Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by progressive memory decline and subsequent loss of broader cognitive functions. Memory decline in the early stages of AD is mostly limited to episodic memory, for which the hippocampus has a crucial role. However, it has been uncertain whether the observed amnesia in the early stages of AD is due to disrupted encoding and consolidation of episodic information, or an impairment in the retrieval of stored memory information. Here we show that in transgenic mouse models of early AD, direct optogenetic activation of hippocampal memory engram cells results in memory retrieval despite the fact that these mice are amnesic in long-term memory tests when natural recall cues are used, revealing a retrieval, rather than a storage impairment. Before amyloid plaque deposition, the amnesia in these mice is age-dependent, which correlates with a progressive reduction in spine density of hippocampal dentate gyrus engram cells. We show that optogenetic induction of long-term potentiation at perforant path synapses of dentate gyrus engram cells restores both spine density and long-term memory. We also demonstrate that an ablation of dentate gyrus engram cells containing restored spine density prevents the rescue of long-term memory. Thus, selective rescue of spine density in engram cells may lead to an effective strategy for treating memory loss in the early stages of AD.

AD is the most common cause of brain degeneration, and typically begins with impairments in cognitive functions. Most research has focused on understanding the relationship between memory impairments and the formation of two pathological hallmarks seen in the late stages of AD: extracellular amyloid plaques and intracellular aggregates of tau protein. The early phases of AD have received relatively less attention, although synaptic phenotypes have been identified as major correlates of cognitive impairments in both human patients and mouse models. Several studies have suggested that the episodic memory deficit of AD patients is due to ineffective encoding of new information. However, since the cognitive measures used in these studies rely on memory retrieval, it is not possible to discriminate rigorously between impairments in information storage and disrupted retrieval of stored information. This issue has an important clinical implication: if the amnesia is due to retrieval impairments, memory could be restored by technologies involving targeted brain stimulation.

A mouse model of AD (hereafter referred to as ‘AD mice’) over-expresses the delta exon 9 variant of presenilin 1 (PS1; also known as PSEN1), in combination with the Swedish mutation of β-amyloid precursor (APP). Consistent with previous reports, 9-month-old AD mice showed severe plaque deposition across multiple brain regions, specifically in the dentate gyrus (DG) and medial entorhinal cortex (EC) (Fig. 1a). However, since the cognitive measures used in these studies rely on memory retrieval, it is not possible to discriminate rigorously between impairments in information storage and disrupted retrieval of stored information. This issue has an important clinical implication: if the amnesia is due to retrieval impairments, memory could be restored by technologies involving targeted brain stimulation.

As expected, these engram-labelled early AD mice were amnesic a day after CFC training (Fig. 1v). But, remarkably, these mice froze on the next day in a distinct context (context B) as robustly as equivalently treated control mice in response to blue light stimulation of the engram cells (Fig. 1w). This light-specific freezing was not observed using on-DOX mice (Extended Data Fig. 2d–f). A natural recall test conducted on the third day in the conditioning context (context A) revealed that the observed optogenetic engram reactivation did not restore memory recall by natural cues in early AD mice (Fig. 1x). This was the case even after multiple rounds of light activation of the engram cells (Extended Data Fig. 3). We replicated the successful optogenetic rescue of memory recall in two other models of early AD: a triple transgenic line obtained by mating c-Fos-tTA mice with double-transgenic APP/PS1 mice (Extended Data Fig. 4a–g) and a widely used triple-transgenic AD model (PS1/APP/tau (also known as MAPT); Extended Data Fig. 4h–m). These data show that DG engram cells in 7-month-old mice models of early AD are sufficient to induce memory recall upon optogenetic reactivation, which indicates a deficit of memory retrievability during early AD-related memory loss.
Figure 1 | Optogenetic activation of memory engrams restores fear memory in early AD mice. a–c, Amyloid-β (Aβ) plaques in 9-month-old AD mice (a), in the DG (b), and in the EC (c). d, Plaque counts in hippocampal sections (n = 4 mice per group). ND, not detected. e, CFC behavioural schedule (n = 10 mice per group). F–i, Freezing levels of 7-month-old AD groups during training (f), STM test (g), LTM test (h) or exposure to neutral context (i). j, c-Fos+ cell counts in the DG of 7-month-old mice after CFC training or LTM test, represented in j (n = 4 mice per group). DAPI, 4′,6-diamidino-2-phenylindole. k–n, Freezing levels of 9-month-old AD mice during training (k), STM test (l), LTM test (m) or exposure to neutral context (n). o, c-Fos+ cell counts in the DG of 9-month-old mice (n = 3 mice per group) after CFC training represented in k. p, Virus-mediated engram labelling strategy using a cocktail of AAVs-c-Fos-tTA.

Reduced dendritic spines have been implicated in memory impairments of AD. In addition, our recent study of protein-synthesis-inhibitor-induced amnesia found reduced engram-cell-specific dendritic spine density. We detected an age-dependent (Extended Data Fig. 5a) decrease in dendritic spine density of DG engram cells in early AD mice (Fig. 2a–c), showing that the long-term memory impairments of early AD correlate with dendritic spine deficits of DG engram cells (Extended Data Fig. 5b). The inability to generate newborn neurons in the DG could play a part in the development of AD-specific cognitive deficits. However, early AD mice showed similar levels of neurogenesis in the DG compared with control mice, which were quantified using doublecortin (DCX) staining (Extended Data Fig. 1l–q). We recently proposed that the persistent cellular connectivity between multiple engram cell ensembles is a fundamental mechanism of memory information.
We labelled putative CFC memory engram cells in both medial EC (MEC) and lateral EC (LEC) with oChIEF20 (a variant of ChR2) and simultaneously labelled CFC memory engram cells in the DG with eYFP (Fig. 2d). With this procedure, perforant path (PP) terminals are also labelled with oChIEF (Fig. 2e, f). One day after footshocks, we optogenetically activated these terminals and quantified the overlap between putative DG engram cells (that is, eYFP+, green) and DG cells in which the endogenous c-Fos (red) had been activated by the optogenetic activation of oChIEF+PP terminals. Both control and early AD mice showed above-chance and indistinguishable levels of c-Fos+/eYFP+ overlap, indicating that the preferential functional connectivity between engram cells is maintained in the early AD mice (Fig. 2g–i).

We then hypothesized that the reversal of dendritic spine deficits in DG engram cells of early AD mice may rescue long-term memory. To investigate this possibility, we took advantage of previous findings that spine formation can be induced rapidly by long-term potentiation (LTP)21,22 and that LTP can be induced in vivo using light activation of oChIEF23. We validated learning-dependent labelling, with oChIEF, of neurons in the MEC (Fig. 3a–c and Extended Data Fig. 6a–c) and LEC (Fig. 3d) as well as PP terminals in the DG (Fig. 3e, f). In vivo extracellular recording upon light stimulation of oChIEF+EC axonal terminals in the DG showed a reliable spiking response of DG cells in anaesthetized control mice (Fig. 3g). Furthermore, in HPC slices from control mice we successfully induced LTP in DG cells using a previously established optical LTP protocol23 (Fig. 3h–j). These biocytin-filled DG cells revealed an increase in spine density after in vitro optical LTP (Extended Data Fig. 6d).

In early AD mice, in vivo application of the engran-specific optical LTP protocol restored spine density of DG engram cells to control levels (AD + 100 Hz group; Fig. 3k, l). Furthermore, this spine restoration in early AD mice correlated with amelioration of long-term memory impairments observed during recall by natural cues (Fig. 3m), an effect that persisted for at least 6 days after training (AD rescue + diphtheria toxin receptor (DTR) + saline group; Fig. 3p). The LTP-induced spine restoration and behavioural deficit rescue were protein-synthesis dependent (Extended Data Fig. 7). The rescued memory was context-specific (Extended Data Fig. 8a). In addition, long-term memory recall of age-matched control mice was unaffected by this optical LTP protocol (Extended Data Fig. 8b). By contrast, applying the optical LTP protocol to a large portion of excitatory PP terminals in the DG (that is, with no restriction to the PP terminals derived from EC engran cells) did not result in long-term memory rescue in early AD mice (Extended Data Fig. 9).

To confirm the correlation between restoration of spine density of DG engran cells and amelioration of long-term memory impairments, which were both induced by the optical LTP protocol, we compared the overlap of natural-recall-cue-induced c-Fos+ cells and CFC-training-labelled DG engran cells after an application of the engran-specific LTP protocol to early AD mice (Fig. 3n). Early AD mice that did not receive the optical LTP protocol showed low levels of c-Fos+/eYFP+ overlap compared with control mice upon natural recall cue delivery. By contrast, early AD mice that went through the optical LTP protocol showed c-Fos+/eYFP+ overlap similar to that of control mice (Fig. 3n). Thus, these data suggest that spine density restoration in DG engran cells contributes to the rescue of long-term memory in early AD mice.

Because of the highly redundant connectivity between the EC and DG24, it is possible that the extensive optical LTP protocol also augmented spine density in some non-engram DG cells. To establish a link between the spine rescue in DG engran cells and the behavioural rescue of early AD mice, we developed an engran-specific ablation25 virus. We confirmed that this DTR-mediated method efficiently ablated DG engran cells after diphtheria toxin (DT) administration (Fig. 3o), while leaving the nearby DG mossy cells intact (Extended Data Fig. 10). By simultaneously labelling axonal terminals of PP with oChIEF and DG engran cells with DTR, we examined the effect of DG engran cell ablation after optical LTP-induced behavioural rescue (Fig. 3p). Within-animal comparisons (test 1 versus test 2) showed a decrease in freezing behaviour of LTP-rected AD mice in which DG engran cells were ablated. These data strengthen the link between DG engran cells with restored spine density and long-term behavioural rescue in early AD mice.

To examine whether the optical LTP-induced behavioural rescue could be applied to DG engran cells from other learning experiences, we labelled memory engrans for inhibitory avoidance or novel object location in early AD mice (Fig. 4a). Early AD mice showed memory impairments in inhibitory avoidance memory and novel object location spatial memory (Fig. 4b, c). Optical LTP-induced spine rescue at the PP→DG engran synapses was sufficient to reverse long-term memory impairments of early AD mice in both behavioural paradigms, thus demonstrating the versatility of our engran-based intervention.

Previous studies that examined the early stages of AD found correlations between memory impairments and synaptic pathology at the...
EC PP input into the DG3,4,6. It has been proposed that these early cognitive deficits are a failure of memory encoding on the basis of behavioural observations in human patients8,9. However, we have shown that optogenetic activation of HPC cells active during learning elicits memory failure in early AD models and may therefore provide a basis for understanding of memory retrieval deficits in several cases of early AD, in which patients show difficulty in memory recall. Our conclusions apply to episodic memory, which involves processing of non-stimulated early AD mice (20.48). Unless specified, statistical comparisons are performed using unpaired t-tests; *P < 0.05, **P < 0.01, ***P < 0.001. Data are presented as mean ± s.e.m.
memory deficits in early AD. Natural rescue of memory recall in early AD mice required the DG engram cells in which synaptic density deficits have been restored by in vivo optical LTP protocols applied to the EC cells activated during learning. By contrast, the application of optical LTP protocols to a much wider array of excitatory EC cells projecting to the DG, which may be analogous to deep brain stimulation, did not rescue memory in AD mice. A potential explanation for this observation is that DG granule cells may contribute to a variety of memories through their partially overlapping engram cell ensembles in a competitive manner, and that activation of a large number of these ensembles simultaneously may interfere with a selective activation of an individual ensemble. Thus, activation of a more targeted engram cell ensemble may be a key requirement for effective retrieval of the specific memory, which is difficult to achieve with the current deep brain stimulation strategy.

Genetic manipulations of specific neuronal populations can have profound effects on cognitive impairments of AD. We propose that strategies applied to engram circuits can support long-lasting improvements in cognitive functions, which may provide insights and therapeutic value for future approaches that rescue memory in AD patients.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Selkoe, D. J. Alzheimer’s disease: genes, proteins, and therapy. Physiol. Rev. 81, 741–766 (2001).
2. Selkoe, D. J. Alzheimer’s disease is a synaptic failure. Science 298, 789–791 (2002).
3. Jacobsen, J. S. et al. Early-onset behavioral and synaptic deficits in a mouse model of Alz103, 5161–5165 (2006).
4. Hsia, A. Y. et al. Plaque-independent disruption of neural circuits in Alzheimer’s disease mouse models. Proc. Natl Acad. Sci. USA 96, 3228–3233 (1999).
5. Mucke, L. et al. High-level neuronal expression of Aβ1−42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J. Neurosci. 20, 4050–4058 (2000).
6. Terry, R. D. et al. Physical basis of cognitive alterations in Alzheimer’s disease: synapse loss is the major correlate of cognitive impairment. Ann. Neurol. 30, 572–580 (1991).
7. Granholm, E. & Butters, N. Associative encoding and retrieval in Alzheimer’s and Huntington’s disease. Brain Cogn. 7, 335–347 (1988).
8. Hodges, J. R., Salmon, D. P. & Butters, N. Differential impairment of semantic and episodic memory in Alzheimer’s and Huntington’s diseases: a controlled prospective study. J. Neurol. Neurosurg. Psychiatry 53, 1089–1095 (1990).
9. Weintraub, S., Wicklund, A. H. & Salmon, D. P. The neuropsychological profile of Alzheimer disease. Cold Spring Harb. Perspect. Med. 2, a006171 (2012).
10. D’Alessandro, J. L. et al. Mutant presenilins specifically elevate the levels of the 42 residue β-amyloid peptide in vivo: evidence for augmentation of a 42-specific γ-secretase. Hum. Mol. Genet. 13, 159–170 (2004).
11. Liu, X. et al. Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature 484, 381–385 (2012).
12. Ramírez, S. et al. Creating a false memory in the hippocampus. Science 341, 387–391 (2013).
13. Redondo, R. L. et al. Bidirectional switch of the valence associated with a hippocampal contextual memory engram. Nature 513, 405–408 (2014).
14. Ryan, T. J., Roy, D. S., Pignattelli, M., Arons, A. & Tonegawa, S. Engram cells retain memory under retrograde amnesia. Science 348, 1007–1013 (2015).
15. Denny, C. A. et al. Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. Neuron 83, 189–201 (2014).
16. Harris, J. A. et al. Transsynaptic progression of amyloid-β-mediated neuronal dysfunction within the entorhinal-hippocampal network. Neuron 68, 428–441 (2010).
17. Hyman, B. T., Van Hoesen, G. W., Kromer, L. J. & Damasio, A. R. Perforant pathway changes and the memory impairment of Alzheimer’s disease. Ann. Neurol. 20, 472–481 (1986).
18. Oddo, S. et al. Triple-transgenic model of Alzheimer’s disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. Neuron 39, 409–421 (2003).
19. Rodríguez, J. J. et al. Impaired adult neurogenesis in the dentate gyrus of a triple transgenic mouse model of Alzheimer’s disease, PLoS One 3, e2935 (2008).
20. Lin, Y. J., Lin, M. Z., Steinbach, P. & Tsien, R. Y. Characterization of engineered channelrhodopsin mutants with improved properties and kinetics. Biophys. J. 96, 1803–1814 (2009).
21. Maletic-Savatic, M., Malinov, R. & Svoboda, K. Rapid dendritic morphogenesis in CA1 hippocampal dentrites induced by synaptic activity. Science 283, 1923–1927 (1999).
22. Engert, F. & Bonhoeffer, T. Dentritic spine changes associated with hippocampal long-term synaptic plasticity. Nature 399, 66–70 (1999).
23. Naito, S. et al. Engineering a memory with LTD and LTP. Nat. Phys. 511, 348–352 (2014).
24. Tamamaki, N. & Nojoy, Y. Projection of the entorhinal layer II neurons in the rat as revealed by intracellular pressure-injection of neurobiotin. Hippocampus 3, 471–480 (1993).
25. Zhan, C. et al. Acute and long-term suppression of feeding behavior by POMC neurons in the brainstem and hypothalamus, respectively. J. Neurosci. 33, 3624–3632 (2013).
26. Tonegawa, S., Liu, X., Ramírez, S. & Redondo, R. Memory engram cells have a finite age of come of age. Neuron 87, 918–931 (2015).
27. Cissé, M. et al. Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. Nature 469, 47–52 (2011).
METHODS

Subjects. The APP/PS1 (ref. 10) double-transgenic AD mice, originally described as Line 85, were obtained from Jackson Laboratory (stock number 004462). Under the control of mouse prion promoter elements, these mice express a chimaeric mouse/human APP transgene containing Swedish mutations (K595N/M596L) as well as a mutant human PS1 transgene (delta exon 9 variant). To label memory engram cells in APP/PS1 mice, we generated a triple-transgenic mouse line by mating c-Fos-Tra112,113 transgenic mice with APP/PS1 double-transgenic mice. The PS1/APP PS1/APP mice were obtained from Jackson Laboratory (stock number 004807). These lines of mice were raised on food containing 40 mg kg−1 DOX for at least 1 week before surgery, and remained on DOX for the remainder of the experiments except for the target-engram labelling days. For in vitro electrophysiology experiments, mice were 24–28 days old at the time of surgery. All experiments were conducted in accordance with US National Institutes of Health (NIH) guidelines and the Massachusetts Institute of Technology Department of Comparative Medicine and Committee of Animal Care. No statistical methods were used to predetermine sample size.

Viral constructs. Our previously established method11,12 for labelling memory engram cells combined c-Fos-Tra transgenic mice with a DOX-sensitive adeno-associated virus (AAV). However, in this study, we modified the method using a double-virus system to label memory engram cells in the early AD mice, which already carry two transgenes. The pAAV-c-Fos-Tra plasmid was constructed by cloning a 1 kb fragment from the c-Fos exon 1 to 35 bp into exon 11 into an AAV backbone using the Kpn1 restriction site at the 5′ terminus and the SpeI restriction site at the 3′ terminus. The AAV backbone contained the rTA-Advanced13 sequence at the SpeI restriction site. The pAAV-TRE-ChR2-eYFP and pAAV-TRE-eYFP constructs were previously described11,12. The pAAV-TRE-oChIEF-tTomato39 plasmid was constructed by replacing the ChR2 fragment from the pAAV-TRE-ChR2-eYFP plasmid using NheI and MfeII restriction sites. The pAAV-CaMKII-oChIEF-tTomato plasmid was constructed by replacing the TRE fragment from the pAAV-TRE-oChIEF-tTomato plasmid using BamHI and EcoRI restriction sites. The pAAV-TRE-DTR-eYFP52 plasmid was constructed by replacing the ChR2 fragment from the pAAV-TRE-ChR2-eYFP plasmid using EcoRI and AgeI restriction sites. AAV vectors were serotyped with AAV5 coat proteins and packaged at the University of Massachusetts Medical School Gene Therapy Center and Vector Core. Viral titres were 1.5 × 1013 genome copy (GC) ml−1 for AAV-c-Fos-Tra, AAV-TRE-ChR2-eYFP and AAVv-TRE-eYFP, 1 × 1013 GC ml−1 for AAVv-TRE-oChIEF-tTomato, 4 × 1013 GC ml−1 for AAVv-CaMKII-oChIEF-tTomato and 2 × 1013 GC ml−1 for AAVv-TRE-DTR-eYFP.

Surgery and optic fibre implants. Mice were anaesthetized with isoflurane or avertin (−1.3 mm mediolateral (ML), −1.9 mm dorsoventral (DV), NEC = −4.7 mm AP, ±3.3 mm ML, −3.3 mm DV) and LEC neurons. Total engram cell reactivation was calculated as ((c-Fos+ eYFP)/(total DAPI)) × 100. Chance overlap was calculated as ((c-Fos+/total DAPI) × (eYFP+/total DAPI)) × 100. Percentage of adult newborn neurons expressing neuronal markers was calculated as ((NeuN+ DCX+)/ (total DAPI−) × 100. DAPI+ counts were approximated from five coronal/sagittal slices using ImageJ. All counting experiments were conducted blind to experimental group. Researcher 1 trained the animals, prepared slices and randomized images, while researcher 2 performed semi-automated cell counting. Statistical comparisons were performed using unpaired t-tests: P < 0.05, **P < 0.01, ***P < 0.001.

Spine density analysis. Engram cells were labelled using c-Fos-Tra driven synthesis of ChR2-eYFP or eYFP alone. The eYFP signal was amplified using immunohistochemistry procedures, after which fluorescence z-stacks were taken by confocal microscopy (Zeiss LSM700) using a ×40 objective. Maximum intensity projections were generated using ZEN Black software (Zeiss). Four mice per experimental group were analysed for dendritic spines. For each mouse, 30–40 dendritic fragments of 10–μm length were quantified (n = 120–160 fragments per group). To measure spine density of DG engram cells with a focus on entorhinal cortical inputs, distal dendritic fragments in the middle-to-outer molecular layer (ML) were selected. For CA3 and CA1 engram cells, apical and basal dendritic fragments were selected. To compute spine density, the number of spines counted on each fragment was normalized by the cylindrical approximation of the surface of the specific fragment. Experiments were conducted blind to experimental group. Researcher 1 imaged dendritic fragments and randomized images, while researcher 2 performed manual spine counting.

In vitro recordings. After isoflurane anaesthesia, brains were quickly removed and used to prepare sagittal slices (300 μm) in an oxygenated cutting solution at 4°C with a vibratome11. Slices were incubated at room temperature in oxygenated artificial cerebrospinal fluid (ACSF) until the recordings. The cutting solution contained (in mM): 115 NaCl, 26 MgCl2, 1.2 NaH2PO4, 1.2 MgSO4, 25 NaHCO3, 1.2 Na2PO4, 10 n-glucose, 230 sucrose, saturated with 95% O2–5% CO2 (pH 7.3, osmolality of 340 mOsm). The ACSF contained (in mM): 124 NaCl, 3 KCl, 2 CaCl2, 1.3 MgSO4, 25 NaHCO3, 1.2 Na2PO4, 10 n-glucose, saturated with 95% O2–5% CO2 (pH 7.3, 300 mOsm). Individual slices were transferred to a submerged experimental chamber and perfused with oxygenated ACSF warmed at 35°C (±0.5°C) at a rate of 3 ml min−1 during recordings. Current or voltage clamp recordings were performed under an IR-DIC microscope (Olympus) with a ×40 water immersion objective (0.8 NA), equipped with four automatic manipulators (Luigs & Neumann) and a CCD camera (Hamamatsu). Borosilicate glass pipettes (Sutter Instruments) were fabricated with resistances of 8–10 MΩ. The intracellular solution (in mM) for current clamp recordings was: 110 K-glucatone, 10 KCl, 10 Hepes, 4 ATP, 0.3 GTP, 10 phosphocreatine, 0.5% bicyno (pH 7.2, 290 mOsm). Recordings used two dual channel amplifiers (Molecular Devices), a 2 kHz filter, 20 kHz digitization and an ADC/DAC module.
When training was complete, mice were switched back to food containing DOX. Mice were kept on regular food without DOX for 24 h before training. Cages using a wheeled cart. The cart and cages remained in an anteroom to habituate them to the journey. For natural memory recall sessions, data were recorded. Data acquisition used an Axon CNS Digidata 1440A system. MATLAB analysis was performed, as previously described. Behavourial assays. Experiments were conducted during the light cycle (7 a.m. to 7 p.m.). Mice were randomly assigned to experimental groups for specific behavourial assays immediately after surgery. Mice were habituated to investigator handling for 1–2 min on three consecutive days. Handling took place in the holding room where the mice were housed. Before each handling session, mice were transferred to the behavourial room and acclimated for 30 min before the training session. On day 1, mice were allowed to explore the chamber with pat-wall and walls. The apparatus was unscented and intermediate lighting was used. All mice had an object (7-cm-tall glass flask filled with metal beads) placed adjacent to the same position as the previous exposure (familiar) or at a novel location based on genotype. On day 4, mice were placed into the chamber with the object either in the DG of mice injected with a cocktail of AAV2−C-Fos-tTA and AAV2−TRE-oChIEF-tdTomato viruses into MEC/LEC. Mice were anaesthetized (10 ml/kg−1) using a mixture of ketamine (100 mg/ml−1)xylazine (20 mg/ml−1) and placed in the stereotactic system. Anaesthesia was maintained by booster doses of ketamine (100 mg kg−1). An optic probe consisting of a tungsten electrode (0.5 MΩ) was attached to an optic fibre (200-μm core diameter), with the tip of the electrode extending beyond the tip of the fibre by 300 μm, was used for simultaneous optical stimulation and extracellular recording. The power intensity of light emitted from the optrode was calibrated to about 10 mW, consistent with the power used in behavourial assays. oChIEF-2 cells were identified by delivering 20-ms light pulses (1 Hz) to the recording site every 50–100 μm. After light-responsive cells were detected, multi-unit activity in response to trains of light pulses (200 ms) at 100 Hz was recorded. Data acquisition used an Axon CNS Digidata 1440A system. MATLAB analysis was performed, as previously described. Contextual fear conditioning. Two distinct contexts were employed. Context A was 29 × 25 × 22 cm chambers with grid floors, opaque triangular ceilings, red lighting, and scented with 1% acetic acid. Four mice were run simultaneously in four identical context A chambers. Context B consisted of four 30 × 25 × 33 cm chambers with perspex floors, transparent square ceilings, bright white lighting, and scented with 0.25% benzaldehyde. Chamber ceilings were customized to hold a rotary joint (Doric Lenses) connected to two 0.32-m patch cords. All mice had patch cords fitted to the optic fibre implant before testing. Two mice were run simultaneously in two identical chambers. ChR2 was stimulated at 20 Hz (15 ms pulse width) using a 473 nm laser (10–15 mW), for the designated epochs. Testing sessions were 12 min in duration, consisting of four 3 min epochs, with the first and third as light-off epochs, and the second and fourth as light-on epochs. At the end of 12 min, the mouse was detached and returned to its home cage. Floors of chambers were cleaned with quatrifide before and between runs. In vivo optical LTP. One day after CFC training and engram labelling (DG plus PP terminals) in control and early AD groups, mice were placed in an open field arena (52 × 25 cm) after patch cords were fitted to the fibre implants. After a 15 min acclimatization period, mice with oChIEF2−PP engram terminals in the DG received the optical LTP protocol (100 blue light pulses of 2 ms each at a frequency of 100 Hz, repeated 5 times every 3 min). This in vivo protocol was repeated 10 times over a 3 h duration. After induction, mice remained in the arena for an additional 15 min before returning to their home cage. To apply optical LTP to a large portion of excitatory MEA neurons, an AAV virus expressing oChIEF-tdTomato under the CaMKII promoter, rather than a C-Fos-TTA/TRE virus (that is, engram labelling), was used. For protein synthesis inhibition experiments, immediately after the in vivo LTP induction protocol mice received 75 mg kg−1 anisomycin (Aniso) or an equivalent volume of saline intraperitoneally. Mice were then returned to their home cages. An hour later, a second injection of Aniso or saline was delivered. Inhibitory avoidance. A 30 × 28 × 34 cm unscented chamber with transparent square ceilings and intermediate lighting was used. The chamber consisted of two sections, one with grid flooring and the other with a white light platform. During the conditioning session (1 min), mice were placed on the light platform, which is the less preferred section of the chamber (relative to the grid section). Once mice entered the grid section of the chamber (all four feet), 0.80 mA shocks of 2 s duration were delivered. On average, each mouse received 2–3 shocks per training session. After 1 min, mice were returned to their home cage. The next day, latency to enter the grid section of the chamber as well as total time on the light platform was measured (3 min test). Novel object location. Spatial memory was measured in a white plastic chamber (28 × 28 cm) that had patterns (series of parallel lines or circles) on opposite walls. The apparatus was unscented and intermediate lighting was used. All mice were transferred to the behavourial room and acclimated for 30 min before the training session. On day 1, mice were allowed to explore the chamber with patterns for 15 min. On days 2 and 3, mice were introduced into the chamber that had an object (7-cm-tall glass flask filled with metal beads) placed adjacent to either patterned wall. The position of the object was counter-balanced within each genotype. On day 4, mice were placed into the chamber with the object either in the same position as the previous exposure (familiar) or at a novel location based on wall patterning. Frequency of visits to the familiar and novel object locations was quantified using an automated detection system (EthoVision XT, Noldus). Total time exploring the object was also measured (nose within 1.5 cm of object). The tracking software plotted heat maps based on exploration time, which was averaged to create representative heat maps for each genotype. Raw data were extracted and analysed using Microsoft Excel.

28. Reijmers, L. G., Perkins, B. L., Matsuo, N. & Mayford, M. Localization of a stable neural correlate of associative memory. Science 317, 1230–1233 (2007).
29. Urringer, S. et al. Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity. Proc. Natl Acad. Sci. USA 97, 7963–7968 (2000).
Extended Data Figure 1 | Characterization of 7-month-old early AD mice. a–d, Images showing hippocampal Aβ+ plaques lacking in control mice (a, b) and 7-month-old AD mice (c), which showed an age-dependent increase in 9-month-old AD mice (d). e, f, Images showing neuronal nuclei (NeuN) staining of DG granule cells in control (e) and 7-month-old AD (f) mice. g, NeuN+ fluorescence intensity of the granule cell layer from control and AD sections shown in e, f (n = 8 mice per group). h, i, Heat maps showing exploratory behaviour in an open field arena from control (h) and 7-month-old AD (i) mice. j, k, Distance travelled (j) and velocity (k) did not differ between control and AD groups (n = 9 mice per group). l, m, Images showing adult newborn neurons (DCX+) in DG sections from control mice (l) that are double positive for NeuN (m). n, Percentage of NeuN+ cells among DCX+ cells (n = 3 mice). o, p, Images showing DCX+ neurons in DG sections from control (o) and AD (p) groups (n = 4 mice per group). q, DCX+ cell counts from control and AD mice. Data are presented as mean ± s.e.m.
Extended Data Figure 2 | Labelling and engram activation of early AD mice on DOX. a, Mice are taken off DOX for 24 h in the home cage (HC) and subsequently trained in CFC. DG sections (n = 3 mice per group) revealed 2.05% ChR2–eYFP labelling in the home cage, consistent with the previously established engram tagging strategy 11. b, Mice were injected with a virus cocktail of AAV9-c-Fos-tTA and AAV9-TRE-ChR2-eYFP. After 1 day off DOX, kainic acid was used to induce seizures. Image showing efficient labelling throughout the DG. c, ChR2–eYFP cell counts from DG sections shown in b (n = 3 mice). d, Behavioural schedule for optogenetic activation of DG engram cells. e, Memory recall 1 day after training (test 1) showed less freezing of AD mice compared with control mice (n = 8 mice per group). f, Engram activation with blue light stimulation (left). Average freezing for the two light-off and light-on epochs (right). Statistical comparisons are performed using unpaired t-tests; **P < 0.01. Data are presented as mean ± s.e.m.
Extended Data Figure 3 | Chronic DG engram activation in early AD mice did not rescue long-term memory. a, Behavioural schedule for repeated DG engram activation experiment. Ctx, context. b, AD mice in which a DG memory engram was reactivated twice a day for 2 days (AD + ChR2) showed increased STM freezing levels compared with memory recall before engram reactivation (ChR2-STM test, n = 9 mice per group). c, Memory recall 1 day after repeated DG engram activations (ChR2-LTM test). NS, not significant. Statistical comparisons are performed using unpaired t-tests; *P < 0.05, **P < 0.01. Data are presented as mean ± s.e.m.
Extended Data Figure 4 | Engram activation restores fear memory in triple-transgenic and PS1/APP/tau models of early AD. a, Triple-transgenic mouse line obtained by mating c-Fos-tTA transgenic mice11,28 with double-transgenic APP/PS1 AD mice10. These mice combined with a DOX-sensitive AAV virus permits memory engram labelling in early AD. b, Triple-transgenic mice were injected with AAV9-TRE-ChR2-eYFP and implanted with an optic fibre targeting the DG. c, Image showing DG engram cells of triple-transgenic mice 24 h after CFC. d, ChR2–eYFP cell counts from control and triple-transgenic AD mice (n = 5 mice per group). e, Behavioural schedule for engram activation. f, Memory recall 1 day after training (test 1) showed less freezing of triple-transgenic AD mice compared with control mice (n = 10 mice per group). g, Engram activation with blue light stimulation (left). Average freezing for the two light-off and light-on epochs (right). Statistical comparisons are performed using unpaired t-tests; *P < 0.05, **P < 0.01. Data are presented as mean ± s.e.m. 

h, 3xTg-AD model knock-in mice + Two transgenes ChR2-eYFP DG

i, Image showing memory engram cells in the DG of 3xTg-AD mice 24 h after CFC. j, ChR2–eYFP cell counts from DG sections of control and 3xTg-AD mice (n = 4 mice per group). k, Behavioural schedule for engram activation. l, Memory recall 1 day after training (test 1) showed less freezing of 3xTg-AD mice compared with control mice (n = 9 mice per group). m, Engram activation with blue light stimulation (left). Average freezing for the two light-off and light-on epochs (right). Statistical comparisons are performed using unpaired t-tests; *P < 0.05, **P < 0.01. Data are presented as mean ± s.e.m.
Extended Data Figure 5 | Dendritic spines of engram cells in 7-month-old early AD mice. a, Average dendritic spine density of DG engram cells showed an age-dependent decrease in 7-month-old APP/PS1 AD mice (n = 7,032 spines) as compared to 5-month-old AD mice (n = 4,577 spines, n = 4 mice per group). Dashed line represents spine density of control mice (1.21). b, Left, average dendritic spine density of CA3 engram cells in control (n = 5,123 spines) and AD mice (n = 6,019 spines, n = 3 mice per group). Right, average dendritic spine density of CA1 engram cells in control (n = 9,120 spines) and AD mice (n = 7,988 spines, n = 5 mice per group). NS, not significant. Statistical comparisons are performed using unpaired t-tests; **P < 0.01. Data are presented as mean ± s.e.m.
Extended Data Figure 6 | High-fidelity responses of oChIEF+ cells and dendritic spines of DG engram cells after in vitro optical LTP.

**a**, EC cells were injected with a virus cocktail containing AAV9-TRE-oChIEF-tdTomato for activity-dependent labelling. **b**, Image showing a biocytin-filled oChIEF+ stellate cell in the EC. **c**, 100 Hz (2-ms pulse width) stimulation of an oChIEF+ cell across 20 consecutive trials. Spiking responses exhibit high fidelity. **d**, Average dendritic spine density of biocytin-filled DG cells showed an increase after optical LTP induction in vitro (n = 1,452 spines, n = 6 cells). Statistical comparisons are performed using unpaired t-tests; *P < 0.05. Data are presented as mean ± s.e.m. © 2016 Macmillan Publishers Limited. All rights reserved.
**Extended Data Figure 7 | Behavioural rescue and spine restoration by optical LTP is protein-synthesis dependent.**

**a,** Modified behavioural schedule for long-term rescue of memory recall in AD mice in the presence of saline or anisomycin (left). Memory recall 2 days after LTP induction followed by drug administration showed less freezing of AD mice treated with anisomycin (AD + 100 Hz + Aniso) compared with saline-treated AD mice (AD + 100 Hz + saline, n = 9 mice per group; right). Dashed line represents freezing level of control mice (48.53).

**b,** Average dendritic spine density in early AD mice treated with anisomycin after LTP induction (n = 4,810 spines) was decreased compared with saline-treated AD mice (n = 6,242 spines, n = 4 mice per group). Dashed line represents spine density of control mice (1.21). Statistical comparisons are performed using unpaired t-tests; *P < 0.05. Data are presented as mean ± s.e.m.
Extended Data Figure 8 | Rescued early AD mouse behaviour in a neutral context and control mouse behaviour after in vivo optical LTP.

a, After the long-term rescue of memory recall in AD mice (test 2; Fig. 3m), animals were placed in an untrained neutral context to measure generalization (n = 10 mice per group). Rescued AD mice (AD + 100 Hz) did not display freezing behaviour. b, Left, average dendritic spine density of DG engram cells from control mice remained unchanged after optical LTP induction in vivo (control + 100 Hz, n = 4,211 spines, n = 3 mice; control data from Fig. 2c). Right, the behavioural rescue protocol applied to early AD mice (Fig. 3m) was tested in age-matched control mice (n = 9 mice per group). Similar freezing levels were observed after optical LTP (test 2) as compared to memory recall before the 100 Hz protocol (test 1). NS, not significant. Statistical comparisons are performed using unpaired t-tests. Data are presented as mean ± s.e.m.
Extended Data Figure 9 | Optical LTP using a CaMKII-oChIEF virus did not rescue memory in early AD mice. **a**, AAV virus expressing oChIEF-tdTomato under a CaMKII promoter. **b**, CaMKII-oChIEF virus injected into MEC and LEC. **c**, **d**, Images showing tdTomato labelling in a large portion of excitatory MEC neurons (**c**) as well as the PP terminals in the DG (**d**). **e**, In vivo optical LTP protocol. **f**, Behavioural schedule for long-term rescue of memory recall in AD mice (left). In contrast to the engram-specific strategy, long-term memory could not be rescued by stimulating a large portion of excitatory PP terminals in the DG (right; n = 9 mice per group). NS, not significant. Statistical comparisons are performed using unpaired t-tests. Data are presented as mean ± s.e.m.
Extended Data Figure 10 | Normal DG mossy cell density after engram cell ablation. a–d, Images showing DG engram cells after saline treatment (a) and the corresponding calretinin positive (CR+) mossy cell axons (b). DTR–eYFP engram cell labelling after DT treatment (c) and the respective CR+ mossy cell axons (d). e, CR+ fluorescence intensity of mossy cell axons from saline- and DT-treated DG sections shown in a–d (n = 8 mice per group). Data are presented as mean ± s.e.m.