combined with roflumilast treatment, animals were able to successfully detect the spatial novelty 2 days after treatment (Figures 5B; for exploration times, Figures S5A and S5B). We also examined whether the pairing of the memory context (i.e., empty arena) was necessary in order to successfully detect the spatial novelty 2 days after the combined engram stimulation and roflumilast treatment. Hence, we repeated the experiment but

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

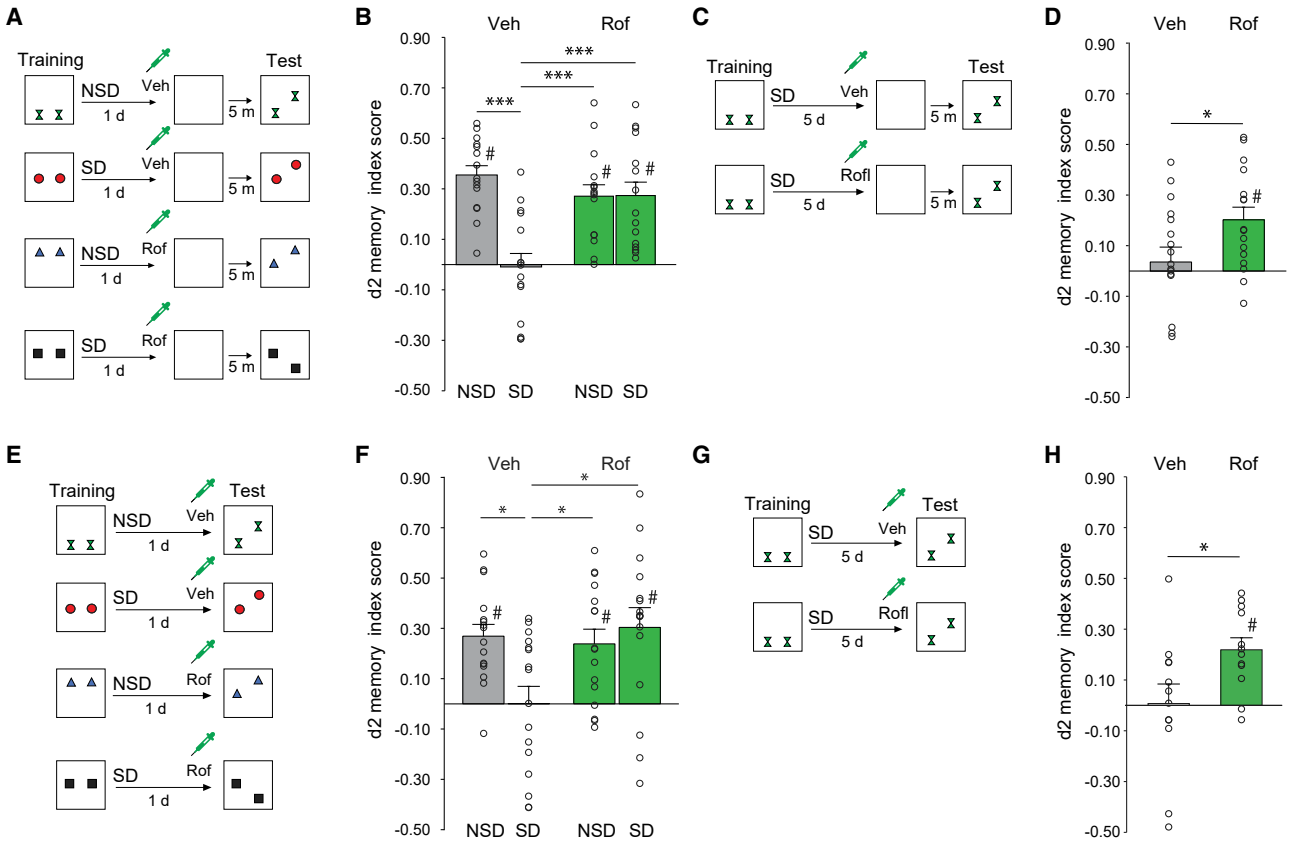

Figure 4. Treatment with roflumilast preceding the testing trial rescues the memory deficits caused by sleep deprivation following training

(A) Following training in the OLM task, wild-type C57BL/6 mice were subjected to 6 h of sleep deprivation (SD) or left undisturbed (NSD). Animals received an intraperitoneal (i.p.) injection with the PDE4 inhibitor roflumilast (rof; 0.03 mg.kg^{-1}) or vehicle solution 30 min before being exposed to the empty arena for 3 min, followed by 5 min home cage, and subsequently the retention test 24 h after training.

(B) Roflumilast treatment preceding the retention test prevents memory retrieval deficits caused by sleep deprivation (SD) following training. All four groups, n = 15, repeated-measures ANOVA of one factor containing four levels ($F_{3,42}$ = 10.364; p < 0.001), followed by pairwise comparisons using Dunnett's post hoc test using "vehicle SD" group as a reference against the other three groups, vehicle SD versus Veh NSD (p < 0.001) versus rof NSD (p < 0.001) versus rof SD (p < 0.001).

(C) Following training in the OLM task, C57BL/6 mice were subjected to 6 h of sleep deprivation (SD). Subsequently, 5 days later, animals received an i.p. injection with roflumilast (rof; 0.03 mg.kg^{-1}) or vehicle solution 30 min before being exposed to the empty arena for 3 min, followed by 5 min home cage.

(D) Roflumilast treatment in combination with exposure to the empty arena preceding the retention test resulted in the proper detection of spatial novelty, 5 days after the training and sleep deprivation (both groups, n = 16; independent-sample t test, t_{30} = 2.206; p = 0.035).

(E) Following training in the OLM task, wild-type C57BL/6 mice were subjected to 6 h of sleep deprivation (SD) or left undisturbed (NSD). Animals received an intraperitoneal (i.p.) injection with the PDE inhibitor roflumilast (rof; 0.03 mg.kg^{-9}) or vehicle solution 30 min before the retention test (24 h after training), without exposure to the empty arena.

(F) Roflumilast treatment preceding the retention test prevents memory retrieval deficits caused by sleep deprivation following training. All four groups, n = 16, repeated-measures ANOVA of one factor containing four levels ($F_{3,45}$ = 3.928; p = 0.014), followed by pairwise comparisons using Dunnett's post hoc test using vehicle SD group as a reference against which the other three groups are compared, vehicle SD versus Veh NSD (p = 0.012) versus rof NSD (p = 0.03) versus rof SD (p = 0.004).

(G) Wild-type C57BL/6 mice were subjected to 6 h of sleep deprivation following training in the OLM task. Animals received an i.p. injection with roflumilast (rof; 0.03 mg.kg^{-1}) or vehicle solution 30 min prior to the retention test, 5 days after training. Important to note is that the animals were not exposed to the empty arena 5 min prior to the testing trial.

(H) Roflumilast treatment preceding the retention test without exposure to the empty arena resulted in a proper detection of spatial novelty, 5 days after the training followed by sleep deprivation (both groups, n = 12; independent-sample t test, t_{22} = 2.347; p = 0.028).

All data are mean ± SEM. *p < 0.05, and ***p < 0.001; # indicates significantly different from zero (i.e., successful detection of spatial novelty).

See also Figure S4 for more information.

optogenetically stimulated the engram cells in the home cage and delivered roflumilast 3 h later (Figure 5C). We found that even without contextual exposure the mice subjected to the combination of optogenetic engram reactivation and roflumilast treatment were able to detect the spatial novelty 2 days later (i.e., 5 days after the initial training trial followed by sleep deprivation) (Figures 5D; for exploration times, Figures S5C and S5D). Thus, both optogenetic engram stimulation or drug treatment, when induced immediately before the retention trial, can provide a short-lasting ability to recall the information stored during sleep

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

CellPress
OPEN ACCESS

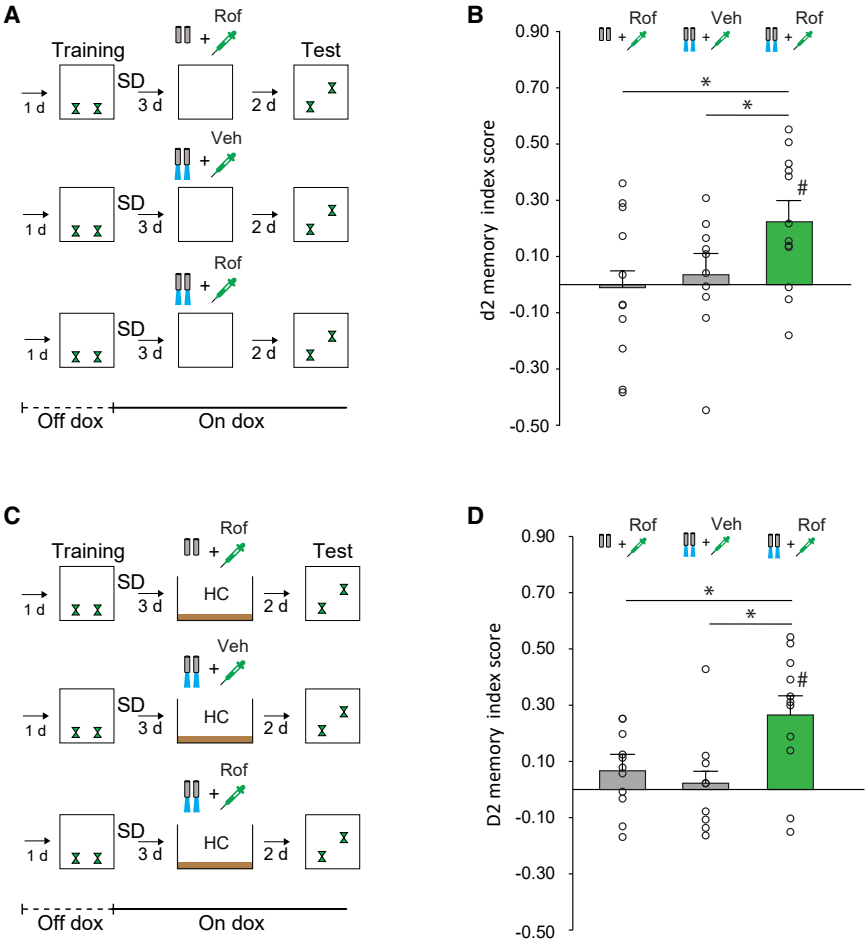

Figure 5. Reversing the access of object-location memories consolidated under sleep deprivation conditions

(A) Mice expressing c-*fos*-tTA were virally injected with AAV9-TRE-ChR2-mCherry and implanted with an optical fiber targeting the dentate gyrus. Mice were taken off dox 1 day before OLM training to enable engram labeling and immediately put on dox after training and subjected to 6 h of sleep deprivation (SD). 3 days thereafter, mice were subjected to (1) optogenetic engram reactivation, (2) treatment with roflumilast (rof; 3 h after optogenetic engram reactivation), or (3) optogenetic engram reactivation in combination with roflumilast treatment. Mice were subjected to the retention test 2 days later (corresponding to 5 days after the training with sleep deprivation).

(B) Combining engram reactivation with roflumilast treatment resulted in the successful detection of spatial novelty, indicative of restored access to object-location memories consolidated under sleep deprivation conditions. Mice subjected to either drug treatment or engram reactivation failed to detect the spatial novelty (laser off + rof, n = 11; laser on + veh, n = 10; laser on + rof, n = 12). One-way ANOVA, $F_{2,30} = 4.748$; p = 0.016; Dunnett's post hoc, laser on + rof versus laser off + rof (p = 0.017), and laser on + veh (p = 0.032).

(C) Similar procedure as in (A), with the exception that the optogenetic engram reactivation, treatment with roflumilast (rof; 3 h after optogenetic engram reactivation), or the combination of both treatments was conducted in the home cage instead of the empty arena.

(D) Combining engram reactivation with roflumilast treatment without exposure to the empty arena resulted in the successful detection of spatial novelty, indicative of restored access to object-location memories consolidated under sleep deprivation conditions. Mice subjected to either drug treatment or engram reactivation only failed to detect the spatial novelty (laser off + rof, n = 11; laser on + veh, n = 9; laser on + rof, n = 11). One-way ANOVA, $F_{2,28} = 4.893$; p = 0.015; Dunnett's post hoc, laser on + rof versus laser off + rof (p = 0.037), and laser on + veh (p = 0.015).

All data are mean ± SEM. *p < 0.05; # indicates significantly different from zero (i.e., successful detection of spatial novelty).

See also Figure S5 for more information.

deprivation (i.e., Figures 1, 2, 3, and 4), whereas only the combination of the two treatments results in a more permanent restoration of access to OLMs days after treatment, without the need for any sort of stimulation or manipulation at the time of retrieval. Importantly, the restoration of OLMs by combining engram reactivation with roflumilast treatment was successful when applied either in the original training context or in the home cage (i.e., Figure 5).

DISCUSSION

In conclusion, our study reveals that deficits in hippocampal OLMs, consolidated under sleep deprivation conditions, are not caused by a loss of information but rather lead to a memory that is inaccessible without optogenetic stimulation or drug treatment. Specifically, we show that OLMs, consolidated under sleep deprivation conditions, can be successfully retrieved by optogenetic stimulation of the OLM engram in the DG or by treatment with the PDE4 inhibitor roflumilast directly preceding the retention test. Intriguingly, these memories could be retrieved with optogenetic stimulation or roflumilast treatment even 5–8 days after the learning event and sleep deprivation episode occurred. Most importantly, while either one of these treatments alone only resulted in a temporary restoration of the OLM, the combination of optogenetic engram stimulation and roflumilast treatment resulted in a longer-lasting restoration of memory and successful retrieval several days thereafter, without the need for subsequent stimulation at the time of retrieval. Moreover, our studies indicate that the optogenetic and pharmacological manipulations used leading to the successful retrieval of memories previously consolidated under sleep deprivation conditions do not require exposure to the original training context to elicit their effects.

While previous experiments have successfully retrieved memories under drug-based and pathologically related conditions,[26,27] our study provides a proof of principle that memories can be successfully retrieved and restored from sleep deprivation-induced amnesia. Indeed, burgeoning literature suggests

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

that memories thought to be "lost" still exist in an inaccessible state and that they can be artificially retrieved and behaviorally expressed.[28,29,43] One possibility is that the amnestic state renders a memory irretrievable without artificial activation, but in a manner that exogenous perturbations (e.g., optogenetics and drug treatment) can bypass, thereby providing evidence of successful memory retrieval. Interestingly, during memory consolidation, engram cell-specific synaptic plasticity processes are taking place that are critical for the natural retrieval of a memory (i.e., retrieval in the absence of optogenetic stimulation or pharmacological treatment).[26,27,44] In particular, engram cells appear to undergo synaptic consolidation involving the facilitation of functional connections between engram cells, evidenced by higher postsynaptic current amplitude and spontaneous excitatory postsynaptic current.[27] In addition, dendritic spines of engram cells tend to increase in number and size after memory formation, indicative of enhanced functional connectivity between engram cells.[45,46] Therefore, a possible mechanism of how sleep deprivation during the consolidation phase leads to an amnesic state of the memory is by misregulation of engram-specific hippocampal structural plasticity. Indeed, sleep deprivation severely impacts synaptic plasticity processes by decreasing synaptic connections in the DG and area CA1 of the hippocampus.[19–21,47] In addition, previous work by Ryan et al.[27] revealed that alterations in synaptic plasticity under conditions of anisomycin-induced amnesia result in less activation of the initial engram (i.e., established during memory acquisition) during the subsequent testing trial. Such a decrease in engram reactivation is considered to underlie the memory deficits. Hence, we predict that sleep deprivation-induced amnesia is also associated with hampered reactivation of the initial training engram. Overall, we propose that sleep deprivation may lead to the suboptimal storage of memories via interference with functional as well as structural engram-specific consolidation processes, indispensable for the natural retrieval of the memory.

Previous studies have also provided insights into the mechanisms through which PDE4 inhibition can improve memory function by studying the spatiotemporal dynamics of cyclic nucleotide signaling (i.e., cAMP) during memory acquisition and consolidation processes.[42,48] Microinfusions of drugs into a specific brain region shortly before the retrieval session can help to elucidate the molecular requirements for memory retrieval. For instance, Szapiro et al. showed that successful memory retrieval during an inhibitory avoidance and/or contextual fear-conditioning paradigm involves the activation of PKA, MAPKs, CAMKII, CREB, glutamate AMPA, NMDA, and metabotropic (mGluR) receptors in the hippocampus.[49] All of these effectors are direct or via crosstalk downstream targets of cAMP.[50,51] With respect to our findings, we hypothesize that roflumilast, known for increasing cAMP levels and activation of these downstream targets, may modulate the excitability of memory engram cells in the hippocampus. This increased baseline excitability level may compensate for the negative effect of sleep loss during memory consolidation. Our findings thus suggest that cAMP-mediated facilitation of the retrieval process is sufficient to rescue the suboptimally stored memory caused by sleep loss. Therefore, roflumilast administration may potentially also be applicable to other deficit models that are known to involve a deficit in the retrieval of a particular memory (e.g., Alzheimer's disease and infantile amnesia).[26,28]

It is important to note that while previous studies successfully reactivated engram cells sufficient to produce fear-related freezing responses,[52] our study probes spatially modulated memories (e.g., novel object locations). It appears that the reactivation of OLMs requires a different experimental approach to obtain the most robust performance in the task. Specifically, we were only able to successfully rescue the spatial memory when the suboptimally stored memory trace was optogenetically reactivated prior (i.e., 5 min) to the testing trial. It will be highly interesting to determine whether optogenetic activation of engrams 5 min prior to the testing trial also produces the proper behavioral response in other non-fear-related tasks. We also demonstrated that the OLMs, consolidated under sleep deprivation conditions, can be optogenetically reactivated either in the original empty arena (i.e., the training context without the objects) or the home cage. These findings are in line with previous fear studies showing that memories can be successfully retrieved, irrespective of the context.[26,27] In addition, from a translational point of view, these findings suggest that spatial memories may be modulated (e.g., rescued or strengthened), irrespective of the environment in which the modulation takes place.

Furthermore, reversing the inaccessibility of the memory engram, and thereby facilitating the retrieval of the memory, has only previously been established via high-frequency optical stimulation of engram cells to artificially induce LTP.[26,44] Therefore, the current study provides a proof of principle for a novel approach via which the inaccessibility of memories can be reversed, even several days after the initial learning episode. By stimulating/targeting the consolidation process of an optogenetically reactivated memory with roflumilast (i.e., roflumilast treatment 3 h after optogenetic engram activation to increase cAMP signaling during its critical consolidation window, Figure 5), the memory trace might be strengthened in such a way that it allows manipulation-free recall to occur several days later. In addition, such memory strengthening may occur irrespective of the context, as context pairing seems not to be necessary for successful strengthening of the memory. Therefore, we believe that this approach may open up intriguing lines of research aiming to perturb such complex mnemonic processing, with a potential therapeutic role for the PDE4 inhibitor roflumilast.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Subjects
- METHOD DETAILS
  - Virus construct and packaging
  - Viral injection and fiber optic implants surgery
  - Optogenetic stimulation protocol
  - Immunohistochemistry

- Cell counting
- Sleep deprivation
- Drug preparation
- Behavioral studies
- Extended description of all behavioral training and testing protocols

● QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.12.006.

ACKNOWLEDGMENTS

We would like to thank members of the Havekes lab for help with the sleep deprivation studies. This work was supported by the Human Frontiers Science Program Organization (HFSP) (grant RGY0063/2017 to R.H.), the Netherlands Organisation for Scientific Research (grant NWA.1228.191.208 to R.H. and S.R.), the US Airforce Office of Scientific Research (AFOSR, grant number FA9550-21-1-0310 to S.R. and R.H.), the Ubbo Emmius foundation (to R.H.), and the FSE Fellowship in Biomedical Sciences (to P.R.A.H. and R.H.).

AUTHOR CONTRIBUTIONS

R.H. conceived the study. Y.G.B., P.R.A.H., M.J.H.K., P.M., S.R., and R.H. designed the experiments. Y.G.B., P.R.A.H., C.P., and S.W. conducted the experiments. Y.G.B. and P.R.A.H. analyzed the data. F.R. and E.L.M. assisted with data collection. Y.G.B., P.R.A.H., M.J.H.K., P.M., S.R., and R.H. interpreted the results. R.H., Y.G.B., and P.R.A.H. wrote the manuscript with input from M.J.H.K., P.M., S.R., and R.H. M.J.H.K. and P.M. supervised the work.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: January 18, 2022
Revised: October 19, 2022
Accepted: December 2, 2022
Published: December 27, 2022

REFERENCES

1. Chattu, V.K., Manzar, M.D., Kumary, S., Burman, D., Spence, D.W., and Pandi-Perumal, S.R. (2018). The global problem of insufficient sleep and its serious public health implications. Healthcare (Basel) *7*, 1. https://doi.org/10.3390/healthcare7010001.
2. Rajaratnam, S.M., and Arendt, J. (2001). Health in a 24-h society. Lancet *358*, 999–1005. https://doi.org/10.1016/S0140-6736(01)06108-6.
3. Abel, T., Havekes, R., Saletin, J.M., and Walker, M.P. (2013). Sleep, plasticity and memory from molecules to whole-brain networks. Curr. Biol. *23*, R774–R788. https://doi.org/10.1016/j.cub.2013.07.025.
4. Areal, C.C., Warby, S.C., and Mongrain, V. (2017). Sleep loss and structural plasticity. Curr. Opin. Neurobiol. *44*, 1–7. https://doi.org/10.1016/j.conb.2016.12.010.
5. Havekes, R., and Aton, S.J. (2020). Impacts of sleep loss versus waking experience on brain plasticity: parallel or orthogonal? Trends Neurosci. *43*, 385–393. https://doi.org/10.1016/j.tins.2020.03.010.
6. Holmer, B.J., Lapierre, S.S., Jake-Schoffman, D.E., and Christou, D.D. (2021). Effects of sleep deprivation on endothelial function in adult humans: a systematic review. GeroScience *43*, 137–158. https://doi.org/10.1007/s11357-020-00312-y.
7. Liew, S.C., and Aung, T. (2021). Sleep deprivation and its association with diseases- a review. Sleep Med. *77*, 192–204. https://doi.org/10.1016/j.sleep.2020.07.048.
8. Meerlo, P., Havekes, R., and Steiger, A. (2015). Chronically restricted or disrupted sleep as a causal factor in the development of depression. Curr. Top. Behav. Neurosci. *25*, 459–481. https://doi.org/10.1007/7854_2015_367.
9. Raven, F., Van der Zee, E.A., Meerlo, P., and Havekes, R. (2018). The role of sleep in regulating structural plasticity and synaptic strength: implications for memory and cognitive function. Sleep Med. Rev. *39*, 3–11. https://doi.org/10.1016/j.smrv.2017.05.002.
10. Havekes, R., Meerlo, P., and Abel, T. (2015). Animal studies on the role of sleep in memory: from behavioral performance to molecular mechanisms. Curr. Top. Behav. Neurosci. *25*, 183–206. https://doi.org/10.1007/7854_2015_369.
11. Klinzing, J.G., Niethard, N., and Born, J. (2019). Mechanisms of systems memory consolidation during sleep. Nat. Neurosci. *22*, 1598–1610. https://doi.org/10.1038/s41593-019-0467-3.
12. Krause, A.J., Simon, E.B., Mander, B.A., Greer, S.M., Saletin, J.M., Goldstein-Piekarski, A.N., and Walker, M.P. (2017). The sleep-deprived human brain. Nat. Rev. Neurosci. *18*, 404–418. https://doi.org/10.1038/nrn.2017.55.
13. Sawangjit, A., Oyanedel, C.N., Niethard, N., Salazar, C., Born, J., and Inostroza, M. (2018). The hippocampus is crucial for forming non-hippocampal long-term memory during sleep. Nature *564*, 109–113. https://doi.org/10.1038/s41586-018-0716-8.
14. Havekes, R., and Abel, T. (2017). The tired hippocampus: the molecular impact of sleep deprivation on hippocampal function. Curr. Opin. Neurobiol. *44*, 13–19. https://doi.org/10.1016/j.conb.2017.02.005.
15. Kreutzmann, J.C., Havekes, R., Abel, T., and Meerlo, P. (2015). Sleep deprivation and hippocampal vulnerability: changes in neuronal plasticity, neurogenesis and cognitive function. Neuroscience *309*, 173–190. https://doi.org/10.1016/j.neuroscience.2015.04.053.
16. Havekes, R., Vecsey, C.G., and Abel, T. (2012). The impact of sleep deprivation on neuronal and glial signaling pathways important for memory and synaptic plasticity. Cell. Signal. *24*, 1251–1260. https://doi.org/10.1016/j.cellsig.2012.02.010.
17. Weiss, J.T., and Donlea, J.M. (2021). Roles for sleep in neural and behavioral plasticity: reviewing variation in the consequences of sleep loss. Front. Behav. Neurosci. *15*, 777799. https://doi.org/10.3389/fnbeh.2021.777799.
18. Vecsey, C.G., Baillie, G.S., Jaganath, D., Havekes, R., Daniels, A., Wimmer, M., Huang, T., Brown, K.M., Li, X.Y., Descalzi, G., et al. (2009). Sleep deprivation impairs cAMP signalling in the hippocampus. Nature *461*, 1122–1125. https://doi.org/10.1038/nature08488.
19. Havekes, R., Park, A.J., Tudor, J.C., Luczak, V.G., Hansen, R.T., Ferri, S.L., Bruinenberg, V.M., Poplawski, S.G., Day, J.P., Aton, S.J., et al. (2016). Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area CA1. eLife *5*, e13424. https://doi.org/10.7554/eLife.13424.
20. Raven, F., Meerlo, P., Van der Zee, E.A., Abel, T., and Havekes, R. (2019). A brief period of sleep deprivation causes spine loss in the dentate gyrus of mice. Neurobiol. Learn. Mem. *160*, 83–90. https://doi.org/10.1016/j.nlm.2018.03.018.
21. Bolsius, Y.G., Meerlo, P., Kas, M.J., Abel, T., and Havekes, R. (2022). Sleep deprivation reduces the density of individual spine subtypes in a branch-specific fashion in CA1 neurons. J. Sleep Res. *31*, e13438. https://doi.org/10.1111/jsr.13438.
22. Wong, L.W., Tann, J.Y., Ibanez, C.F., and Sajikumar, S. (2019). The p75 neurotrophin receptor is an essential mediator of impairments in hippocampal-dependent associative plasticity and memory induced by sleep deprivation. J. Neurosci. *39*, 5452–5465. https://doi.org/10.1523/JNEUROSCI.2876-18.2019.
23. Yang, G., Lai, C.S., Cichon, J., Ma, L., Li, W., and Gan, W.B. (2014). Sleep promotes branch-specific formation of dendritic spines after learning. Science *344*, 1173–1178. https://doi.org/10.1126/science.1249098.

24. Tudor, J.C., Davis, E.J., Peixoto, L., Wimmer, M.E., van Tilborg, E., Park, A.J., Poplawski, S.G., Chung, C.W., Havekes, R., Huang, J., et al. (2016). Sleep deprivation impairs memory by attenuating mTORC1-dependent protein synthesis. Sci. Signal. *9*, ra41. https://doi.org/10.1126/scisignal.aad4949.

25. Ramirez, S., Liu, X., Lin, P.A., Suh, J., Pignatelli, M., Redondo, R.L., Ryan, T.J., and Tonegawa, S. (2013). Creating a false memory in the hippocampus. Science *341*, 387–391. https://doi.org/10.1126/science.1239073.

26. Roy, D.S., Arons, A., Mitchell, T.I., Pignatelli, M., Ryan, T.J., and Tonegawa, S. (2016). Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. Nature *531*, 508–512. https://doi.org/10.1038/nature17172.

27. Ryan, T.J., Roy, D.S., Pignatelli, M., Arons, A., and Tonegawa, S. (2015). Memory. Engram cells retain memory under retrograde amnesia. Science *348*, 1007–1013. https://doi.org/10.1126/science.aaa5542.

28. Guskjolen, A., Kenney, J.W., de la Parra, J., Yeung, B.A., Josselyn, S.A., and Frankland, P.W. (2018). Recovery of "lost" infant memories in mice. Curr. Biol. *28*, 2283–2290.e3. https://doi.org/10.1016/j.cub.2018.05.059.

29. Ryan, T.J., and Frankland, P.W. (2022). Forgetting as a form of adaptive engram cell plasticity. Nat. Rev. Neurosci. *23*, 173–186. https://doi.org/10.1038/s41583-021-00548-3.

30. Oliveira, A.M., Hawk, J.D., Abel, T., and Havekes, R. (2010). Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. Learn. Mem. *17*, 155–160. https://doi.org/10.1101/lm.1625310.

31. Havekes, R., Bruinenberg, V.M., Tudor, J.C., Ferri, S.L., Baumann, A., Meerlo, P., and Abel, T. (2014). Transiently increasing cAMP levels selectively in hippocampal excitatory neurons during sleep deprivation prevents memory deficits caused by sleep loss. J. Neurosci. *34*, 15715–15721. https://doi.org/10.1523/JNEUROSCI.2403-14.2014.

32. Heckman, P.R.A., Roig Kuhn, F., Meerlo, P., and Havekes, R. (2020). A brief period of sleep deprivation negatively impacts the acquisition, consolidation, and retrieval of object-location memories. Neurobiol. Learn. Mem. *175*, 107326. https://doi.org/10.1016/j.nlm.2020.107326.

33. Inostroza, M., Binder, S., and Born, J. (2013). Sleep-dependency of episodic-like memory consolidation in rats. Behav. Brain Res. *237*, 15–22. https://doi.org/10.1016/j.bbr.2012.09.011.

34. Raven, F., Heckman, P.R.A., Havekes, R., and Meerlo, P. (2020). Sleep deprivation-induced impairment of memory consolidation is not mediated by glucocorticoid stress hormones. J. Sleep Res. *29*, e12972. https://doi.org/10.1111/jsr.12972.

35. Liu, X., Ramirez, S., Pang, P.T., Puryear, C.B., Govindarajan, A., Deisseroth, K., and Tonegawa, S. (2012). Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature *484*, 381–385. https://doi.org/10.1038/nature11028.

36. Heckman, P.R.A., Roig Kuhn, F., Raven, F., Bolsius, Y.G., Prickaerts, J., Meerlo, P., and Havekes, R. (2020). Phosphodiesterase inhibitors Roflumilast and vardenafil prevent sleep deprivation-induced deficits in spatial pattern separation. Synapse *74*, e22150. https://doi.org/10.1002/syn.22150.

37. Vanmierlo, T., Creemers, P., Akkerman, S., van Duinen, M., Sambeth, A., De Vry, J., Uz, T., Blokland, A., and Prickaerts, J. (2016). The PDE4 inhibitor Roflumilast improves memory in rodents at non-emetic doses. Behav. Brain Res. *303*, 26–33. https://doi.org/10.1016/j.bbr.2016.01.031.

38. Van Duinen, M.A., Sambeth, A., Heckman, P.R.A., Smit, S., Tsai, M., Lahu, G., Uz, T., Blokland, A., and Prickaerts, J. (2018). Acute administration of Roflumilast enhances immediate recall of verbal word memory in healthy young adults. Neuropharmacology *131*, 31–38. https://doi.org/10.1016/j.neuropharm.2017.12.019.

39. Blokland, A., Van Duinen, M.A., Sambeth, A., Heckman, P.R.A., Tsai, M., Lahu, G., Uz, T., and Prickaerts, J. (2019). Acute treatment with the PDE4 inhibitor Roflumilast improves verbal word memory in healthy old individuals: a double-blind placebo-controlled study. Neurobiol. Aging *77*, 37–43. https://doi.org/10.1016/j.neurobiolaging.2019.01.014.

40. Pignatelli, M., Ryan, T.J., Roy, D.S., Lovett, C., Smith, L.M., Muralidhar, S., and Tonegawa, S. (2019). Engram cell excitability state determines the efficacy of memory retrieval. Neuron *101*, 274–284.e5. https://doi.org/10.1016/j.neuron.2018.11.029.

41. Argyrousi, E.K., Heckman, P.R., van Hagen, B.T., Muysers, H., van Goethem, N.P., and Prickaerts, J. (2020). Pro-cognitive effect of upregulating cyclic guanosine monophosphate signalling during memory acquisition or early consolidation is mediated by increased AMPA receptor trafficking. J. Psychopharmacol. *34*, 103–114. https://doi.org/10.1177/0269881119885262.

42. Bollen, E., Puzzo, D., Rutten, K., Privitera, L., De Vry, J., Vanmierlo, T., Kenis, G., Palmeri, A., D'Hooge, R., Balschun, D., et al. (2014). Improved long-term memory via enhancing cGMP-PKG signaling requires cAMP-PKA signaling. Neuropsychopharmacology *39*, 2497–2505. https://doi.org/10.1038/npp.2014.106.

43. Ryan, T.J., Ortega-de San Luis, C.O., Pezzoli, M., and Sen, S. (2021). Engram cell connectivity: an evolving substrate for information storage. Curr. Opin. Neurobiol. *67*, 215–225. https://doi.org/10.1016/j.conb.2021.01.006.

44. Abdou, K., Shehata, M., Choko, K., Nishizono, H., Matsuo, M., Muramatsu, S.I., and Inokuchi, K. (2018). Synapse-specific representation of the identity of overlapping memory engrams. Science *360*, 1227–1231. https://doi.org/10.1126/science.aat3810.

45. Choi, J.H., Sim, S.E., Kim, J.I., Choi, D.I., Oh, J., Ye, S., Lee, J., Kim, T., Ko, H.G., Lim, C.S., and Kaang, B.-K. (2018). Interregional synaptic maps among engram cells underlie memory formation. Science *360*, 430–435. https://doi.org/10.1126/science.aas9204.

46. Choi, D.I., Kim, J., Lee, H., Kim, J.I., Sung, Y., Choi, J.E., Venkat, S.J., Park, P., Jung, H., and Kaang, B.K. (2021). Synaptic correlates of associative fear memory in the lateral amygdala. Neuron *109*, 2717–2726.e3. https://doi.org/10.1016/j.neuron.2021.07.003.

47. Acosta-Peña, E., Camacho-Abrego, I., Melgarejo-Gutiérrez, M., Flores, G., Drucker-Colín, R., and García-García, F. (2015). Sleep deprivation induces differential morphological changes in the hippocampus and prefrontal cortex in young and old rats. Synapse *69*, 15–25. https://doi.org/10.1002/syn.21779.

48. Akkerman, S., Blokland, A., and Prickaerts, J. (2016). Possible overlapping time frames of acquisition and consolidation phases in object memory processes: a pharmacological approach. Learn. Mem. *23*, 29–37. https://doi.org/10.1101/lm.040162.115.

49. Szapiro, G., Galante, J.M., Barros, D.M., Levi de Stein, M., Vianna, M.R., Izquierdo, L.A., Izquierdo, I., and Medina, J.H. (2002). Molecular mechanisms of memory retrieval. Neurochem. Res. *27*, 1491–1498. https://doi.org/10.1023/a:1021648405461.

50. Heckman, P.R.A., Blokland, A., Bollen, E.P.P., and Prickaerts, J. (2018). Phosphodiesterase inhibition and modulation of corticostriatal and hippocampal circuits: clinical overview and translational considerations. Neurosci. Biobehav. Rev. *87*, 233–254. https://doi.org/10.1016/j.neubiorev.2018.02.007.

51. Argyrousi, E.K., Heckman, P.R.A., and Prickaerts, J. (2020). Role of cyclic nucleotides and their downstream signaling cascades in memory function: being at the right time at the right spot. Neurosci. Biobehav. Rev. *113*, 12–38. https://doi.org/10.1016/j.neubiorev.2020.02.004.

52. Josselyn, S.A., and Tonegawa, S. (2020). Memory engrams: recalling the past and imagining the future. Science *367*, eaaw4325. https://doi.org/10.1126/science.aaw4325.

53. Tuscher, J.J., Luine, V., Frankfurt, M., and Frick, K.M. (2016). Estradiol-mediated spine changes in the dorsal hippocampus and medial prefrontal cortex of ovariectomized female mice depend on ERK and mTOR activation in the dorsal hippocampus. J. Neurosci. *36*, 1483–1489. https://doi.org/10.1523/JNEUROSCI.3135-15.2016.

54. Meerlo, P., de Bruin, E.A., Strijkstra, A.M., and Daan, S. (2001). A social conflict increases EEG slow-wave activity during subsequent sleep. Physiol. Behav. *73*, 331–335. https://doi.org/10.1016/s0031-9384(01)00451-6.

55. Hagewoud, R., Whitcomb, S.N., Heeringa, A.N., Havekes, R., Koolhaas, J.M., and Meerlo, P. (2010). A time for learning and a time for sleep: the effect of sleep deprivation on contextual fear conditioning at different times of the day. Sleep *33*, 1315–1322. https://doi.org/10.1093/sleep/33.10.1315.

56. Ruskin, D.N., Dunn, K.E., Billiot, I., Bazan, N.G., and LaHoste, G.J. (2006). Eliminating the adrenal stress response does not affect sleep deprivation-induced acquisition deficits in the water maze. Life Sci. *78*, 2833–2838. https://doi.org/10.1016/j.lfs.2005.11.003.

57. Tiba, P.A., Oliveira, M.G., Rossi, V.C., Tufik, S., and Suchecki, D. (2008). Glucocorticoids are not responsible for paradoxical sleep deprivation-induced memory impairments. Sleep *31*, 505–515. https://doi.org/10.1093/sleep/31.4.505.

58. van der Borght, K., Ferrari, F., Klauke, K., Roman, V., Havekes, R., Sgoifo, A., van der Zee, E.A., and Meerlo, P. (2006). Hippocampal cell proliferation across the day: increase by running wheel activity, but no effect of sleep and wakefulness. Behav. Brain Res. *167*, 36–41. https://doi.org/10.1016/j.bbr.2005.08.012.

59. Blokland, A., Heckman, P., Vanmierlo, T., Schreiber, R., Paes, D., and Prickaerts, J. (2019). Phosphodiesterase type 4 inhibition in CNS diseases. Trends Pharmacol. Sci. *40*, 971–985. https://doi.org/10.1016/j.tips.2019.10.006.

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
mCherry [rabbit anti-mCherry, 1:1000]	Invitrogen	RRID: AB_TB254421
goat anti-rabbit Alexa 555	Thermo-fisher	RRID: AB_A32730
Bacterial and virus strains		
AVV9-TRE- ChR2-mCherry, titer: $3.75e^{14}$, 200 nL	Penn Vector Core, Philadelphia, USA	N/A
Chemicals, peptides, and recombinant proteins		
PDE4 inhibitor roflumilast	Sigma Aldrich (Merck), Zwijndrecht, the Netherlands	SKU: SML1099
Experimental models: Organisms/strains		
Hemizygous *c-fos-tTA/c-fos-shEGFP* male mice on a C57BL/6J background	Bred in house but original breeding pair from Jackson Labs	Strain #018306 (Jackson)

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact: Dr. Robbert Havekes (r.havekes@rug.nl).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Subjects

For the engram tagging studies (Figures 1, 2, 3, and 5), we used hemizygous *c-fos-tTA/c-fos-shEGFP* male mice on a C57BL/6 background, which were bred in our facility (first breeding pairs from Jackson Labs). Our previous studies examining the molecular underpinnings of sleep deprivation-induced deficits in memory synaptic plasticity were conducted in male mice, because the estrous cycle has a major impact on structural plasticity.[53] Our transgenic male mice express a tetracycline-transcriptional-trans-activator (tTA) under the control of a c-fos promoter, originally developed by Dr. Mark Mayford. Pharmacological studies without light stimulation (Figure 4) were done with C57BL/6 (Charles River labs). All mice were maintained on a 12-hrs light/12-hrs dark cycle and had *ad libitum* access to food and water. C-*fos-tTA/c-fos-shEGFP* were group housed prior to surgery and were individually housed afterwards. Non-surgery C57BL/6 mice were group housed until one week prior to behavioral testing when they were individually housed. All mice were 2-5 months old during testing, which always took place at the beginning of the light phase. All mice initially received regular mouse chow (Altromin). However, one week prior to surgery, the c-*fos-tTA/c-fos-shEGFP mice* were put on food containing 40 $mg.kg^{-1}$ doxycycline (dox) (Envigo). They remained on the dox diet until one day before the occurrence of the engram labelling. Mice were put back on regular chow 24 hrs prior to training in the learning task or exposure to the alternative context. Subsequently, the food was changed to a high dose dox chow (1 gkg^{-1}) to prevent any further labelling and the animals were maintained on this diet throughout the experiment. All procedures were approved by the national Central Authority for Scientific Procedures on Animals (CCD) and the Institutional Animal Welfare Body (IvD, University of Groningen, The Netherlands), and conform Directive 2010/63/EU.

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

METHOD DETAILS

Virus construct and packaging

In order to label memory engrams, we bilaterally injected an adeno-associated virus (AVV_9-TRE-ChR2-mCherry, titer: $3.75e^{14}$, 200 nL; Penn Vector Core, Philadelphia, US) in the dentate gyrus (DG) of *c-fos-tTA/c-fos-shEGFP* mice using the following coordinates, -2.0 mm anterior-posterior (AP), 1.3 mm ± medial-lateral (ML), -1.8 mm dorsal-ventral (DV). Channelrhodopsin-2 (ChR2) and mCherry expression was controlled by the tetracycline response element (TRE). In this way, FOS expression would drive the expression of tTA leading to the transcription of ChR2 and mCherry. The transcription of ChR2 and mCherry could be suppressed by keeping animals on a dox-containing chow (*i.e.*, c-fos expression would only lead to ChR2 and mCherry expression in the absence of dox).

Viral injection and fiber optic implants surgery

All surgeries were conducted using a stereotaxic apparatus (Kopf instruments). Mice were anesthetized with isoflurane (1.8–2.0%) and remained on a heating pad throughout the surgery. At the start of the surgery, mice were subcutaneously injected with carprofen (0.1 mL/10g), and 0.1 mL lidocaine was applied locally (subcutaneously) at the surgery site on top of the skull. Small bilateral holes were drilled in the skull using a 0.5 mm diameter microdrill at the appropriate locations. A 10 μL Hamilton micro syringe attached to a nanofil 33G needle (WPI) was loaded with virus and slowly lowered to the pocket site where it remained for one minute (see below for all coordinates). The needle was raised to the injection site where it again remained for one min before injecting started using a flow rate of 70 $nL.min^{-1}$ and controlled by a microinjection pump (Micro4; WPI). A total of 200 nL virus was injected. After the viral injection, the needle remained at the target site for two min before it was slowly retracted from the brain. Optical fibers were mounted (see coordinates below), and stabilized with cement (C&B metabond) and two screws (0.96 mm diameter). At the end of the surgery, mice were injected with saline 0.1 mL (0.9%). All animals were allowed to recover for at least one week before starting behavioral experiments. The coordinates for the DG injections and optic fiber mounting were as follows, -2.0 mm AP, 1.3 mm ± ML, -1.8 mm DV, and a pocket was made at -1.9 mm DV. The pocket site refers to a small area underneath the placement of the needle during the viral injection. This pocket site allows the virus to easily spread in the dentate gyrus minimizing the pressure build-up. The optic fiber was mounted at -2.0 mm AP and ± 0.13 mm ML, and the glass fiber was -0.175 mm long.

Optogenetic stimulation protocol

DG engram cells were optogenetically stimulated with 15 ms pulses of 20 Hz (473 nm) in accordance with previous work.[25] Stimulation was induced by means of a laser (CrystaLaser) which was driven by a TTL input (Doric lenses). The power delivered at the DG was 15-10 mW. All optogenetic laser stimulation was conducted in an open rectangular arena (empty OLM test arena) of 40 cm x 30 cm x 30 cm (length x width x height).

Immunohistochemistry

Animals were transcardially perfused with 0.9% NaCl + Heparin and 4% paraformaldehyde in PBS (0.01M) followed by a 48 hr post fixation at 4°C. Coronal brain sections were cut at a thickness of 20 microns. All sections were rinsed twice with PBS and incubated with 5% normal goat serum (NGS) in PBST (0.2% TritonX100) for one hour, followed by a 24 hrs incubation with primary antibodies at 4°C. After antibody incubation, all the sections were washed three times for 10 min with PBST and incubated for two hrs with the secondary antibodies diluted in NGS. A final incubation of 3 min with DAPI (1:1000, Thermo Fisher Scientific) was done, followed by three washing steps with PBST. Slices were mounted on glass slides and covered with mowiol (Sigma) to be visualized under a fluorescent microscope (Leica DMI6000). The following antibodies or combination of antibodies were used, mCherry [rabbit anti-mCherry, 1:1000, Invitrogen, LOT: TB254421; goat anti-rabbit Alexa 555, Thermo-fisher, LOT: A32730].

Cell counting

For the quantification of the ChR2-mCherry expression in the DG, the number of positive mCherry cells were counted from 3-4 coronal hippocampal slices per animal. The coronal slices were taken from the dorsal hippocampus surrounding the coordinates covered by the optical fiber implants (-2.0 mm AP). Fluorescent images were acquired on a Leica DMI6000 using 10x magnification. Manual cell counting analysis was performed using ImageJ software. The cell body layer of dentate granule cells was marked as a region of interest based on the DAPI signal in each slice. For each section, the area was measured and the number of mCherry-positive cells was counted after subtraction of background fluorescence. Counting was performed blind to experimental the condition.

Sleep deprivation

Mice were sleep deprived during the first six hrs of the light phase, directly following the training trial of the object-location memory (OLM) task, using the gentle stimulation method (*e.g.*, Havekes et al.,[19] Raven et al.,[34] and Heckman et al.[36]). In detail, animals were kept awake by gently tapping or shaking the cage. Shaking only occurred once tapping the cage was no longer sufficient. This sleep deprivation method has been extensively validated both at the behavioral level as well as through EEG recordings and has indicated that this procedure successfully omits all REM sleep and approximately 95% of all NREM sleep (*e.g.,* Meerlo et al.[54]), and work by us and others showed that behavioral and plasticity phenotypes associated with sleep deprivation were not caused by elevated plasma corticosterone levels or the gentle stimulation method itself (*e.g.,* Vecsey et al.,[18] Hagewoud et al.,[55] Ruskin et al.,[56] Tiba et al.,[57] and

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

van der Borght et al.[58]). More recently, we reported that blocking the synthesis and release of corticosterone in mice selectively during the SD period does not prevent sleep deprivation-induced memory deficits in the OLM task.[34]

Drug preparation

The phosphodiesterase type 4 (PDE4) inhibitor roflumilast (Sigma Aldrich, Zwijndrecht, the Netherlands) was dissolved in vehicle solution containing 98% methyl cellulose Tylose (Sigma Aldrich, Zwijndrecht, the Netherlands) and 2% Tween80 (Sigma Aldrich, Zwijndrecht, the Netherlands) on the day of the behavioral studies and injected in a volume of 2 $mL.kg^{-1}$. Roflumilast was administered intraperitoneally at a dose of 0.03 $mg.kg^{-1}$. Dose, injection volume, and injection schemes are based on our experience with the current drug in the OLM paradigm (*e.g*, Heckman et al.[36] and Blokland et al.[59]). Specifically, the timing of injections was based on the pharmacokinetics of roflumilast in mice,[37] and the exact time window during which cAMP is important for specific memory processes.[42] Furthermore, roflumilast shows a peak plasma and brain concentration 30 min after administration, has a half-life in rodents of about 6 hrs, and after 21 hrs roflumilast can no longer be detected in the mouse brain when using a dose of 0.03 $mgkg^{-1}$.[37]

Behavioral studies

Handling and habituation

Before the behavioral experiments started, all mice were handled and habituated for three consecutive days. On the first day, they were handled for 4 min/per mouse; on the second day mice were again handled 4 min/per mouse, and were habituated to scruff handling; on the third day, mice were brought to the experimental room and allowed to explore the empty arena for 5 min. Mice involved in the optogenetic experiments, in which the engram reactivation took place in the empty arena, were connected to the optic fiber cable and allowed to explore the empty arena for the full 5 min of the habituation trial with the laser turned on (15-10 mW, 15 ms pulses of 20 Hz). Mice that were optogenetically stimulated in the home cage during the training day were also stimulated in the home cage during habituation and were allowed to explore the empty arena afterward. Important to note is that animals were kept on dox during the whole habituation period, to prevent the tagging of engram cells. As such, all mice were habituated to exploring the empty arena while being on dox, and connected to a fiber optic cable, emitting blue light either in the empty arena or in the home cage depending on the experiment.

Object-location memory (OLM) paradigm

This spatial learning task requires the hippocampus and is based on the innate preference for spatial novelty. The OLM task was conducted in a rectangular arena (40 cm x 30 cm x 30 cm) with two spatial cues at the short walls on opposite sides of the arena. The spatial cues cover the full short walls and consist out of a black and white checkerboard pattern (total length x width: 25.4 cm x 21 cm, with black and white checkerboards of 1.5 cm x 1.5 cm) or alternating black and white striping (total length x width: 30 cm x 21 cm, with horizontal stripes that are 2.6 cm wide). The OLM task consisted of 10 min training and testing trials, during which the animals were allowed to freely explore two identical objects. All the animals entered the arena from the same side of the arena. Animals were placed into the arena facing the wall and away from the objects. However, the starting position of the animals with respect to the locations of objects in the arena was randomized as the locations of the objects were randomly and in a counterbalanced fashion assigned to each animal. During the training trial, the objects were placed symmetrically on a horizontal line, approximately 7.5 cm from the wall. In the testing trial, one of the objects was displaced along a straight line to a position that was 15 cm away from the training trial location. The combination of the side (left or right) and direction (up or down) of displacement of the objects in the testing trial was randomized and, during repeated testing, counterbalanced to avoid any place preferences. Object preferences were prevented by using different sets of objects for each animal in every experiment. It should be noted that any carry-over effects from using the same arena and distal cues cannot be excluded. Between animals and trials, the objects and arena were cleaned with a 70% ethanol solution to avoid the presence of olfactory cues. The exploration times per mouse for each object during the training and test trial were manually scored using custom software (ORT v2; Maastricht, The Netherlands) by an experimenter blind to treatment. Directing the nose to the object at a distance of no more than 1 cm and/or touching the object with the nose was considered exploratory behavior. From these exploration times, the relative measure of discrimination was calculated controlling for total exploration time: the d2 index. To calculate the d2 index, the full 10 min of the test trial was used. This relative discrimination index is calculated from the raw object exploration times during the test trial using the following equation: (exploration time object 1 at novel location - exploration time object 2 at original location) / (exploration time object 1 at novel location + exploration time object 2 at original location). Of note, animals would be excluded from the analysis if they did not explore both objects at least once during the training trial and test trial. In practice, this did not happen. For a detailed description of the experimental design of each individual study, see supplemental information.

Extended description of all behavioral training and testing protocols

Sleep deprivation, laser stimulation, and the object-location memory task (Figures 1D, 1E, S1A, and S1B)

In this experiment, we examined whether sleep deprivation following training attenuated memory performance during the test trial 24 hrs after learning. Moreover, we investigated whether optogenetic stimulation hampers the normal learning and formation of object-location memories. Mice were kept on a dox diet (40 $mgkg^{-1}$) throughout the whole experiment to prevent any tagging. First, mice were handled and habituated according to our handling and habituation protocol (see the STAR Methods section). They underwent an object-location memory (OLM) training trial, and were immediately sleep deprived (SD) or left undisturbed (NSD) depending on their experimental condition (Figure 1D). In the testing procedure 24 hrs later, mice were first connected to the fiber optic cable

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

and placed back into their home cage for 90 sec. Next, they were placed into the empty arena *without objects* for three minutes. During these three mins, animals received laser stimulation. Subsequently, after the laser stimulation, the mice were placed back into their home cage for 5 min, before being placed into the arena *with objects* for 10 min (testing trial). In Figure 1D, optogenetic stimulation took place in the training context without objects, and 5 min before the testing trial. Two days after the testing procedure, we repeated the whole experiment including training and test trial in the same batch of animals, as the within design has more power and required fewer animals. However, during this second experiment, we switched experimental groups in such a way that animals that were sleep deprived during the previous session were now left undisturbed, and *vice versa*. Data from both experiments were pooled so that every mouse constitutes its own control (*i.e.*, sleep deprivation versus normal sleep). The order of treatment was always included as a factor in the statistical analysis of the experiments (see the quantification and statistical analysis section of the STAR Methods).

Engram reactivation 24 hours after the learning episode followed by sleep deprivation (Figures 1F, 1G, S1C, and S1D)

For this experiment, we used the same mice as in the *optogenetic stimulation without engram labeling* study, described above. Importantly, different objects and locations were used to avoid any carry-over effects. In this experiment, the engram of the training trial was tagged by replacing the dox diet (40 $mg.kg^{-1}$) with normal chow 24 hrs before the training trial. To prevent any tagging after the training trial, we exchanged the normal chow with a high-dose dox diet (1g kg^{-1}) immediately after the training trial. During the training trial, animals were allowed to explore two similar objects for ten min. Immediately following training, all animals were sleep deprived for six hrs. Twenty-four hours after the training trial, animals underwent a test trial procedure in which they were first connected to the fiber optic cable and placed back into their home cage for 90 sec. Next, they were placed into the empty arena *without objects* for three minutes. During these three mins, half the animals received laser stimulation, while the other half received no laser stimulation. Subsequently, after the laser stimulation, the mice were placed back into their home cage for 5 mins, before being placed into the arena *with objects* for ten min (testing trial). For the home cage reactivation experiment (Figures 1H, 1I, S1E, and S1F), mice underwent a similar training and testing procedure, except that the optogenetic reactivation took place in the home cage instead of the empty arena.

Engram reactivation 5 & 8 days after learning episode followed by sleep deprivation (Figures 2A–2D, S2A, and S2B)

In order to investigate whether the memory engram could still be optogenetically reactivated after a prolonged period, we used the same mice as in the 24 hrs reactivation experiment described above. Upon completion of the testing trial of the 24 hrs reactivation experiment, animals were left undisturbed for four days. Thus, 5 days after the training trial followed by sleep deprivation, mice underwent an additional delayed testing trial. The procedures for the current testing trial were similar to those described for the testing trial of the 24 hrs reactivation experiment with the important distinction that the group receiving laser stimulation now received no laser stimulation, and vice versa. Three days after this delayed reactivation trial, we subjected the mice to another testing trial, during which we also interchanged laser stimulation conditions. For the experiments in Figures 2E and 2F (Figures S2C and S2D) we used new mice that were only subjected to a single testing trial 5 days after the training trial followed by sleep deprivation.

Reactivation OLM engram versus alternative context engram (Figures 3A–3C, S3A, and S3B)

This experiment was conducted to demonstrate that our behavioral data was induced specifically due to engram reactivation of the tagged learning episode, and is not induced by activation of an engram from an unrelated learning episode. Mice were first habituated according to our habituation protocol (see the STAR Methods section). After habituation, they were divided into two groups: training context versus alternative context. Both groups were subjected to similar experimental procedures and only differed in their "tagged context", which was accomplished by taking the mice off dox food at different time points during exposure to different contexts. The alternative context consisted of a different and smaller box compared to the original OLM arena. The alternative context had different dimensions (54.5 cm x 44.0 cm x 55.0 cm), and spatial cues (*i.e.*, mosaic patterned and vertically striped).

Both groups were first exposed to an alternative context (day 1), followed by the exposure to an empty arena 24 hrs later (day 2). On day 3, they underwent the OLM training trial. Whereas one group was taken off dox 24 hrs before the OLM training trial, the other was taken off dox 24 hrs before exposure to the alternative context (Figure 3A). Both groups were deprived of sleep after the OLM training trial, and transferred back to the housing room thereafter where they were left undisturbed for the next 5 days. On day 5, the tagged engram was reactivated and tested according to the other optogenetic testing protocol in which the engram was reactivated in the empty arena. All groups were divided in such a way that 'reactivation' or 'no reactivation' conditions were balanced in both the training context and alternative context group. Three days following the testing trial, all animals were subjected to another retention trial, however, the reactivation conditions were reversed between groups in a cross-design fashion.

Treatment with roflumilast after OLM training (Figures 4A–4H and S4A–S4H)

For the current experiment, we first handled the animals according to our handling protocol (see the STAR Methods section) with the addition of an IP injection with saline (0.03 mL per animal) during the last day of handling. On experimental day 1, mice underwent a training trial in which they explored two similar objects for 10 min. Thereafter, all the groups were sleep deprived for 6 hrs. After 24 hrs (Figures 4A, 4B, 4E, 4F, S4A, S4B, S4E, and S4F) or after 5 days (Figures 4C, 4D, 4G, 4H, S4C, S4D, S4G, and S4H), the testing trial took place. In the experiments of Figures 4A–4D, mice were first exposed to the box *without objects* and placed back in the home cage. Next, they were subjected to a testing trial in which one of the objects was relocated to a novel location. The experiments of Figures 4E–4H had a similar procedure without exposure to the empty arena. The mice were injected with vehicle solution or roflumilast (depending on the experimental group) 30 min before the testing trial.

Please cite this article in press as: Bolsius et al., Recovering object-location memories after sleep deprivation-induced amnesia, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.12.006

Optogenetic engram reactivation in combination with roflumilast treatment to restore the accessibility of spatial memories under sleep-deprived conditions (Figures 5 and S5)

Similar to all previously described studies, mice were first handled and habituated (see the STAR Methods section). At the end of the third habituation day and 24 hrs before the training trial, mice were taken off dox food. On experimental day 1, mice underwent the learning trial in which they were allowed to explore two similar objects for 10 min. After the training trial, we immediately changed the diet to a 1 gkg^{-1} dox diet, and subjected them to 6 hrs of sleep deprivation. For the next 3 days, the animals were left undisturbed. On day 4, the reactivation session took place, and the mice were divided into three experimental conditions: 'laser on + vehicle', 'laser off + roflumilast', or 'laser on + roflumilast'. For the experiment in Figures 5A and 5B, the reactivation session was in the OLM arena *without objects* and lasted three mins. For the experiment in Figures 5C and 5D, the reactivation session was in the home cage and lasted three mins. During these three min the laser was turned on in the laser on experimental groups. Three hrs after the reactivation we injected roflumilast or vehicle IP. Two days later mice were subjected to a ''normal'' testing session in which one of the objects was moved to a novel location. During this test session, no drugs or laser treatment was applied.

QUANTIFICATION AND STATISTICAL ANALYSIS

IBM SPSS Statistics 26 was used to analyze all data. All statistical tests were two-tailed. All data was checked for normality. In addition, where applicable, Levene's tests were first run to check for equality of variances. None of the conducted Levene's tests were significant. For all repeated measures ANOVA, the sphericity assumption was always checked using Mauchly's sphericity test. Sphericity assumption turned out not to be violated in all analyses, meaning sphericity was assumed when inspecting tables showing tests of within-subject effects.

Data from Figures 1G and 2D as well Figures 4C, 4D, S1C, S1D, and S1F was analyzed using an independent-samples t-test. Data from Figures 1E, 2B, S1A, S1B, and S1E were analyzed using paired-samples t-tests.

Data from Figure 1I was analyzed by means of repeated measures ANOVA using 'laser' (on vs off) as 'within subject factor' and 'order' (day 5 vs day 8) as between-subject factor. No significant 'laser*order' interaction effect was found. Subsequent analysis of main effects showed a main effect for 'laser'. An additional paired samples t-test confirmed this effect of 'laser on vs off'.

For Figure 1K, A repeated measures ANOVA was conducted using 'laser' (on vs off) as within subject factor and 'context' (OLM vs Alternative context (Alt Ctx)) as well as 'order' (laser 'on-then-off' vs 'off-then-on') as between subject factors. No three-way interaction was revealed, nor a laser*order interaction, both indicating that the order of laser stimulation had no effect and that the data of day 5 and 8 could validly be pooled. Finally, a significant laser*context interaction was shown. Next, we conducted pairwise comparisons using the Dunnett test with 'OLM-context laser ON' as reference against which all other conditions are compared: 1) against 'OLM laser OFF', 2) against 'Alt Ctx laser ON', and 3) against 'Alt Ctx laser off'.

Data of Figures 2B, S4A, and S4B was analyzed by means of repeated measures ANOVA using one within factor containing four levels (*i.e.*, the four experimental conditions). After showing significance of the overall ANOVA for Figure 2B, we continued with a pairwise comparisons by means of Dunnett post hoc test using 'vehicle SD' group as reference against which the other three groups are compared, 'veh SD' vs 'Veh NSD', vs 'rof NSD', vs 'rof SD'.

For Figures 3, S5A, and S5B, a one-way ANOVA was used with 'experimental condition' as between-subject factor containing three levels. After showing significance of the overall ANOVA for the data in Figure 3, additional post hoc analyses using Dunnett showed that both 'laser off+rof', and 'laser on+veh' differed from the reference group 'laser on+rof'.

Figure S1G was analyzed using repeated measures ANOVA using 'laser' (on vs off) as within subject factor and 'context' (OLM vs Alt Ctx) as between subject factor. No significant interaction or main effect was observed.