ARTICLES

Recovery of learning and memory is associated with chromatin remodelling

Andre Fischer[1,2,3]†, Farahnaz Sananbenesi[1,2,3]†, Xinyu Wang[1,2,3], Matthew Dobbin[1,2,3] & Li-Huei Tsai[1,2,3]

Neurodegenerative diseases of the central nervous system are often associated with impaired learning and memory, eventually leading to dementia. An important aspect in pre-clinical research is the exploration of strategies to re-establish learning ability and access to long-term memories. By using a mouse model that allows temporally and spatially restricted induction of neuronal loss, we show here that environmental enrichment reinstated learning behaviour and re-established access to long-term memories after significant brain atrophy and neuronal loss had already occurred. Environmental enrichment correlated with chromatin modifications (increased histone-tail acetylation). Moreover, increased histone acetylation by inhibitors of histone deacetylases induced sprouting of dendrites, an increased number of synapses, and reinstated learning behaviour and access to long-term memories. These data suggest that inhibition of histone deacetylases might be a suitable therapeutic avenue for neurodegenerative diseases associated with learning and memory impairment, and raises the possibility of recovery of long-term memories in patients with dementia.

Brain atrophy occurs during normal ageing and is an early feature of neurodegenerative diseases associated with impaired learning and memory. However, owing to animal-model limitations, pre-clinical research has barely explored strategies to recover impaired learning or lost memories after substantial neuronal loss had taken place. Only recently have mouse models with extensive neurodegeneration in the forebrain been reported[1–3]. One of these models is the bi-transgenic CK-p25 Tg mouse in which expression of p25, a protein implicated in various neurodegenerative diseases[4], is under the control of the *CamKII* promoter and can be switched on or off with a doxycycline diet[3,5].

We previously showed that post-natal induction of p25 expression for six weeks caused learning impairment in the fear conditioning and water maze paradigm that was accompanied by severe synaptic and neuronal loss in the forebrain[3]. Because these tests depend on motor function and emotional state, we emphasize that CK-p25 mice p25-induced for six weeks display normal basal anxiety levels and locomotor activity[3]. In this study, we used the CK-p25 Tg mouse model to test therapeutic strategies aimed at restoring learning and the access to long-term memory after synaptic and neuronal loss had already occurred. We hypothesized that a potential mechanism to reinstate learning and memory in a degenerated brain would be to upregulate the plasticity and function of the remaining neurons. A well-established but poorly understood approach to increase synaptic function in rodents is exposure to an enriched environment (environmental enrichment; EE)[6]. Exposure of wild-type mice to EE facilitated their learning ability and caused elevated levels of marker proteins for synaptic integrity and plasticity, indicating dendritic branching and synaptogenesis[6] (Supplementary Figs 1 and 2).

EE restores learning after neuronal loss

To investigate the effect of EE on learning behaviour after neuronal loss had already occurred, p25 was induced in 11-month-old CK-p25 Tg mice for six weeks, followed by EE for four weeks (Fig. 1a). We found that despite a comparable extent of brain atrophy (Fig. 1b, Supplementary Fig. 3a), EE-treated CK-p25 Tg mice showed markedly increased associative and spatial learning when compared to the non-enriched CK-p25 Tg mice (Fig. 1c, d). This suggests that EE can reinstate learning ability in mice with severe neurodegeneration. When brain weight was plotted against the freezing behaviour of individual mice we found that improved learning but not brain weight is associated with EE in CK-p25 Tg mice (data not shown).

Consistently, the neuronal marker protein NeuN, used to assess neuronal density in the brain, was similarly reduced in EE-treated and untreated CK-p25 Tg mice. Notably, levels of synaptic marker proteins and synaptophysin and MAP-2 immunoreactivity, were significantly higher in EE-treated CK-p25 Tg mice when compared to non-enriched CK-p25 Tg mice (Fig. 1e, f; Supplementary Fig. 3b). This result indicates that EE promoted growth of new dendrites and synapses in CK-p25 Tg mice. Thus, despite the substantial loss of neurons, EE induced the refinement of the synaptic network, which may be the cause of improved learning in the CK-p25 Tg mice.

EE re-establishes access to long-term memories

Although 'learning and memory' is a commonly used phrase, individuals suffering from neurodegenerative diseases can display impairments that distinguish between learning and memory. For example, while patients have difficulty learning new information, they also suffer from inability to recognize close relatives and other attributes of long-term memory. Because we can control the onset and extent of neurodegeneration in CK-p25 Tg mice, we were able to address the fate of consolidated long-term memories experimentally. It was previously shown that a single fear-conditioning trial results in a stable long-term memory that is initially encoded in the hippocampus but is probably transferred to the cortical network after three to four weeks[7]. A similar time window is reported for human long-term memories[8].

To establish an experimental model that allows investigation of the fate of long-term memories, 11-month-old uninduced CK-p25 Tg

[1]Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, [2]Howard Hughes Medical Institute, [3]Riken-MIT Neuroscience Research Center, Vassar Street, Bldg 46, Cambridge 02139, USA. †Present address: European Neuroscience Institute (ENI), Medical School University Goettingen, Max Planck Society, Grisebach Strasse 5, Goettingen 37077, Germany.

and control mice were trained in the fear-conditioning paradigm and returned to their home cages for four weeks to allow the consolidation of hippocampus-independent long-term memories. Afterwards, p25 expression was induced for either three or six weeks before the mice were subjected to the memory test (Fig. 2a). These time points were chosen because, unlike six-week induction, after three weeks of p25 expression no overt pathology was observed[3].

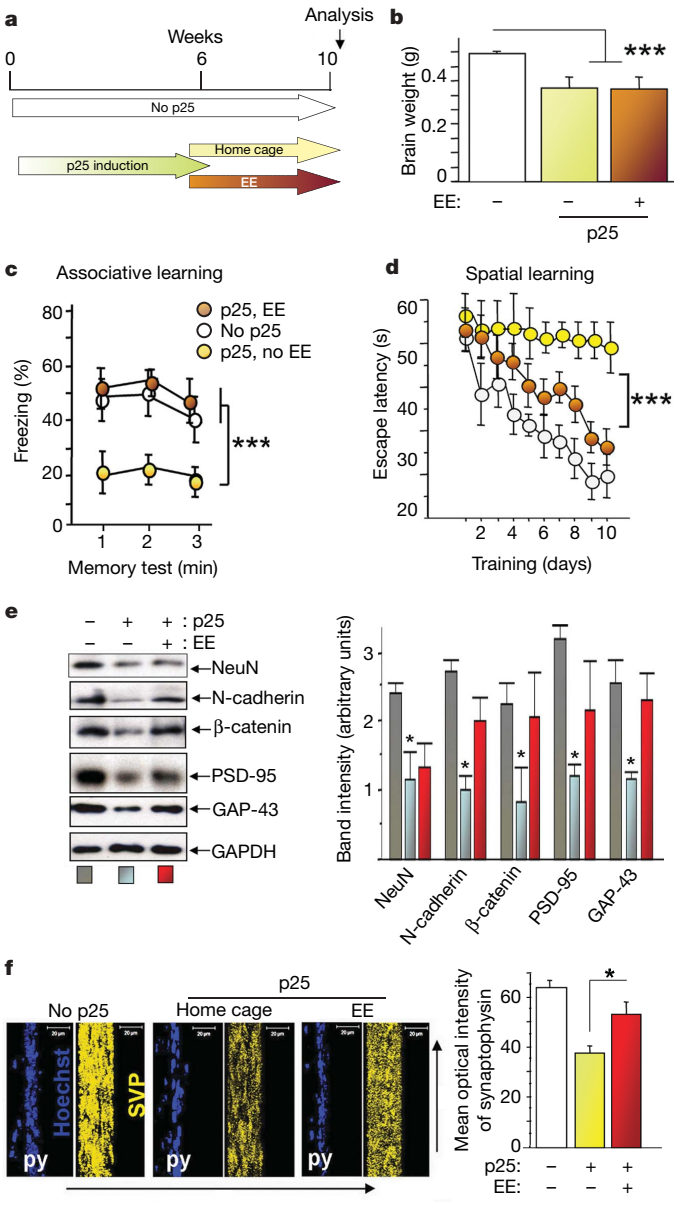

Figure 1 | Environmental enrichment reinstates learning in CK-p25 Tg mice after neurodegeneration. **a**, Experimental design ($n = 8$ per group). The experiment starts with the induction of p25 in CK-p25 Tg mice (0 weeks). **b**, Enriched and non-enriched CK-p25 Tg mice displayed similar brain atrophy (***$P < 0.0001$ versus control). **c**, Non-enriched CK-p25 Tg mice displayed impaired associative learning ($P = 0.0337$ versus control), whereas enriched CK-p25 Tg mice were improved when compared to the non-enriched group (***$P < 0.0001$). **d**, Enriched CK-p25 Tg mice performed significantly better in the water maze test than non-enriched CK-p25 mice ($F_{1,568} = 77.167$; ***$P < 0.0001$ versus control), but were still inferior to the no-p25 group ($F_{1,568} = 49.453$; $P < 0.0001$). **e**, Hippocampal lysates were analysed for neuronal and synaptic protein levels (*$P < 0.05$ enriched versus non enriched CK-p25 Tg, $n = 3$). **f**, Enriched CK-p25 Tg mice displayed significantly increased hippocampal synaptophysin immunoreactivity when compared to non-enriched CK-p25 Tg mice (*$P = 0.0304$). Scale bar, 20 μm. py, pyramidal cell layer; SVP, synaptophysin. Error bars indicate s.e.m.

CK-p25 Tg mice induced for three weeks showed similar levels of freezing behaviour, indicative of learned fear, when compared to the control mice, demonstrating the retrieval of consolidated long-term memories in these animals (Fig. 2b; experiment 1). Conversely, little freezing behaviour was observed in six-week-induced CK-p25 Tg mice (Fig. 2b; experiment 2). This suggests that the access to long-term memories has been lost. The loss of consolidated long-term memory was also evident in the water maze paradigm (Supplementary Fig. 5a, b)

Notably, it was not clear whether memories were lost, or whether they became inaccessible owing to synaptic and neuronal loss. In the latter case, it might be possible to re-establish access to such memories if sufficient refinement of the neuronal network can be achieved by the remaining neurons. To test this hypothesis, uninduced CK-p25 Tg and control mice were trained in the fear-conditioning

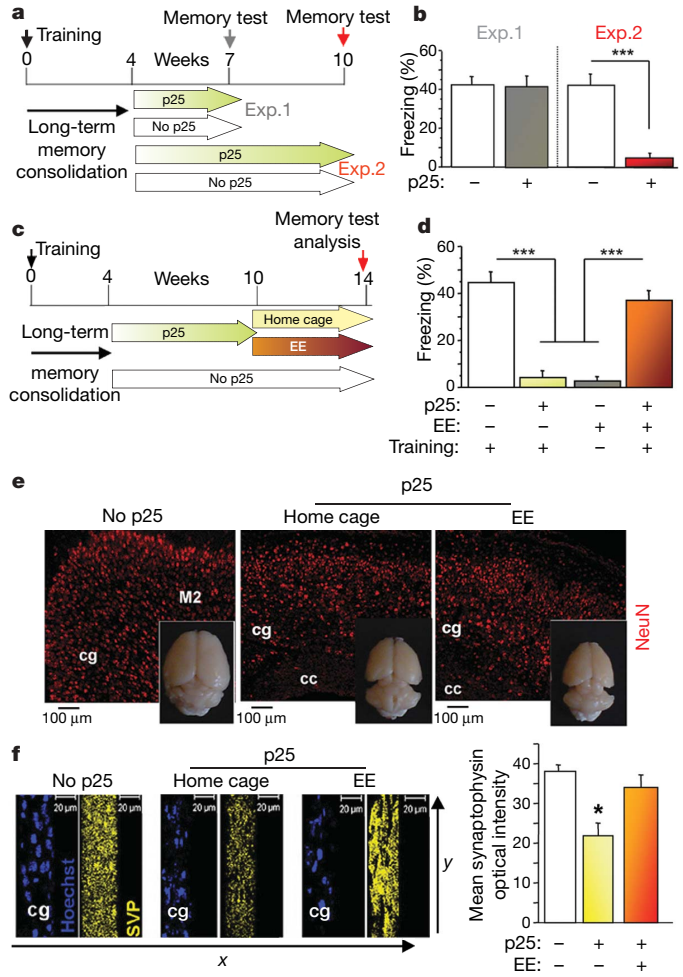

Figure 2 | Environmental enrichment re-establishes access to long-term memories. **a**, Experimental design. **b**, In experiment 1, three-week-induced CK-p25 Tg mice ($n = 8$) showed freezing behaviour similar to that of control mice ($n = 9$; $P = 0.863$). In experiment 2, six-week-induced CK-p25 Tg mice ($n = 9$) showed significant reduction in freezing behaviour (***$P < 0.0001$ versus control, $n = 16$). **c**, Experimental design. **d**, Non-enriched CK-p25 Tg mice ($n = 9$) displayed decreased freezing (***$P < 0.0001$ versus control, $n = 22$). Enriched CK-p25 Tg mice ($n = 13$) performed significantly better (***$P < 0.0001$ versus non-enriched CK-p25 group). An additional control group consisted of an enriched group that did not express p25 and were not subjected to the training ($n = 6$). **e**, NeuN staining and brain atrophy in the ACC. **f**, Enriched CK-p25 Tg mice displayed increased hippocampal synaptophysin immunoreactivity when compared to non-enriched CK-p25 mice ($P = 0.0251$). Scale bar, 20 μm. *$P < 0.05$ versus non-enriched group, $n = 3$. cg, cingulate cortex; M2, motor cortex 2; cc, corpus callosum. Error bars indicate s.e.m.

paradigm and returned to their home cages for four weeks followed by six weeks of p25 induction. Next, the mice were either subjected to EE or kept in their home cages for an additional four weeks (Fig. 2c). Afterwards, all mice were subjected to the memory test. Whereas non-enriched CK-p25 Tg mice showed significantly impaired freezing behaviour, indicating the loss of long-term memories, enriched CK-p25 Tg mice displayed much improved freezing behaviour, indicating a marked recovery of long-term memories (Fig. 2d). Importantly, enriched and non-enriched CK-p25 Tg mice have a similar extent of neuronal loss and brain atrophy (Fig. 2e). Evidence for the recovery of long-term memories was also found by using the water maze paradigm (Supplementary Fig. 5a, c). The fact that long-term memories can be recovered by EE supports the idea that the apparent 'memory loss' is really a reflection of inaccessible memories. These findings are in line with the phenomenon known as 'fluctuating memories' in which demented patients experience temporary time periods of apparent clarity[9,10].

The anterior cingulate cortex (ACC) of the brain is implicated in the consolidation and encoding of long-term memories[11]. CK-p25 Tg mice with no EE treatment displayed reduced synaptophysin immunoreactivity in the ACC when compared to control mice (Fig. 2f). In contrast, cortical levels of synaptic marker proteins and synaptophysin and MAP-2 immunoreactivity were higher in enriched than in non-enriched CK-p25 Tg mice (Fig. 2f, Supplementary Fig. 3c, d). These data suggest that EE leads to the recovery of long-term memories by re-establishing the synaptic network.

EE induces histone modification

Other than a few genes involved in synaptic function, relatively little is known about the mechanism underlying EE[12–14]. We speculated that EE might induce a transcriptional program that leads to activation of plasticity genes. Histone acetylation, which has been implicated in transcriptional regulation of gene expression via chromatin modification, has recently been implicated in synaptic plasticity and learning behaviour[15–18]. Here we found that EE induced hippocampal and cortical acetylation and methylation of histones 3 and 4 (H3, H4) as soon as three hours after treatment (Fig. 3a, b). In addition, intraperitoneal and intracerebroventricular injections of the histone deacetylase (HDAC) inhibitors sodium butyrate (SB) or trichostatin A significantly facilitated associative learning in wild-type mice (Supplementary Fig. 4a, b)[15]. To investigate whether inhibition of HDACs mimics the effects of EE, we administered SB daily intraperitoneally into wild-type mice for four weeks (Fig. 3c). The *in vivo* effect of SB was confirmed by a robust increase in H3 and H4 acetylation in the hippocampus (Fig. 3d).

When trained in the fear-conditioning paradigm, SB-injected mice showed significantly facilitated associative learning (Fig. 3e). Similarly, spatial learning in the water maze paradigm was facilitated, as indicated by shorter escape latencies (Fig. 3f) and improved target-quadrant preference in a probe trail performed after day 6 (data not shown). In addition we observed increased hippocampal MAP-2 and synaptophysin immunoreactivity and increased levels of synaptic and dendritic marker proteins in SB-injected mice (Supplementary Fig. 4d–f). Locomotor activity or basal anxiety was not altered in SB-injected mice (Supplementary Fig. 4c). Importantly, enrichment and SB-treatment did not upregulate the protein levels of GAPDH or actin. We also did not see activation of the Erk1/2 signalling pathway, which has been implicated in learning and memory (Supplementary Fig. 4g). This is consistent with gene-array findings that application of non-selective HDAC inhibitors to cells upregulates only 8–20% of all genes analysed[19–21]. Thus, enrichment and HDAC inhibition are likely to regulate relatively specific transcriptional programs.

HDAC inhibition restores learning and memory

Next we wished to examine whether sustained inhibition of HDACs would reinstate learning behaviour and recover access to long-term

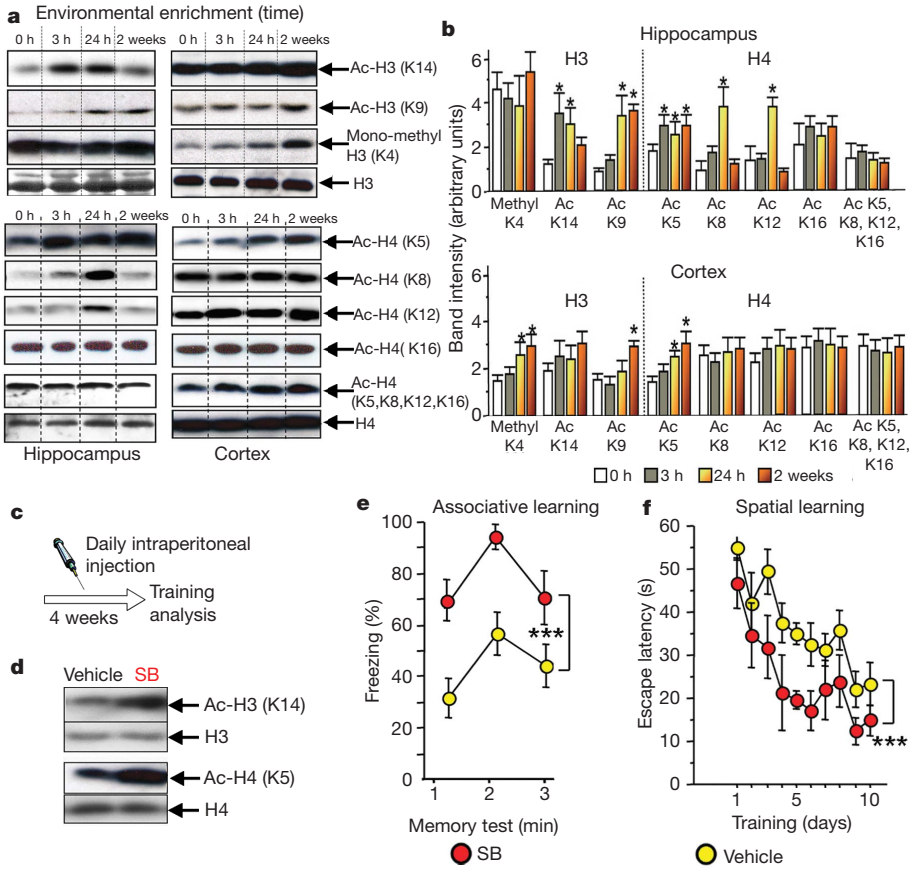

Figure 3 | Environmental enrichment induces chromatin modifications, and histone-deacetylase inhibitors facilitate learning behaviour. **a**, Wild-type mice were subjected to EE. Hippocampal and cortical brain lysates were obtained 3 h, 24 h or 2 weeks later ($n = 3$ per group) and probed with antibodies detecting histone-tail modification. Increases in hippocampal H3 (K9, K14) and H4 (K5, K8, K12) and in cortical H3 (K9) and H4 (K5) acetylation were observed. In addition, increased methylation (methyl) of H3 (K4) was observed in cortical lysates. **b**, Quantification of **a**. Ac, acetylation. $^{*}P < 0.05$ versus 0 h. **c**, Experimental design. **d**, Daily injection intraperitoneally with SB ($1.2\ g\ kg^{-1}$) increased acetylation of H3 and H4 in hippocampal lysates. **e**, This treatment improved associative learning ($^{***}P < 0.0001$ versus vehicle). **f**, Similarly, SB-injected mice displayed a shorter escape latency in the water maze paradigm ($F_{1,138} = 24.119$; $^{***}P < 0.0001$ versus vehicle). Error bars indicate s.e.m.

memories in CK-p25 Tg mice that had developed synaptic and neuronal loss. To this end, p25 expression was induced in 11-month-old CK-p25 mice for six weeks, before one group was injected daily for four weeks with SB while the control group received saline injection (vehicle, Fig. 4a). Compared to the vehicle group, SB-treated CK-p25 Tg mice showed significantly enhanced associative and spatial learning (Fig. 4b, c) and increased levels of synaptic marker proteins (Fig. 4d, e; Supplementary Fig. 3f, h). Importantly, SB- and vehicle-injected CK-p25 Tg mice displayed similar extents of brain atrophy and hippocampal neuronal loss (Supplementary Fig. 3e). These findings suggest that increased histone acetylation using the HDAC inhibitor SB can reinstate learning ability in mice exhibiting severe neurodegeneration.

We next evaluated the effect of HDAC inhibition on the recovery of inaccessible long-term memories. CK-p25 Tg mice were trained in the fear-conditioning paradigm and returned to their home cages for four weeks to allow the consolidation of long-term memories after which p25 was induced for six weeks. Mice were then injected daily intraperitoneally with either SB or vehicle for four weeks (Fig. 4f). Vehicle-injected CK-p25 Tg mice showed impaired access to long-term memory, as revealed by the markedly reduced freezing behaviour compared to the control mice that did not express p25 (Fig. 4g). Despite a similar degree of brain atrophy and neuronal loss (Supplementary Fig. 3g), SB-injected CK-p25 Tg mice showed significantly increased freezing behaviour and elevated levels of synaptic marker proteins when compared to the vehicle CK-p25 Tg group (Fig. 4g–i; Supplementary Fig. 3g, h). Similarly, SB administration also leads to the recovery of long-term spatial memories (Supplementary Fig. 5a, d). Thus, chronic injection of SB led to the recovery of memories in CK-p25 Tg mice that had developed severe neuronal loss.

Discussion

Here we have showed that EE enabled the recovery of impaired learning and lost long-term memories after animals had developed severe neurodegeneration and synaptic loss. Our results suggest that the effect of EE is likely to be mediated, at least in part, by elevated acetylation of histones H3 and H4, which initiates 'rewiring' of the neural network.

It is important to note that the effect of HDAC inhibitors on learning and memory could be a combination of modifications on chromatin and non-histone proteins, because it has been reported that some HDACs also target non-histone substrates[22]. Moreover, it is possible that different classes of HDACs are distinctly involved in synaptic plasticity. For example, it has been shown that HDAC5 is associated with downregulation of certain brain-derived

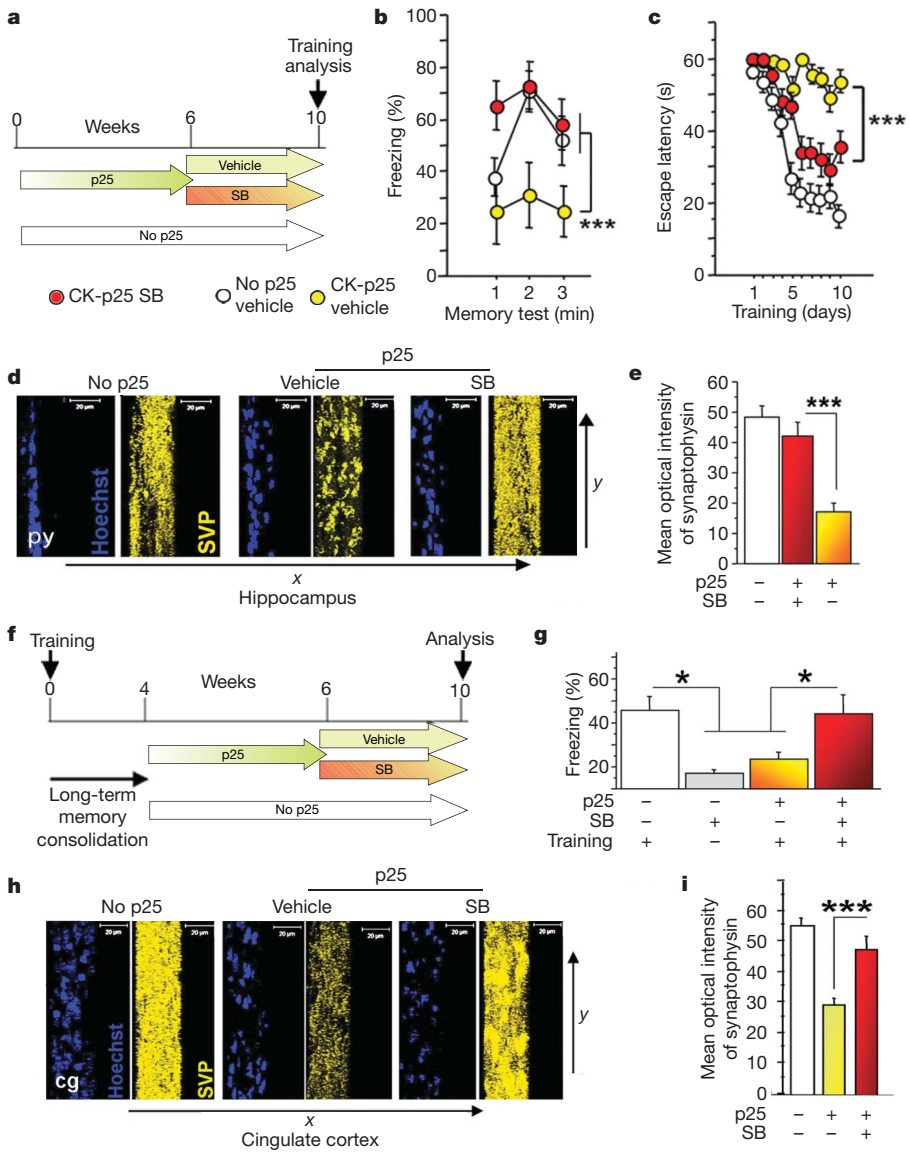

Figure 4 | Sodium butyrate facilitates learning and re-establishes the access to long-term memories in CK-p25 Tg mice. **a**, Experimental design. **b**, **c**, SB-injected ($1.2\,g\,kg^{-1}$) CK-p25 Tg mice ($n = 9$) displayed more freezing behaviour (**b**) when compared to saline (vehicle)-injected CK-p25 Tg mice ($P = 0.009$; $n = 9$) and performed better (**c**) in the water maze paradigm when compared to the CK-p25 Tg vehicle group ($F_{1,538} = 87.484$; $P < 0.0001$; $n = 9$). **d**, Increased hippocampal synaptophysin immunoreactivity in CK-p25 Tg mice injected with SB. Scale bar, 20 μm. **e**, Quantification of **d** ($P < 0.0001$ versus vehicle). **f**, Experimental design. **g**, When compared to the trained control group ($n = 15$), saline-injected CK-p25 Tg mice ($n = 12$) showed a significantly reduced freezing behaviour ($^*P < 0.0001$). SB-injected (daily; $1.2\,g\,kg^{-1}$) CK-p25 Tg mice ($n = 14$) performed significantly better than vehicle-injected CK-p25 Tg mice ($^*P = 0.0496$). **h**, Increased synaptophysin immunoreactivity in the ACC of SB-injected CK-p25 Tg mice ($P < 0.0001$ versus vehicle). Scale bar, 20 μm. **i**, Quantification of **h**. Error bars indicate s.e.m. **b**, **c**, **e**, **i**, $^{***}P < 0.05$, SB versus vehicle-treated CK-p25 Tg mice.

neurotrophic factor (BDNF) transcripts in a social defeat paradigm, a phenomenon that can be reversed with HDAC inhibitor treatment[23]. Future work will aim to determine the specific HDACs that regulate distinct forms of synaptic plasticity, learning and memory.

Interestingly, spontaneous 'rewiring' of the brain and recovery of memories was recently reported in a brain-injured man who was in a minimally conscious state for 20 years[24]. Thus, re-establishment of a neural network may allow recovery of long-term memories not only in rodents, but also in humans. Therefore, it is possible that HDAC inhibitors would be capable of re-establishing neural networks in human brains. If so, this suggests that using small molecules that target HDACs in patients with dementia could facilitate access to long-term memories.

Although our data suggest that dendritic sprouting and new synaptogenesis play important roles in the recovery of learning and memory, we cannot exclude the possibility that increased neurogenesis in the dentate gyrus[25] may also contribute to this process. Moreover, our data support computational models that showed that memory performance of a theoretical network diminishes on loss of connectivity but that this performance recovers once connectivity of this network is restored[26–28].

METHODS SUMMARY

Environmental enrichment. Up to four mice were continuously housed in a cage that contained two wheels for voluntary running and a variety of toys (obtained from Petco) to create tunnels, and climbing devices. Food and water was *ad libitum*. The food was hidden within the bedding. Toys and running wheels were changed on a daily basis.

Learning tests. All behavioural testing was performed as described before[3].

Cannulation and injection. Microcannula were inserted into the lateral brain ventricles as previously described[29]. Sodium butyrate (Sigma) was dissolved in artificial cerebrospinal fluid. A stock solution of trichostatin A (Sigma) was dissolved in DMSO and diluted with artificial cerebrospinal fluid before injection.

Immunoblotting and staining. Lysates for immunoblotting were prepared as previously described[3]. To isolate histones, brain tissue was homogenized in TX-buffer (50 mM Tris HCl, 150 mM NACl, 2 mM EDTA, 1% Triton-100) and incubated at 4 °C for 15 min before centrifugation at 2,000 r.p.m. (400*g*) for 10 min. After a wash-step in TX-buffer the pellet was dissolved in TX-buffer containing 0.2 M HCl and incubated on ice for 30 min, before a second centrifugation at 10,000 r.p.m. (9,300*g*) for 10 min. The supernatant was either dialysed or directly used for immunoblotting. Immunoblot data were quantified by measuring the band intensity using NIH imaging software and UN-SCAN-it gel digitizing software (Silk Scientific) by employing a Fujifilm LAS-1000 or 3100 imager whenever appropriate. Immunostaining was performed as described previously[3] using LSMeta10 software and a confocal microscope (Zeiss).

Statistical analysis. The data were analysed by unpaired Student's *t*-test and one-way ANOVA (analyis of variance). One-way ANOVA followed by post-hoc Scheffe's test was employed to compare means from several groups. Error bars present s.e.m.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 24 October 2006; accepted 23 March 2007.
Published online 29 April 2007.

1. Andorfer, C. *et al.* Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. *J. Neurosci.* **25,** 5446–5454 (2005).
2. Santacruz, K. *et al.* Tau suppression in a neurodegenerative mouse model improves memory function. *Science* **309,** 476–481 (2005).
3. Fischer, A., Sananbenesi, F., Pang, P. T., Lu, B. & Tsai, L. H. Opposing roles of transient and prolonged expression of p25 in synaptic plasticity and hippocampus-dependent memory. *Neuron* **48,** 825–838 (2005).
4. Cruz, J. C. & Tsai, L. H. Jekyll and Hyde kinase: roles for Cdk5 in brain development and disease. *Curr. Opin. Neurobiol.* **14,** 390–394 (2004).
5. Cruz, J. C., Tseng, H. C., Goldman, J. A., Shih, H. & Tsai, L. H. Aberrant Cdk5 activation by p25 triggers pathological events leading to neurodegeneration and neurofibrillary tangles. *Neuron* **40,** 471–483 (2003).
6. Nithianantharajah, J. & Hannan, A. J. Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Rev. Neurosci.* **7,** 697–709 (2006).
7. Kim, J. J. & Fanselow, M. S. Modality-specific retrograde amnesia of fear. *Science* **256,** 675–677 (1992).
8. Scoville, W. B. & Milner, B. Loss of recent memory after bilateral hippocampal lesions. *Neuropsychiatry Clin. Neurosci. 2000* **1,** 103–113 (1957).
9. Bradshaw, J., Saling, M., Hopwood, M., Anderson, V. & Brodtmann, A. Fluctuating cognition in dementia with Lewy bodies and Alzheimer's disease is qualitatively distinct. *J. Neurol. Neurosurg. Psychiatry* **75,** 382–387 (2004).
10. Palop, J. J., Chin, J. & Mucke, L. A network dysfunction perspective on neurodegenerative diseases. *Nature* **443,** 768–773 (2006).
11. Frankland, P. W., Bontempi, B., Talton, L. E., Kaczmarek, L. & Silva, A. J. The involvement of the anterior cingulate cortex in remote contextual fear memory. *Science* **304,** 881–883 (2004).
12. Need, A. C. & Giese, K. P. Handling and environmental enrichment do not rescue learning and memory impairments in αCamKII^{T286A} mutant mice. *Genes Brain Behav.* **2,** 132–139 (2003).
13. Tang, Y. P., Wang, H. S., Feng, M., Kyin, Y. Z. & Tsien, J. Z. Differential effects of enrichment on learning and memory function in NR2B transgenic mice. *Neuropharmacology* **41,** 779–790 (2001).
14. Rampon, C. *et al.* Effects of environmental enrichment on gene expression in the brain. *Proc. Natl Acad. Sci. USA* **97,** 12880–12884 (2000).
15. Levenson, J. M. *et al.* Regulation of histone acetylation during memory formation in the hippocampus. *J. Biol. Chem.* **279,** 40545–40559 (2004).
16. Kumar, A. *et al.* Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* **48,** 303–314 (2005).
17. Alarcon, J. M. *et al.* Chromatin acetylation, memory, and LTP are impaired in CBP$^{+/-}$ mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. *Neuron* **42,** 947–959 (2004).
18. Korzus, E., Rosenfeld, M. G. & Mayford, M. CBP histone acetyltransferase activity is a critical component of memory consolidation. *Neuron* **42,** 961–972 (2004).
19. Li, R. W. & Li, C. Butyrate induces profound changes in gene expression related to multiple signal pathways in bovine kidney epithelial cells. *BMC Genomics* **14,** 1–14 (2006).
20. Iacomino, G., Tecce, M. F., Grimaldi, C., Tosto, M. & Russo, G. L. Transcriptional response of a human colon adenocarcinoma cell line to sodium butyrate. *Biochem. Biophys. Res. Commun.* **285,** 1280–1289 (2001).
21. Tabuchi, Y. *et al.* Genetic networks responsive to sodium butyrate in colonic epithelial cells. *FEBS Lett.* **580,** 3035–3041 (2006).
22. Yuan, Z. L., Guan, Y. J., Chatterjee, D. & Chin, Y. E. Stat3 dimerization regulated by reversible acetylation of a single lysine residue. *Science* **307,** 217–218 (2005).
23. Tsankova, N. M. *et al.* Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nature Neurosci.* **9,** 519–525 (2006).
24. Voss, H. U. *et al.* Possible axonal regrowth in late recovery from the minimally conscious state. *J. Clin. Invest.* **116,** 2005–2011 (2006).
25. van Praag, H., Kempermann, G. & Gage, F. H. Neuronal consequences of environmental enrichment. *Nature Rev. Neurosci.* **1,** 191–198 (2000).
26. Horn, D., Ruppin, E., Usher, M. & Hermann, M. Neural network modeling of Alzheimer's Disease. *Neural Comput.* **5,** 736–749 (1993).
27. Ruppin, E., Reggia, J. A. & Horn, D. Pathogenesis of schizophrenic delusions and hallucinations: a neural model. *Schizophr. Bull.* **22,** 105–123 (1996).
28. Horn, D., Levy, N. & Ruppin, E. Neuronal-based synaptic compensation: a computational study in Alzheimer's disease. *Neural Comput.* **8,** 1227–1243 (1996).
29. Fischer, A., Sananbenesi, F., Schrick, C., Spiess, J. & Radulovic, J. Cyclin-dependent kinase 5 is required for associative learning. *J. Neurosci.* **22,** 3700–3707 (2002).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank B. Samuels, M. Bear, M. Wilson, W. Fischle and A. Schwienhorst for reading the manuscript and critical discussion, and all members of the Tsai laboratory for advice. We also thank S. Eimer and K. Schwarze for technical help. L-H.T. is an investigator of Howard Hughes Medical Institute. This work is partially supported by an NIH grant to L-H.T. This work was also partially supported by a Humboldt fellowship to A.F. and a German research foundation (DFG) fellowship to F.S., and by funds from the ENI Goettingen to A.F. The ENI is jointly funded by the Medical School University Goettingen and the Max Planck Society.

Author Contributions The studies were conceived and designed by A.F. and L.-H.T. A.F., F.S., X.W. and M.D. contributed to the experiments in this work. The paper was written by A.F. and L.-H.T.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to L.H.-T. (lhtsai@mit.edu) or A.F. (andre.fischer@mpi-mail.mpg.de).

METHODS

Fear conditioning. To measure associative learning we employed the contextual fear-conditioning paradigm. The training consisted of a single exposure to the conditioning context (3 min) followed by a single electric foot shock. In experiments where we investigated the acquisition of fear memories, the shock intensity was 0.7 mA (constant current, 2 s) and the memory test was performed 24 h later. In experiments where we investigated the retrieval of long-term memories the shock intensity was 1.5 mA (constant current, 2 s). After the training, mice were allowed to develop long-term memories for 4 weeks. The memory test was performed as indicated in the text.

Water maze. The water maze training was performed in a circular tank (diameter 1.2 m) filled with opaque water. A platform (11 cm × 11 cm) was submerged below the water's surface in the centre of the target quadrant. The swimming path of the mice was recorded by a video camera and analysed by Videomot 2 software (TSE). For each training session, the mice were placed into the maze from four random points of the tank and were allowed to search for the platform for 60 s. If the mice did not find the platform within 60 s, they were gently guided to it. Mice were allowed to remain on the platform for 15 s. During the memory test (probe trial) the platform was removed from the tank and the mice were allowed to swim in the maze for 60 s. For experiments where we investigated learning, the probe trial was performed within 24 h after the last training session. For experimental detail about the employment of the water maze paradigm to investigate long-term memories, please see the legend of Supplementary Fig. 5. At the end of each experiment, the ability of mice to find a visible platform was performed to control for possible locomotor deficits. For the visible platform test no significant difference among experimental groups was found (data not shown).

Immunoblotting. Antibodies were used in 1:1,000 concentrations for immunoblotting and 1:500 for immunostaining. All antibodies detecting histones and anti-PSD-95 were from Upstate. Antisynaptophysin (SVP38) was from Sigma. Anti-neuronal nuclei (neuN) and anti-growth associated protein (Gap43) were from Chemicon and anti-N-cadherin, anti-(R)-catenin were from Santa Cruz. Anti-MAP-2 antibody was from Sigma (1:200 dilution). For quantitative immunoblot analysis equal amounts of proteins were loaded to each lane. To confirm equal loading, blots were reprobed with corresponding pan-antibodies or antibodies for house-keeping proteins such as GAPDH or actin. For quantification we always used a signal in the linear range.

Immunostaining. Synaptophysin staining was used to analyse synaptic plasticity. Synaptophysin staining is commonly used to analyse synaptic plasticity in animal models for neurodegeneration or post-mortem tissue from human Alzheimer's disease patients. Confocal images (1 μm) were scanned and subjected to three-dimensional reconstruction. The pictures are displayed as seen from the *x* axis. LSMeta10 software (Zeiss) was used to calculate the mean synaptophysin intensity. Brain sections with the strongest intensity were scanned first. All other images included in the analysis were scanned using the same microscope setting. Staining was quantified using LSMeta10 software (Zeiss).