

**Memory trace interference impairs recall in a mouse model of Alzheimer's disease**

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## Abstract

In Alzheimer's disease (AD), hippocampus-dependent memories underlie an extensive decline. The neuronal ensemble encoding a memory, termed engram, is partly recapitulated during memory recall. Artificial activation of an engram can restore memory in a mouse model of early AD, but its fate and the factors that render the engram nonfunctional are yet to be revealed. Here we used repeated two-photon *in vivo* imaging in fosGFP transgenic mice that performed a hippocampus-dependent memory task. We found that the partial reactivation of the CA1 engram during recall is preserved under AD-like conditions. However, we identified a novelty-like ensemble that interfered with the engram and thus compromised recall. Mimicking a novelty-like ensemble in healthy mice was sufficient to affect memory recall. In turn, reducing the novelty-like signal rescued the recall impairment under AD-like conditions. These findings suggest a novel mechanistic process that contributes to the deterioration of memories in AD.

## Introduction

Memories are the results of our past experiences and crucial for our everyday life. The presence of neural correlates underlying and serving memory formation during learning was hypothesized already almost a century ago<sup>1</sup>. These neuronal correlates - termed engram or memory trace<sup>2</sup> - are partly reactivated during memory recall<sup>3,4</sup>. Memory traces are identified<sup>5</sup> with the help of immediate early gene (IEG) expression that serves as a correlate of neuronal activity<sup>6-8</sup> and activity-dependent plasticity<sup>9</sup>. The manipulation of memory traces has led to valuable insights in the relevance of these neuronal ensembles for memories and associated behavior in various brain regions<sup>2</sup>.

In Alzheimer's disease (AD), the most common form of dementia<sup>10,11</sup>, declarative memories are subject to decline<sup>12,13</sup>. This symptom is associated with severe pathophysiological changes in the hippocampal CA1 region in AD<sup>14</sup>. CA1 serves as the main output region of the hippocampus and is crucial for contextual memory formation and retrieval<sup>15</sup>. It is furthermore proposed to be involved in novelty detection in rodents and humans, due to its ability to act as a comparator between past and present experiences<sup>16-18</sup>. In mouse models with AD-like pathology, CA1 neurons show aberrant activity in close proximity to amyloid- $\beta$  plaques (A $\beta$  plaques)<sup>19,20</sup>, a pathological hallmark of the disease. In addition, aging, a major risk factor of AD, leads to altered contextual representations in CA1<sup>21</sup>. The artificial silencing of a CA1 engram impairs memory recall<sup>22</sup> and results in the emergence of an alternative engram that presumably neutralized a learned association<sup>23</sup>. The artificial reactivation of a contextual engram was sufficient to induce the recall of the memory<sup>24</sup>, even under amnesic<sup>25</sup> and AD-like conditions<sup>26</sup>. However, the actual fate of the hippocampal CA1 engram in the context of AD-related memory loss and the underlying mechanisms are still unknown.

Here we used repetitive hippocampal two-photon *in vivo* imaging in IEG reporter mice during hippocampus-dependent learning and memory. We revealed an intact contextual

engram under AD-like conditions in hippocampal CA1 and identified an additional ensemble interfering with memory recall. Artificial activation of an interfering ensemble impaired memory in healthy mice, whereas suppression of the interfering ensemble rescued memory deficits under AD-like conditions.

## Results

### FosGFP expression revealed two sub-populations of CA1 pyramidal neurons

To investigate cellular correlates of memory under healthy and AD-like conditions through the IEG *Fos*, we crossbred fosGFP<sup>27</sup> mice with the APP<sup>swe</sup>/PSEN1<sup>dE9</sup><sup>28</sup> (APP/PS1) mouse model of AD. To monitor fosGFP expression changes in CA1 pyramidal neurons of the dorsal hippocampus upon memory acquisition and recall, we implanted a chronic cranial window above this region and carried out two-photon *in vivo* imaging as previously described<sup>29</sup> (Fig. 1a). We assessed fosGFP expression in the same population of neurons daily, first without behavioral stimulus (BL, baseline fosGFP expression) and then upon contextual fear conditioning (cFC) and subsequent memory recall (test), a hippocampus-dependent learning and memory task (A-A and A-B, conditioning in A and test in either A or B) (Fig. 1b). Neurons that showed an increase in fosGFP expression were associated with higher neuronal activity during initial novel context exposure (~ 90 min before), compared to neurons that showed a decrease or no change in fosGFP expression (Extended Data Fig. 1). This is in line with a recent study showing that fosGFP expression is correlated with learning-related activity changes<sup>30</sup>. Only ~1% of all fosGFP-positive (fosGFP<sup>+</sup>) neurons were inhibitory (GAD67<sup>+</sup>) suggesting that the majority of fosGFP<sup>+</sup> neurons within the CA1 pyramidal layer were excitatory (Fig. 1c-e). Even though we probably slightly overestimated endogenous proto-oncogene c-Fos (c-Fos) by measuring fosGFP expression - possibly due to the longer half-life of GFP compared to c-Fos - we rarely detected endogenous c-Fos



expressing cells that were fosGFP-negative (Extended Data Fig. 2a-d). This indicates that fosGFP represents the majority of endogenous c-Fos expression. In fosGFP-APP/PS1 mice the density of fosGFP<sup>+</sup> neurons was comparable to healthy littermates, whereas the fluorescence intensity was altered in proximity to A $\beta$  plaques (Extended Data Fig. 2e-m). This is in line with previous work showing aberrant expression levels of IEGs in different mouse models of AD<sup>31,32</sup> as well as in human AD brains<sup>33,34</sup>. However, daily fosGFP expression changes under baseline conditions in fosGFP-APP/PS1 mice were comparable with those of healthy littermates, indicating no disturbance of the CA1 neuronal network on this level (Fig. 1f-i). A large fraction (~60%) of fosGFP<sup>+</sup> neurons maintained their expression from one day to the other (CON = continuous expression), whereas the remaining fraction switched expression on a daily basis (ON = expression switched on, OFF = expression switched off) (Fig. 1f-h). Both populations, CON and ON/OFF, remained constant during the baseline period, underscoring the stability of the hippocampal network in both genotypes (Fig. 1g,h). Our data suggest the existence of two neuronal sub-populations in CA1: these could either represent stable (CON) and varying (ON, OFF) contextual or spatial aspects of the environment<sup>35</sup>, or incorporate temporal information that is coded by CA1 over different scales, ranging from seconds and minutes to days<sup>36</sup>.

#### **Learning-induced fosGFP expression is intact in CA1 of fosGFP-APP/PS1 mice**

In accordance with previous studies<sup>37,38</sup> APP/PS1 transgenic mice displayed significantly reduced contextual fear memory (Fig. 1j), associated with impaired memory acquisition on the behavioral level (Extended Data Fig. 3a,b). We excluded differences in exploratory behavior between fosGFP and fosGFP-APP/PS1 mice by measuring the travelled distance in context A before conditioning (pre-shock) (Fig. 1k and Extended

Data Fig.3b). After conditioning in context A, exposure of both fosGFP and fosGFP-APP/PS1 mice to a novel, but similar context (context B) led to low freezing behavior, validating the context specificity of memory recall (Fig. 1l). We hypothesized that the impairment in memory acquisition and recall of fosGFP-APP/PS1 mice was reflected on the neuronal network level by altered fosGFP expression. In contradiction to our hypothesis we found a twofold increase of ON neurons upon memory acquisition in both, fosGFP and fosGFP-APP/PS1 mice (Fig. 1m,n). This indicates that learning-induced fosGFP expression is intact in CA1 under AD-like conditions. Similarly, endogenous c-Fos expression in CA1 did not reflect the short-term memory deficit of APP/PS1 mice (Extended Data Fig. 3c-f). On the day after memory acquisition, we detected an increase of OFF neurons in fosGFP and fosGFP-APP/PS1 mice, suggesting that the network activity returned to baseline levels (Fig. 1m,n). Upon memory recall in context A, the number of ON neurons was not altered compared to the day before conditioning (d0-1) in fosGFP mice (Fig. 1m and Supplementary Table 1). This finding is in accordance with previous results showing that c-Fos expression in CA1 is mainly elevated in response to exploration of novel rather than familiar environments or to exposure to aversive stimuli alone<sup>39</sup>. Indeed, exposing fosGFP and fosGFP-APP/PS1 mice to a novel context B on the test day led to a twofold increase of ON neurons, comparable to the increase after conditioning (Fig. 1o,p). Interestingly, re-exposing fosGFP-APP/PS1 mice to the conditioned and thus familiar context A resulted in a similar increase of their ON population (Fig. 1n), showing a tendency in the inter-group comparison (Extended Data Fig. 4). The total number of ON neurons was increased in mice exposed to a novel context B and fosGFP-APP/PS1 mice tested in context A (Fig. 1q). The number of CON neurons remained stable over time in all experimental groups. We further tested whether the population of ON cells detected upon cFC represents a memory trace. Indeed, optogenetic activation of this CA1 ensemble that

expressed c-Fos upon learning was sufficient to artificially induce freezing in APP/PS1 mice to the same extent as in wild-type mice (Extended Data Fig. 5). This is in line with, and further extends, previous findings showing that CA1 activity is necessary<sup>15,22</sup> and sufficient<sup>25</sup> for recent and remote memory recall. Moreover, this validates that CA1 neurons expressing c-Fos upon learning comprise a neuronal ensemble relevant for memory recall. In addition, our *in vivo* fosGFP data revealed an intact c-Fos expression increase in CA1 upon learning in APP/PS1 mice.

### **Sparse CA1 engram occurs independent of memory**

We then sought to describe the fate of the memory-relevant ensemble during “natural” recall and investigate the relevance of individual neurons' activity history. For this, we calculated the relative frequency of all possible fosGFP expression patterns based on the data in Fig. 1 (Fig. 2a-f). The expression of fosGFP in neurons that started to express fosGFP upon cFC (ON) did not persist on the day following behavioral testing for the majority of this population, and was also not induced by memory recall (Fig. 2g). Only a fifth of the initially activated neurons were reactivated during recall. Only a tenth of neurons that had been fosGFP-positive the day before conditioning (CON) were reactivated (Fig. 2h). Counterintuitively, the reactivated ensembles that expressed fosGFP upon learning was of similar size under healthy and under AD-like conditions, comprising around 15% of the initially active ensemble (Fig. 2i). Moreover, the relative frequency of reactivated neurons (pattern C and I) increased in all four experimental groups (Fig. 2j), indicating that potential engram cells were present regardless of memory performance in the three experimental groups that exhibited decreased freezing (G<sub>2</sub>, fosGFP-APP/PS1 A-A; G<sub>3</sub>, fosGFP A-B and G<sub>4</sub>, fosGFP-APP/PS1 A-B) as well as in mice with intact memory recall (G<sub>1</sub>, fosGFP A-A) (Fig. 1j,l, Fig. 2j). Hence, we hypothesized that memory deficits in fosGFP-APP/PS1 mice might be reflected on the

network level by neuronal ensembles interfering with the memory trace during recall. Therefore, we analyzed the fosGFP expression history of neurons that were fosGFP<sup>+</sup> upon recall and thus constituted the recall network (RN) (Fig. 3a). Among the possible fosGFP expression patterns, three patterns (C, F, I) displayed a clear change in the relative frequency between baseline (BL) and the learning and memory period (A-A/B) (Fig. 3a,b). Pattern C and I, representing reactivated neurons, increased in relative frequency to a similar extent in all four experimental groups confirming our previous finding that potential engram cells are present independent of memory (Fig. 3c). Surprisingly, pattern F, comprising neurons with first-time fosGFP expression upon memory recall, was only increased in mice with impaired memory (G<sub>2</sub>, fosGFP-APP/PS1 A-A) and those exposed to a novel context (G<sub>3</sub>, fosGFP A-B and G<sub>4</sub>, fosGFP-APP/PS1 A-B), compared to those successfully recalling the context (G<sub>1</sub>, FosGFP A-A) (Fig. 3d). Since these neurons potentially encoded novel context information (test in context B), we termed these neurons novelty-like cells. Next, we determined the ratio of engram cells to novelty-like cells for each group in order to assess engram cell frequency relative to novelty-like cell frequency. In accordance with the behavioral performance of the mice, this ratio was significantly increased only in G<sub>1</sub> (fosGFP A-A), but not in G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub> (Fig. 3e). Comparing the complete recall network of the four experimental groups by calculating a correlation coefficient matrix of their relative pattern frequency changes revealed that the neuronal network composition of fosGFP-APP/PS1 A-A mice (G<sub>2</sub>) displayed a configuration similar to fosGFP mice exposed to a novel context B (G<sub>3</sub>) (Fig. 3f and Supplementary Table 2). Our finding suggests that fosGFP-APP/PS1 mice perceive the familiar context A as novel, resulting in the interference of novelty-like ensembles with the otherwise intact engram and thus impeding memory recall (Fig. 3g).

### **Reducing novelty-like activity ameliorates memory recall in APP/PS1 mice**

To test whether novelty-like ensemble activity in CA1 was sufficient to affect memory recall, we induced activity with DREADDS (designer receptors exclusively activated by designer drugs) in excitatory neurons. The artificial activation of glutamatergic neurons in CA1 during memory recall decreased freezing to the conditioned context (Extended Data Fig. 6). Instead of artificially activating a large subset of CA1 neurons, we next mimicked a novelty-like cell ensemble by activating only neurons that encoded a novel context (context B). Indeed, artificial activation of neurons encoding a novel context B decreased freezing in the conditioned context A (Fig. 4a-f and Extended Data Fig. 7). Moreover, silencing neurons encoding a novel context B (Extended Data Fig. 8), to reduce novelty-like cell activity during recall, rescued memory deficits of APP/PS1 mice (Fig. 4g-k), excluding any unspecific effects of CNO (clozapine-N-oxide) or tagging alone (Extended Data Fig. 9 and Extended Data Fig. 10). In summary, our results indicate that novelty-like activity in CA1 interferes with memory recall. Accordingly, the reduction of novelty-like activity restored memory in APP/PS1 mice.

## Discussion

Our analysis of fosGFP expression dynamics in hippocampal CA1 revealed two subpopulations of pyramidal neurons - one maintaining fosGFP expression on a daily basis and another switching it on and off. This suggests a different role of both populations in information processing, in line with the fact that CA1 is increasingly recognized as heterogeneous population of neurons<sup>40</sup>.

According to this, we found the potential contextual engram to be part of both subpopulations by constituting 15% of the population active during learning. Our finding of a sparse and transient engram cell ensemble supports the hypothesis that memory traces in hippocampal CA1 are rapidly refined, thereby increasing the efficiency of information processing<sup>2,41,42</sup>. Although we did not investigate reactivation during remote memory

recall, we suspect the engram to be sparser and hardly visible on the level of CA1 fosGFP expression. This hypothesis is based on the memory index theory, which postulates that the hippocampus serves as an indexing system for complex representations of context manifested in cortical networks<sup>35</sup>.

In our study, impairment of hippocampus-dependent memory under AD-like conditions was associated with the presence of novelty-like cells in the recall network, presumably interfering with engram cells, rather than with the absence of the memory engram. Although we found the memory trace to be intact on the level of fosGFP expression, we cannot exclude impairments beyond the reporters' temporal and spatial resolution, for example in coordinated firing of CA1 pyramidal neurons or downstream connectivity. Accordingly, novelty-like cells found under AD-like conditions might resemble an alternative engram that was observed during optogenetic inhibition of the retrieved engram in CA1<sup>23</sup>. Here, the alternative engram could act as a competing memory trace. It was shown before that the artificial activation of a competing memory trace was sufficient to interfere with recall of the conditioned context<sup>43</sup>. In this study<sup>43</sup> the memory trace was artificially activated across all brain regions, in contrast to our CA1-specific study.

By mimicking novelty-like activity, we cannot exclude an associated "safety" or "non-aversive" component within the artificially activated ensemble that reduced freezing. However, our data argue against this possibility as memory interference worked equally well with a randomly activated set of neurons without any associated context or valence (Extended Data Fig. 6). This is in line with previous findings that even a slight stimulation of the hippocampal formation was sufficient to impair spatial memory<sup>44</sup>. We thus conclude that the novelty-like ensemble and not the associated valence is the dominating factor that interferes with recall.

Reducing potential novelty-like ensemble activity had a beneficial effect on memory

under AD-like conditions. A different interpretation of Fig.4g-k would be that exposure to context A elicited both, recall of the safety-associated context B and fear-associated context A and thus, silencing of context B ensembles resulted in an increased freezing response due to the dominating fear recall. However, our freezing data in Fig. 1l as well as the cellular data in Fig. 3f indicate that APP/PS1 mice discriminate context A and context B very well, despite their impairment in recalling context A. Therefore, we conclude that inhibition of the context B ensemble decreases the possibility of novelty-like activity and thereby increases engram contrast. Vice versa, we propose that novelty-like neural activity in the recall network interferes with successful recall on the behavioral level, presumably by perturbing information flow. This mechanism might even apply for fosGFP mice exposed to the novel context B ( $G_3$ ) that as well show context A engram cells during recall in context B. The presence of a novelty-like ensemble in a familiar environment would hence indicate a defect in CA1's function as a comparator between past and present contextual experiences under AD-like conditions. Our data suggest that CA1 wrongly detect a mismatch between the perceived context during recall and the memory of the past experience during conditioning, mistakenly categorizing a known context as novel (Fig. 2f). A similar mis-classification phenotype was shown in CA1 of aged mice<sup>21</sup>. Here, two visits of the same environment resulted in less similar CA1 representations compared to young mice. Hence the question is what mechanism underlies the mis-classification phenotype? Previous studies showed that the concerted arrival of precisely timed inputs from CA3 and entorhinal cortex (EC) at different dendritic compartments of CA1 neurons is indispensable for successful information flow<sup>45</sup> and most probably for performing this computation<sup>46,47</sup> (Fig. 3g). It is tempting to speculate that impaired connectivity of CA1 inputs and resulting impairments in CA1 multimodal input synchrony caused this mis-classification phenotype. The medial EC for example is affected early during the pathology of AD<sup>10,48</sup> and optogenetic stimulation of connections

270 between dentate gyrus (DG) and EC was sufficient to rescue memory deficits in a  
271 mouse model of AD<sup>26</sup>. Furthermore, CA3 input to CA1 neurons might be affected. An  
272 impaired dendritic computation of CA1 neurons due to structural and physiological  
273 synaptic deficits<sup>49</sup> may also contribute to the observed mis-classification. However, this  
274 needs to be further investigated. Our results reveal the neuronal network dysfunction in  
275 CA1 as a contributing factor to memory deficits under AD-like conditions and provide  
276 relevant evidence that might facilitate the development of a novel strategy to counteract  
277 AD-related memory loss.



## **Acknowledgements**

This work was supported by the DZNE, grants from the Deutsche Forschungsgemeinschaft (SFB 1089 C01 and B06), Centres of excellence in Neurodegeneration (CoEN 3018), the ERA-NET MicroSynDep and MicroSchiz project. We thank P. Thevenaz and Erik Meijering for the development of the ImageJ plugins “stackreg” and “TurboReg”. We acknowledge William Wisden for providing the Addgene plasmids #66794 and #66795. We thank Bryan Roth for providing the Addgene plasmid #50475 and the Addgene viral prep #44361. Addgene plasmid #26973 was a gift from Karl Deisseroth. We acknowledge Susanne Schoch and Lioba Dammer for cloning and preparation of viral particles. We thank Simon Wiegert, Stefan Remy, Gabor Petzold, Rafał Czajkowski and Laura Ewell for helpful discussion on the manuscript and the Light Microscope and Animal Facilities of DZNE for constant support.

## **Author contributions**

Conceptualization, M.F. and S.P.; Methodology, S.P. and L.C.J.; Formal Analysis, S.P., M.M., F.M. and J.W.; Investigation, S.P., L.C.J., J.S., M.M. and E.A.G.; Resources, L.D., S.S., B.S., W.S.J. and D.E.; Writing – Original Draft, M.F. and S.P.; Writing – Review & Editing, M.F., S.P., E.A.G.; Visualization, S.P.; Supervision, M.F.; Funding Acquisition, M.F.

## **Competing interests**

The authors declare no competing interests.

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## Figure titles and legends

**Fig. 1 Accessing fosGFP expression *in vivo* revealed two sub-populations of CA1 neurons under healthy and AD-like conditions.** **a**, Schematic of hippocampal *in vivo* imaging under healthy (fosGFP) and AD-like conditions (fosGFP-APP/PS1 mice). **b**, Timeline and experimental groups to investigate baseline (BL) and learning- and memory-induced (A-A and A-B) fosGFP expression; cFC, contextual fear conditioning; test, memory recall. **c-e**, Two-photon *in vivo* fosGFP expression (**c**, left) and confocal images (**c**, right) plus quantification (**d,e**) of fosGFP- and GAD67-positive neurons in CA1. In **d** and **e**, data from n=3 fosGFP mice. **f**, Illustration of fosGFP expression changes: neurons that gained (ON), lost (OFF) or kept (CON) their fosGFP expression in a day-to-day interval are color-coded in green, magenta and blue, respectively. **g,h**, Color-coded images (left) and quantification (right) of ON, OFF and CON in a day-to-day interval neurons during BL in fosGFP (**g**) and fosGFP-APP/PS1 mice (**h**). **i**, Average percentage of ON, OFF and CON neurons during BL in fosGFP and fosGFP-APP/PS1



mice. **j**, Freezing during memory test in the conditioned context A. **k**, Travelled distance  
 in context A before conditioning, pre-shock. **l**, Freezing in a novel context B. **m-p**, Color-  
 coded images (left) and quantification (right) of fosGFP expression changes in fosGFP  
 and fosGFP-APP/PS1 mice trained in context A, and tested either in context A (**m,n**) or  
 B (**o,p**). **q**, Number of ON cells upon d3-4 during BL and A-A/B. Data in **g-i** and **m-q**  
 from n=7 fosGFP A-A ( $G_1$ ), n=6 fosGFP-APP/PS1 A-A ( $G_2$ ), n=6 fosGFP A-B ( $G_3$ ) and  
 n=3 fosGFP-APP/PS1 A-B ( $G_4$ ) mice; BL data (**g-i**) comprise measurements from 3745  
 ( $G_1$ ), 4195 ( $G_2$ ), 2983 ( $G_3$ ) and 2025 ( $G_4$ ) cells, respectively; data including BL and A-A/B  
 imaging (**m-q**) comprise measurements from 4322 ( $G_1$ ), 5099 ( $G_2$ ), 3755 ( $G_3$ ) and 2195  
 ( $G_4$ ) cells, respectively; statistics were done over mice; data in **j** and **l** from n=13 fosGFP  
 and n=14 fosGFP-APP/PS1 mice; data in **k** from n=15 fosGFP and n=11 fosGFP-  
 APP/PS1 mice (see also Extended Data Fig. 3a,b); **g-i** and **m-o**, two-way ANOVA with  
 Holm-Sidak's multiple comparison test; **q**, three-way ANOVA with Holm-Sidak's multiple  
 comparison test; **m-p**, d0-1 (reference interval) is compared d1-2, d2-3 and d3-4,  
 statistical significant results are marked by asterisks; **j-l**, two-sided unpaired t-test; <sup>n.s.</sup>p <  
 0.05; \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; for exact p-values see Supplementary Table 1.  
 Data in **e,g-q** are presented as mean  $\pm$  SEM; line plots in **g,h** and **m-p** represent the  
 means (bold lines), SEM (shadings) and individual mouse values (fine lines) of the  
 consecutively measured data at d0-1, d1-2, d2-3 and d3-4; **e**, **i-l**, **q**, each data point  
 depicts the value of an individual mouse. Scale bars, 250  $\mu$ m (**c**, left), 50  $\mu$ m (**c**, right  
 and **g**).

**Fig. 2 Presence of engram cells independent of memory.** **a**, Scheme visualizing  
 fosGFP expression patterns (randomly named from A to O) during four days, either of  
 the baseline period (BL) or the learning and memory period (A-A/B). **(b)** Relative

frequencies of fosGFP expression patterns during A-A/B, comparing experimental groups G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub>. **c-f**, Relative frequencies of fosGFP expression patterns during BL and A-A/B for all experimental groups: G<sub>1</sub> (**c**), G<sub>2</sub> (**d**), G<sub>3</sub> (**e**), G<sub>4</sub> (**f**). **g,h**, Scheme and pie chart visualizing the expression fate of neurons that gained (ON, **g**) and those that kept (CON, **h**) fosGFP expression upon contextual fear conditioning (cFC); pie charts depict the average fraction of the respective fosGFP expression patterns in relation to the total number of ON or CON neurons, respectively; fosGFP expression patterns C, L, H, A (**g**) and I, J, E, O (**h**) correspond to those shown in (**a**). **i**, Inter-group comparison of the reactivated fraction of ON and CON neurons; i.e. frequency of pattern C and I divided by all neurons that expressed fosGFP upon cFC (ON and CON). **j**, Relative frequency of engram cells (**j**, pattern C and I) during the baseline (BL) and learning and memory period (A-A/B). Data according to Fig. 1 from n=7 fosGFP A-A (G<sub>1</sub>), n=6 fosGFP-APP/PS1 A-A (G<sub>2</sub>), n=6 fosGFP A-B (G<sub>3</sub>) and n=3 fosGFP-APP/PS1 A-B (G<sub>4</sub>) mice; data including BL and A-A/B imaging (**m-q**) comprise measurements from 4322 (G<sub>1</sub>), 5099 (G<sub>2</sub>), 3755 (G<sub>3</sub>) and 2195 (G<sub>4</sub>) cells, respectively; statistics were done over mice. **i**, two-way ANOVA with Holm-Sidak's multiple comparisons test; **j** three-way ANOVA with Holm-Sidak's multiple comparisons test; <sup>n.s.</sup>p<0.05, \*p<0.05, \*\*p<0.01, for exact p-values see Supplementary Table 1. Data are presented as mean ± SEM; each data point depicts the value of an individual mouse.

**Fig. 3 Interference of novelty-like cells with memory engram cells impairs recall. a**, Scheme visualizing fosGFP expression patterns during imaging sessions d1-d4 that depict a possible expression history of neurons composing the recall network (fosGFP<sup>+</sup> on d4). FosGFP expression patterns are randomly named by letters from A to O (see also Extended Data Fig. 6); BL, baseline period; A-A/A, learning and memory period; RN, recall network; fosGFP<sup>+</sup>, fosGFP-positive; fosGFP<sup>-</sup>, fosGFP-negative. **b**, Changes of

the relative frequency ( $\Delta f_{d4}$ ) of fosGFP expression patterns among RN neurons visualized by a color code; columns represent experimental groups ( $G_1$ ,  $G_2$ ,  $G_3$  and  $G_4$ ), whereas rows show fosGFP expression patterns corresponding to **a, c, d**, Inter-group comparisons of the change of the relative frequency ( $\Delta f_{d4}$ ) of fosGFP expression patterns C and I (**c**, engram cells) and pattern F (**d**, novelty-like cells) among RN neurons. **e**, Quantification of engram to novelty-like cell ratio. **f**, Weighted Pearson correlation coefficients for pairwise comparisons between the  $\Delta f_{d4}$  values of experimental groups  $G_1$ ,  $G_2$ ,  $G_3$ , and  $G_4$  of data shown in **b**, visualized by a color code (see Supplementary Table 2 for exact values and statistics). **g**, Scheme of CA1 within the hippocampal circuitry (left). Model visualizing the CA1 recall network (RN) under healthy and under AD-like conditions (right). Data according to Fig. 1 from  $n=7$   $G_1$ ,  $n=6$   $G_2$ ,  $n=6$   $G_3$  and  $n=3$   $G_4$  mice; data comprise measurements from 3467 ( $G_1$ ), 4377 ( $G_2$ ), 2549 ( $G_3$ ) and 1766 ( $G_4$ ) RN cells, respectively; statistics were done over mice; **c, d** two-way ANOVA with Holm-Sidak's multiple comparisons test; **f**, two-sided Pearson correlation with post-hoc t-test with Holm-Bonferroni multiple comparison correction; <sup>n.s.</sup> $p < 0.05$ ; \* $p < 0.05$ ; \*\* $p < 0.01$ ; for exact p-values see Supplementary Table 1 and 2. Data are presented as mean  $\pm$  SEM; each data point depicts the value of an individual mouse.

**Fig. 4 Inducing novelty-like activity impaired memory recall whereas reducing it ameliorated memory impairment. a, b**, Scheme of the experimental setting (**a**) and timeline (**b**) for inducing novelty-like activity (ensemble of neurons active in context B) during memory recall of the conditioned context A. **c, d**, Overview (left) and zoom (right) of exemplary confocal images showing CA1 targeted expression of hM3D(Gq)-mCherry in coronal brain slices and stained endogenous c-Fos in vehicle- (**c**) and CNO-treated (**d**) mice; for each mouse, four brain slices were stained and analyzed, with similar results. **e**, Density of c-Fos-positive (c-Fos<sup>+</sup>) neurons in vehicle- (black) and CNO-treated

(yellow) mice; data from n=7 vehicle-treated and n=8 CNO-treated mice; the value of each mouse represents an average of four analyzed brain slices. **f**, Freezing behavior of vehicle- and CNO-treated mice; data from n=12 vehicle- and n=12 CNO-treated mice. **g,h**, Scheme of the experimental setting (**g**) and timeline (**h**) for reducing novelty-like activity (ensemble of neurons active in context B) during memory recall of the conditioned context A. **i,j**, Overview (left) and zoom (right) of exemplary confocal images of coronal brain sections showing CA1 targeted expression of hM4D(Gi)-mCherry and MeXO4-stained A $\beta$  plaques; for each mouse, four brain slices were stained and quantified, with similar results; for analysis of hM4D(Gi)-mCherry activation on Fos expression see Extended Data Figure 8. **k**, Freezing behavior of mice, with (+) and without (-) hM4D(Gi)-tagged neurons. Data in **k** from n=4 APP/PS1(-), n=12 APP/PS1(+), n=4 wild-type(-) and n=13 wild-type (+); **e,f**, two-sided unpaired t-test; **k**, two-way ANOVA with Holm-Sidak's multiple comparison test; \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001 for exact p-values see Supplementary Table 1. Data are presented as mean  $\pm$  SEM; each data point depicts the value of an individual mouse. Scale bars, 500  $\mu$ m (**c**, left), 25  $\mu$ m (**c**, right).

**Extended Data Fig. 1 *In vivo* fosGFP expression is associated with elevated neuronal activity.** **a**, Scheme of the experimental setting (left) and an exemplary two-photon *in vivo* image showing jRGECO1a and fosGFP expression (right). **b**, Fractions of jRGECO1a-expressing cells that are fosGFP-positive (fosGFP<sup>+</sup>) or -negative (fosGFP<sup>-</sup>). **c**, Event frequency of fosGFP<sup>+</sup> and fosGFP<sup>-</sup> neurons. Data in **b** and **c** from 1953 jRGECO1a-expressing cells in n=4 mice. **d**, Experimental timeline to assess fosGFP expression and its underlying neuronal activity, approximated by the Ca<sup>2+</sup> event frequency. CE, novel context exposure; CTR, control. **e-j**, Exemplary two-photon images (left) and quantification (right) of UP (**e,f**), DOWN (**g,h**) and no change cells (**i,j**). **k,l**,

Exemplary two-photon image of jRGECO1a-expressing CA1 neurons (**k**) and respective  $\text{Ca}^{2+}$  traces (**l**) of identified UP, DOWN and no change neurons marked in **k**. **m,n**,  $\text{Ca}^{2+}$  event frequency of UP, DOWN and no change neurons in the group of mice exposed to a novel context (CE, **m**) and those under continuous anesthesia (CTR, **n**). **o**, Experimental timeline to determine the onset of fosGFP expression upon contextual fear conditioning (cFC). **p**, Exemplary binary two-photon images (left) and quantification (right) of fosGFP expression onset (ON) and decay (OFF). Data in **d-n** from n=4 CE and n=6 CTR mice; **c**, two-sided Mann-Whitney test; **f,h,j**, two-sided unpaired t-test; **m,n**, one-way ANOVA with Holm-Sidak's multiple comparison test; <sup>n.s.</sup>p > 0.05; \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001 for exact p-values see Supplementary Table 1. Data in **f,h** and **j** are presented as mean  $\pm$  SEM; each data point depicts the value of an individual mouse. Data in **c m** and **n** show the median (bold line), 25%- and 75%-quartiles (dashed lines), minimum and maximum values (upper and lower end of violins). Scale bars, 50  $\mu\text{m}$  (**a**, right), 5  $\mu\text{m}$  (**e, g, i**), 25  $\mu\text{m}$  (**k**, right).

**Extended Data Fig. 2 Characterization of fosGFP expressing neurons.** **a**, Coronal mouse brain section showing the analyzed part (framed) of dorsal CA1. **b**, Quantification of endogenous c-Fos-positive (c-Fos<sup>+</sup>) and fosGFP-positive (fosGFP<sup>+</sup>) neurons in wild-type and fosGFP mice, respectively, and APP/PS1 and fosGFP-APP/PS1 mice; data from n=9 wild-type, n=6 fosGFP, n=5 APP/PS1 and n=9 fosGFP-APP/PS1 mice. **c**, Representative confocal images of c-Fos<sup>+</sup> and fosGFP<sup>+</sup> neurons in CA1 of fosGFP and fosGFP-APP/PS1 mice; for each mouse, five brain slices were stained and analyzed (see **d**), with similar results. **d**, Fraction of endogenous c-Fos<sup>+</sup> nuclei that were simultaneously positive for fosGFP; data from n=3 fosGFP and n=3 fosGFP-APP/PS1 mice. **e-g**, Method to convert 8-bit raw data into binary images. Exemplary measurement of background fluorescence intensity (BG) in a fosGFP raw data image with manually

606 placed circular masks (red,  $\varnothing$  7.45  $\mu$ m) (**e**). Exemplary fosGFP raw data (**f**, left) and  
 607 corresponding binary image (**f**, right). Circular masks (white) were manually placed  
 608 above putatively fosGFP expressing nuclei to measure their fluorescence intensity (**f**,  
 609 left). Nuclei with a fosGFP fluorescence intensity above or below threshold (TH) were  
 610 defined as fosGFP<sup>+</sup> or fosGFP<sup>-</sup>, respectively (**g**). **h**, Images showing the density of  
 611 fosGFP<sup>+</sup> neurons around MeXO4-stained A $\beta$ -plaques (yellow) and randomly placed  
 612 virtual A $\beta$ -plaques in fosGFP-APP/PS1 and fosGFP mice, respectively; 250 x 250  $\mu$ m  
 613 excerpts of a 1.9 x 1.9 mm field of view; images were acquired once for each mouse;  
 614 other excerpts show similar results. **i,j**, Density of fosGFP<sup>+</sup> neurons in CA1 irrespective  
 615 of A $\beta$  plaque distance (**i**), and in the proximity (<50  $\mu$ m, near) or distant (>50  $\mu$ m, far) to  
 616 MeXO4-stained A $\beta$ -plaques (**j**) comparing fosGFP-APP/PS1 and fosGFP mice,  
 617 respectively. **k**, Exemplary images of fosGFP-expressing neurons' fluorescence  
 618 intensities in CA1 of fosGFP and fosGFP-APP/PS1 mice; 250 x 250  $\mu$ m excerpts of a  
 619 1.9 x 1.9 mm field of view; image was acquired once, other excerpts show similar  
 620 results; white circles (radius=50  $\mu$ m) in **h** and **k** depict the area close to a MeXO4-  
 621 positive A $\beta$ -plaque (in fosGFP-APP/PS1 mice) or a virtual plaque (in fosGFP mice),  
 622 respectively. **l,m**, Fluorescence intensity of fosGFP-expressing neurons in fosGFP and  
 623 fosGFP-APP/PS1 mice regardless of A $\beta$  plaque distance (**l**), and in proximity (<50  $\mu$ m,  
 624 near) or distant (>50  $\mu$ m, far) to MeXO4 stained A $\beta$  plaques (**m**); data in **h-m** from n=8  
 625 fosGFP and n=6 fosGFP-APP/PS1 mice; statistics were done over mice, except for **l** and  
 626 **m**; data in **l** are from 2873 (fosGFP) and 2092 (fosGFP-APP/PS1) cells; data in **m** are  
 627 from 1373 (fosGFP – near), 674 (fosGFP-APP/PS1-near), 1500 (fosGFP – far) and 1418  
 628 (fosGFP-APP/PS1 – far) cells; **d,i**, two-sided unpaired t-test; **j**, two-way ANOVA with  
 629 Holm-Sidak's correction for multiple comparisons; **l**, two-sided Mann-Whitney test; **m**,  
 630 Kruskal-Wallis test with Dunn's correction for multiple comparisons; \*p <0.05,

\*\*p < 0.01, \*\*\*p < 0.001, for exact p-values see Supplementary Table 1. Data in **b,d,i** and **j** are presented as mean  $\pm$  SEM; each data point depicts the value of an individual mouse. Data in **l** and **m** show the median (bold line), 25%- and 75%-quartiles (dashed lines), minimum and maximum values (upper and lower end of violins). Scale bars, 40  $\mu$ m (**c**), 20  $\mu$ m (**e,f**), 50  $\mu$ m (**h,k**).

**Extended Data Fig. 3 Impaired learning and short-term memory in APP/PS1 mice.**

**a**, Experimental protocol for fear conditioning in context A: two minutes of exploration (pre-shock) are followed by three 0.5 mA foot shocks spaced by one minute intervals. Mouse was removed from the chamber one minute after the last foot shock (see also methods section). **b**, Freezing behavior of wild-type and APP/PS1 mice during conditioning. **c**, Experimental paradigm for testing short term memory (STM). **d**, Freezing behavior of wild-type and APP/PS1 mice during STM test, one hour after conditioning. **e,f**, Fraction of c-Fos-positive (c-Fos<sup>+</sup>) per DAPI-positiv (DAPI<sup>+</sup>) neurons (**e**) and density of DAPI<sup>+</sup> neurons upon STM test (**f**). **a,b**, Data from n=17 wild-type and n=18 APP/PS1 mice; **c-f**, data from n=4 wild-type and n=4 APP/PS1 transgenic mice; **b**, two-way ANOVA with Holm-Sidak's correction for multiple comparisons; **d,f**, two-sided unpaired t-test; **e**, two-sided Mann-Whitney test; \*\*p < 0.01, for exact p-values see Supplementary Table 1. Data are presented as mean  $\pm$  SEM; each data point depicts the value of an individual mouse.

**Extended Data Fig. 4 ON cells upon memory recall.** Inter-group comparison of the fold change of ON cells upon memory recall (d3-4 in A-A/B), corresponding to Fig. 1m-p. Data according to Fig.1, n=7 fosGFP A-A (G<sub>1</sub>), n=6 fosGFP-APP/PS1 A-A (G<sub>2</sub>), n=6 fosGFP A-B (G<sub>3</sub>) and n=3 fosGFP-APP/PS1 A-B (G<sub>4</sub>) mice; data include BL and A-A/B imaging, comprising measurements from 4322 (G<sub>1</sub>), 5099 (G<sub>2</sub>), 3755 (G<sub>3</sub>) and 2195 (G<sub>4</sub>)

cells, respectively; statistics were done over mice; two-way ANOVA with Holm-Sidak's multiple comparisons test; <sup>n.s.</sup>p>0.05, for exact p-values see Supplementary Table 1. Data are presented as mean ± SEM; each data point depicts the value of an individual mouse.

**Extended Data Fig. 5 Optogenetic CA1 memory trace activation induces recall in APP/PS1 mice.** **a**, AAV-c-Fos-tTA and AAV-PTRE-tight-hChR2-EYFP were injected bilaterally into the hippocampus of APP/PS1 mice and wild-type littermates (left) before optical fibers were implanted to target CA1 (right). **b**, Experimental timeline. **c**, Representative confocal images showing hChR2-EYFP expression in wild-type (upper) and APP/PS1 mice (lower), respectively; for each mouse, four brain slices were stained and analyzed (see **i,j**), with similar results. **d,e**, Freezing during the stimulation protocol in context C, before (control, black) and after tagging (hChR2, green) of the same set of APP/PS1 (**d**, left) and wild-type mice (**e**, left). Average freezing during intervals without (off) and with (on) light stimulation in hChR2-tagged APP/PS1 (**d**, right) and wild-type (**e**, right) mice, respectively. **f,g**, Freezing during test I (**f**) and test II (**g**) comparing wild-type and APP/PS1 mice. **h**, Comparison of net freezing during light stimulation (on - off, average freezing during light on periods minus average freezing during light off periods) between wild-type and APP/PS1 mice. **i,j**, Density of hChR2-positive (hChR2<sup>+</sup>) cells regardless of Aβ plaque distance (**i**), and in proximity (<50 μm, near) or distant (>50 μm, far) to MeXO4 stained Aβ plaques in wild-type and APP/PS1 mice (**j**). Data from n=6 wild-type and n=8 APP/PS1 mice; **d** (right), **e** (right), two-sided paired t-test; **f-j**, two-sided unpaired t-test; \*\*\*\*p<0.0001, \*\*\*p<0.001, for exact p-values see Supplementary Table 1. Data are presented as mean ± SEM; each data point depicts the value of an individual mouse.



**Extended Data Fig. 6 Mimicking novelty-like activity in CA1 impaired recall performance.** **a,b**, Experimental approach. **c,d**, Overview (left) and zoom (right) of exemplary confocal images of coronal brain sections showing CA1 targeted expression of hM3D(Gq)-mCherry and c-Fos in vehicle- (**c**) and CNO-treated mice (**d**); for each mouse, four brain slices were stained and analyzed (see **e**), with similar results. **e**, Density of c-Fos-positive (c-Fos<sup>+</sup>) neurons in vehicle- and CNO-treated mice; the value of each mouse represents an average of four analyzed brain slices. **f**, Freezing behavior of vehicle- and CNO-treated mice. Data from n=5 CNO-treated and n=4 saline-treated mice; **e,f**, two-sided unpaired t-test; \*\*\*\*p<0.0001, \*p<0.05, for exact p-values see Supplementary Table 1. Data are presented as mean ± SEM; each data point depicts the value of an individual mouse. Scale bars, 500 µm (**c**, left), 50 µm (**c**, right).

**Extended Data Fig. 7 Presence of doxycycline prevents DREADD expression.** **a,b**, Experimental approach. **c**, Density of c-Fos-positive (c-Fos<sup>+</sup>) neurons after memory retrieval; the value of each mouse represents an average of four analyzed brain slices. **d**, Freezing behavior of non-treated and non-labeled mice during recall on d8 (test). **e**, Overview (left) and zoom (right) of an exemplary confocal image of a coronal brain sections showing CA1 lacking expression of hM3D(Gq)-mCherry; for each mouse, four brain slices were stained and analyzed (see **c**), with similar results. Data from n=5 wild-type mice. Data are presented as mean ± SEM; each data point depicts the value of an individual mouse. Scale bars, 500 µm (**e**, left), 50 µm (**e**, right).

**Extended Data Fig. 8 Reduced endogenous c-Fos expression in hM4D(Gi)-positive CA1 pyramidal neurons.** **a,b**, Scheme of the experimental setting (**a**) and timeline (**b**) for reducing noise-like activity (ensemble of neurons active in context B) during memory recall of the conditioned context A (see Fig. 3g-k). **c,d**, Intensity distribution of

endogenous c-Fos measured in the nuclei of hM4D(Gi)-mCherry-negative (mCherry<sup>-</sup>) and hM4D(Gi)-mCherry-positive (mCherry<sup>+</sup>) cells in both APP/PS1 (**c**) and wild-type mice (**d**). **e**, Quantification of hM4D(Gi)-mCherry expression in wild-type and APP/PS1 mice. Data from n=5 wild-type and n=3 APP/PS1 mice; **c,d**, two-sided Mann-Whitney test; **e**, two-sided unpaired t-test; <sup>n.s.</sup>p>0.05; \*\*\*\*p<0.0001, for statistical details and exact p-values see Supplementary Table 1. Data in **c** and **d** show the median (bold line), 25%- and 75%-quartiles (dashed lines), minimum and maximum values (upper and lower end of violins). Data in **e** are presented as mean ± SEM; each data point depicts the value of an individual mouse.

**Extended Data Fig. 9 CNO alone has no effect on exploratory behavior or memory recall.** **a**, Travelled distance in context B (left) and freezing in the conditioned context A (right) comparing vehicle- and CNO-treated wild-type mice expressing hM4D(Gi). Data in **a** from n=4 vehicle- and n=8 CNO-treated wild-type mice. **b**, Travelled distances in context B comparing wild-type and APP/PS1 mice expressing hM4D(Gi) (left) or lacking DREADD expression (right); data in **b** (left) from n=8 wild-type and n=10 APP/PS1 mice; data in **b** (right) from n=4 wild-type and n=4 APP/PS1 mice. **c**, Experimental timeline (left). Travelled distance in context B (middle) and freezing in the conditioned context A (right) comparing vehicle- and CNO-treated wild-type mice. Data in **c** from n=6 vehicle-treated and n=7 CNO-treated wild-type mice. **a** (left), two-sided Mann-Whitney test; **a** (right), **b** and **c**, two-sided unpaired t-test; <sup>n.s.</sup>p>0.05, for exact p-values see Supplementary Table 1. Data are presented as mean ± SEM; each data point depicts the value of an individual mouse.

**Extended Data Fig. 10 AAV injection and DREADD expression alone have no effect on memory recall.** **a,b**, Experimental setup (**a**) and timeline (**b**) to determine the influence of either hM4D(Gi) expression or sham injection (just AAV-c-Fos-tTA) on

memory recall. **c**, Exemplary confocal images showing hM4D(Gi)-mCherry expression in groups A, B and C; for each mouse, four brain slices were stained, with similar results. **d**, Freezing behavior of groups A, B and C during memory test. Data from n=6 group A, n=6 group B and n=6 group C mice. **d**, one-way ANOVA with Holm-Sidak's multiple comparison test; <sup>n.s.</sup>p>0.05, for exact p-values see Supplementary Table 1. Data are presented as mean ± SEM; each data point depicts the value of an individual mouse. Scale bars, 500 µm (**c**, left), 50 µm (**c**, right).

## METHODS

### Experimental animals

FosGFP and APP<sub>swe</sub>/PSEN1dE9 (APP/PS1) mice were obtained from The Jackson Laboratory (stock number: 014135) and the Mutant Mouse Regional Resource Center (Stock number: 034832; formerly JAX stock number: 005864), respectively. Both mouse lines were maintained on a C57/BL6 background. FosGFP mice express enhanced green fluorescent protein (EGFP) under the *Fos* promoter resulting in a fosGFP fusion protein. APP/PS1 transgenic mice express human amyloid precursor protein with a Swedish mutation APP<sub>K595N/M695L</sub> under the mouse prion protein promoter and a mutant human presenilin1 with a deletion of exon 9 (PS1-dE). Heterozygous fosGFP<sup>tg/wt</sup> and APP/PS1<sup>tg/wt</sup> were crossbred. FosGFP<sup>tg/w</sup>,APP/PS1<sup>wt/wt</sup> (fosGFP) and FosGFP<sup>tg/w</sup>,APP/PS1<sup>tg/wt</sup> (fosGFP-APP/PS1) were selected for experiments, carried out at an age of 13-18 months. Wild-type mice were on a C57/BL6 background and 3-5 month of age. APP/PS1 transgenic mice and wild-type littermates were 14-19 month of age. *Slc17a6*<sup>tm2(cre)Lowl</sup>/J (VGlut2-ires-cre) mice (The Jacksons Laboratory, stock number: 016963) were on a 129S4 background and 6-8 months of age. A summary of the experimental animals used in the manuscript can be found in the Life Sciences Reporting Summary. Mice were group-housed in colonies of up to 5 mice, separated by sex in IVC cages under specific pathogen free (SPF) conditions with unlimited access to food and water. The tap water is preprocessed in three steps to reduce the risk of bacterial and fungal growth: 1. microfiltration, 2. UV decontamination and 3. mild acidification to pH5.5. The light and dark cycle was 12h/12h and the temperature was kept constant at 22°C. Equal numbers of male and female mice were randomly assigned to the experimental groups. Behavioral experiments and imaging was carried out at the light cycle. All procedures were in accordance with an animal protocol approved by the DZNE and the government of North-Rhine-Westphalia.

## **Hippocampal window surgery**

We surgically implanted a unilateral cranial window ( $\varnothing$  3 mm) on top of the dorsal hippocampus as previously described<sup>29</sup>, centered around -1.90 mm (AP) and +2.10 mm (ML) from bregma. The mice were anesthetized with an intraperitoneal injection of ketamine/xylazine (0.13/0.01 mg/g body weight). Additionally, an anti-inflammatory (dexamethasone, 0.2 mg/kg) and an analgesic drug (buprenorphine hydrochloride 0.05 mg/kg; TEMGESIC®, Reckitt Benckiser Healthcare (UK) Ltd., Great Britain) were subcutaneously administered immediately before surgery. The analgesic was applied for three consecutive days after surgery as post-operative treatment. To avoid an experimental bias caused by inflammatory processes, mice were given at least four weeks to completely recover from surgery before *in vivo* imaging started.

## **Hippocampus two-photon *in vivo* imaging**

*Fos*-driven EGFP expression data were acquired using an upright Zeiss (Carl Zeiss Microscopy GmbH, Jena, Germany) Axio Examiner LSM7MP setup, equipped with a Coherent Cameleon Ultra2 two-photon laser (Coherent, Dieburg, Germany). A 16x water immersion objective with a numerical aperture of 0.8 (Nikon) was used. Enhanced green fluorescent protein (EGFP) was excited at 920 nm. Fluorescence emission was separated by a dichroic mirror (LP555), detecting the green (BP 500-550) and red emitted light (BP 575-610) with non-descanned detectors (NDDs). Image acquisition was performed with ZEN2010 (Carl Zeiss Microscopy GmbH). For each imaging session mice were anesthetized with ketamine/xylazine (0.13/0.01 mg/g body weight). To record *fosGFP* expression in the dorsal CA1 region of the hippocampus we acquired a tile scan consisting of 3 x 3 separate z-stacks of 120  $\mu$ m depth with 3  $\mu$ m z-spacing (0.5  $\mu$ m/pixels), starting at the surface of the *stratum pyramidale*, thereby spanning a large part of the dorsal CA1 area. For each mouse a similar area of around 0.25 mm<sup>2</sup>

was analyzed. Repetitive scanning of the same positions over time was achieved by orienting to the vascular pattern under reflected light illumination using a GFP filter set and a metal halide lamp HXP100 (Carl Zeiss Microscopy GmbH, Jena, Germany). Visualization of MeXO4-stained A $\beta$  plaques was achieved by exciting at 780 nm and detecting emission with a BP 450/60 filter. We acquired another 3 x 3 tile scan of 220  $\mu$ m depth with 5  $\mu$ m z-spacing during the first imaging session (d0), which was aligned to the fosGFP data in x,y and z, but exceeding it in z, 50  $\mu$ m below and above. Contextual fear conditioning and memory test were conducted two hours before imaging sessions to ensure induction of fosGFP expression. To avoid handling-evoked fosGFP expression mice were accustomed to the experimenter and to the involved transport between holding and behavior rooms on three consecutive days before the first imaging session. For *in vivo* imaging, mice were anaesthetized and let recover from anesthesia inside their home cage. FosGFP-APP/PS1 transgenic mice received an intraperitoneal dose of 50  $\mu$ g MeXO4 (0,5  $\mu$ g/ $\mu$ L MeXO4, 10% DMSO, 45% 1,2-Propanediol)<sup>50</sup> during the handling sessions. FosGFP mice received the same volume of the vehicle (solvent without MeXO4). Each mouse underwent ten imaging sessions: five during the baseline period (BL, d0-d4), and five during the learning and memory period (A-A/B, d0-d4), separated by at least two weeks.

For the correlation of fosGFP expression with neuronal activity, the *Fos*-driven EGFP signal and jRGECO1a signal were acquired using a galvo-resonant scanner (Thorlabs, Newton, USA) on a custom-built two-photon microscope equipped with a titanium sapphire (Ti:Sa) 80 MHz Cameleon Ultra II two-photon laser (Coherent, Dieburg, Germany). A 16x water immersion objective with a numerical aperture of 0.8 (Nikon) was used. EGFP was excited at 920 nm; the jRGECO1a calcium indicator was excited as 980 nm. Fluorescence emission was separated by a dichroic mirror (LP562), detecting the green (BP 525/50) and red emitted light (BP 607/70). The emission signal was

collected using a GaAsP PMT (Thorlabs, Newton, USA). ThorImageLS software (Thorlabs, version 2.1) was used to control image acquisition. 3-5 time series (640 x 343  $\mu\text{m}$ , 0.715  $\mu\text{m}/\text{pixel}$ ) á 2:50 minutes were acquired at 32.3 Hz.

### **Correlation of fosGFP expression changes with neuronal activity**

Mice were anesthetized using 5% isoflurane, directly after taking them out of their home cage. While being under anesthesia, the mice were head-fixed under the microscope and placed on a custom-built linear treadmill. After the localization of the CA1 pyramidal cell layer, an initial recording of the *Fos*-driven EGFP signal was performed. Then, the anesthesia was removed and the mice were allowed to slowly wake up and experience the treadmill for the first time (CE, context exposure). This moment was defined as minute zero. Recording of jRGECO1a signal was started directly upon removal of anesthesia and performed for the next 15 minutes. Subsequently, another recording of EGFP signal was performed at 60 and 90 minutes. As a control, the same procedure was performed while maintaining a constant anesthesia of 1% isoflurane (CTR, control).

### **Contextual fear conditioning**

The training and the recall for contextual fear conditioning were conducted in a chamber (22 x 20 x 40 cm) composed of transparent plastic walls, a stainless steel grid floor connected to an aversive stimulator/scrambler (Med associates Inc.) and bright light conditions (context A). For training mice were placed in the chamber for 120 seconds before the first foot shock was delivered (0,75 mA, 2 seconds). With an inter-shock interval of 60 seconds two more shocks were applied and mice were returned to the home cage 60 seconds after the third shock. For contextual memory recall mice were placed in the conditioning chamber 2 days after the fear conditioning and were allowed to explore the context for 5 minutes. After every trial the chamber was cleaned by 70%

ethanol. Context B was located in a different room than the conditioning chamber. It was composed of red transparent plastic walls (21 x 21 x 40 cm), a white soft plastic floor and dim light conditions. Here, inter-trial cleaning was conducted with pine flavored 70% ethanol. Context C was composed of white non-transparent MDF (medium density fiber) walls and floor (25 x 25 x 40 cm). For the optogenetic experiment, mice explored context C and context A under fiber-attached conditions and including light stimulation. To assess short-term memory (STM) mice underwent the same training protocol as mentioned above, but were tested 1 hour after conditioning. During training and test mice were video recorded from above by using a camcorder. An experimenter blind to the experimental groups determined the cumulative duration of freezing behavior during the first minute before the first foot shock was applied (pre-shock), and the first 4 minutes of the recall session, manually and offline by using EthoVision XT (Noldus). Freezing was defined as the complete absence of movement, except for breathing.

#### **Adeno-Associated Virus (AAV)**

AAV2-DIO-hM3D(Gq)-mCherry (titer:  $6.1 \times 10^{12}$  virus genomes/mL) was a gift from Bryan Roth (Addgene viral prep #44361). The plasmids pAAV-c-Fos-tTA-pA (Addgene plasmid #66794) and pAAV-PTRE-tight-hM3D(Gq)-mCherry (Addgene plasmid #66795) were a gift from William Wisden. The plasmids pAAV-PTRE-tight-hM4D(Gi)-mCherry and pAAV-PTRE-tight-hChR2(H134R)-EYFP were prepared by cloning the reading frame of hM4D(Gi)-mCherry and hChR2(H134R)-EYFP obtained from pAAV-hSyn-hM4D(Gi)-mCherry (Addgene plasmid #50475, a gift from Bryan Roth) and pAAV-hSyn-hChR2(H134R)-EYFP (Addgene plasmid #26973, a gift from Karl Deisseroth), respectively into the pAAV-PTRE-tight-hM3D(Gq)-mCherry, thereby replacing the hM3D(Gq)-mCherry. Cloning and preparation of viral particles were conducted by Lioba Dammer (kindly coordinated by Susanne Schoch).



873

#### 874 **AAV injection**

875 For bilaterally injecting AAVs, mice were anesthetized, the hair was removed at the site  
876 of incision before wiping it with 70% ethanol. A small incision was made along the  
877 midline to expose bregma and the sites of injection by carefully pushing the skin aside.  
878 Small holes were drilled and the opening of the dura mater was ascertained. From here  
879 the needle was lowered to the depth of interest from brain surface. A volume of 0.5  $\mu$ l  
880 virus per hemisphere was injected with a speed of 0.1  $\mu$ l/minute. After injecting the virus  
881 the needle was left at the site of injection for 10 minutes to allow the virus to diffuse into  
882 the tissue. The skin was closed by stitches and mice were given three weeks to recover  
883 before experiments started. For targeting CA1 the virus combinations of AAV1/2-c-Fos-  
884 tTA with either AAV1/2-PTRE-tight-hChR2(H134R)-EYFP, AAV1/2-PTRE-tight-  
885 hM3D(Gq)-mCherry) or AAV1/2-PTRE-tight-hM4(Gi)-mCherry were injected 1:2 at -1.95  
886 mm (AP),  $\pm$  1.50 mm (ML), -1.15 mm (DV, from brain surface) from bregma. Coordinates  
887 for targeting CA1 in VGlut2-ires-cre mice with AAV2-DIO-hM3Dq-mCherry slightly  
888 differed to account for the age and background difference of the mice: -1.85 mm (AP),  $\pm$   
889 1.50 mm (ML), -1.10 mm (DV, from brain surface) from bregma.

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891

#### 892 **Tagging and manipulating neuronal ensembles**

893 Doxycycline (DOX) was delivered in the preprocessed tap water at 2 mg/ml and  
894 5% sucrose from the day of injection<sup>51</sup> in order to prevent tTA-mediated expression. For  
895 tagging of neuronal ensembles, mice received preprocessed tap water without DOX for  
896 two days before they were exposed to the appropriate context for five minutes. DOX  
897 treatment was continued immediately after context exposure. To control for DOX  
898 efficiency in preventing the tTA-mediated expression, a group of mice received DOX

continuously, while being involved in the same experimental procedures. For activating hM3D(Gq) and hM4D(Gi), 3 µg/g bodyweight CNO (0.4 µg/µl CNO in 0.9% saline, 1% DMSO)<sup>52</sup> was injected intraperitoneally, 40 minutes before start of the behavioral task. Control animals received just the solvent (vehicle).

### **Immunohistochemistry**

Mice were transcardially perfused with phosphate buffered saline (PBS) pH7.4 followed by 4% paraformaldehyde (PFA) for 5 minutes. Brains were fixed over night in 4% PFA in PBS. Coronal slices of 100 µm thickness were cut on a vibratome (Leica VT 1200, Leica Germany) ranging from -1.60 to -2.60 mm (AP, from bregma). Free-floating slices were permeabilized over night in 0.8% Triton-X100™ and blocked with 4% normal goat serum (ThermoFisher Scientific) plus 4% bovine serum albumin (Carl Roth). To assess fosGFP and endogenous c-Fos expression post-mortem, mice were perfused 120 minutes and 90 minutes, respectively, after the behavioral paradigm started. FosGFP fluorescence was enhanced by using an antibody against GFP (chicken anti-GFP 1:1000; ab13970, abcam). Endogenous proto-oncogene c-Fos (c-Fos) was labeled with an antibody against c-Fos (rabbit anti-c-Fos 1:500; sc-52, Santa Cruz Biotechnology). This antibody recognizes the N-terminus of both fosGFP and endogenous c-Fos protein, hence does not allow distinguishing between both. However, neurons that just express endogenous c-Fos can be identified in fosGFP mice if counterstained by a fluorophore different from GFP (see also Extended Data Fig. 2c,d). Inhibitory interneurons were stained with an antibody targeting GAD67 (mouse anti-GAD67 1:1000; MAB5406, Merck Millipore). mCherry fluorescence was enhanced by using an antibody against mCherry (rat anti-mCherry 1:10000; M-11217, ThermoFisher Scientific). Secondary labeling was performed with an Alexa Fluor® 488 goat anti-rabbit (A-11008, ThermoFisher Scientific) and goat anti-chicken (A-11039), and Alexa Fluor® 594 goat anti-rabbit (A-11012,

ThermoFisher Scientific) and goat anti-mouse (R37121), respectively (all 1:400). DAPI was applied 1:5000 during the last 20 minutes of incubation of the secondary antibody. Further details can be found in the Life Sciences Reporting Summary.

## **Confocal microscopy**

Confocal images were acquired using an inverted LSM700 microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) with a 20x air objective. Alexa Fluor® 488 and fosGFP were excited at 488 nm and detected using a 490-555 nm bandpass filter. Alexa Fluor® 594 was excited at 555 nm and detected using a 603-627 nm bandpass filter. MeXO4 and DAPI were excited at 405 nm and detected at a wavelength of 490 nm upwards. The pinhole was set to an airy unit of one. Stacks were acquired with a resolution of 0.62 µm/pixel and a z-spacing of 2 µm.

## **QUANTIFICATION AND STATISTICAL ANALYSIS**

### **Analysis of two-photon *in vivo* images**

Raw data z-stacks were processed with the open source software Fiji (ImageJ 1.46j). Autofluorescence was reduced by subtracting the red from the green channel. Z-stacks spanning the dorsal hippocampal CA1 area (30 slices with 3 µm z-spacing) were maximum intensity projected. Maximum intensity projections of every imaging session were aligned in x-y-dimension using the “TurboReg” plugin<sup>53</sup>. Fluorescence intensities of the background (BG) and of fosGFP-positive (fosGFP<sup>+</sup>) nuclei were measured within circular masks (Ø 7.45 µm) that were manually placed besides and above potentially fosGFP<sup>+</sup> nuclei, respectively. Masks were consecutively numbered, thereby enabling to track and identify fosGFP fluorescence intensity of individual nuclei throughout the experiment. Neurons were categorized as fosGFP<sup>+</sup> or fosGFP<sup>-</sup> if the signal intensity was above or below threshold ( $\geq 6$  SD above the mean BG fluorescence), respectively.

Obtained binary images were used to calculate the density of fosGFP<sup>+</sup> neurons. Density and fosGFP fluorescence intensities near and far from MeXO4-stained A $\beta$  plaques were measured in the data obtained from the first imaging session (BL, d0). To distinguish between fosGFP<sup>+</sup> neurons in proximity and distant to a MeXO4-stained A $\beta$  plaques, a circular mask (radius = 50  $\mu$ m) was centered on the A $\beta$ -plaque, identified in the maximum intensity projection of the respective image. Neurons inside the mask were defined as proximal (<50  $\mu$ m, near) to the A $\beta$  plaque, neurons outside as distant (>50  $\mu$ m, far). We randomly applied the masks of fosGFP-APP/PS1 mice to fosGFP mice, to allow for defining fosGFP<sup>+</sup> neurons proximal and distant to virtual A $\beta$  plaques in fosGFP mice.

#### **Visualization of fosGFP expression changes**

FosGFP expression changes were defined on a day-to-day interval (d0-1, d1-2, d2-3, d3-4). Changes were categorized in ON (from fosGFP<sup>-</sup> to fosGFP<sup>+</sup>), OFF (from fosGFP<sup>+</sup> to fosGFP<sup>-</sup>) and CON (staying fosGFP<sup>+</sup>). The fractions of ON, OFF and CON neurons were calculated by dividing the total number of neurons in each category by the sum of ON, OFF and CON neurons per day-to-day interval. To calculate the fold change of each category during the learning and memory period (A-A/B), the obtained value was divided by the average baseline value of the corresponding category. Here, d0-1 was used as a reference point for statistical comparisons within genotypes, as no behavioral stimulation preceded. Images containing ON, OFF and CON neurons were generated by pseudo-coloring the circular masks (see also Analysis of two-photon *in vivo* images) with green, magenta and blue, respectively.

#### **fosGFP expression pattern and network similarity analysis**

For expression pattern analysis we determined the fosGFP expression on four consecutive days from d1 to d4 during both, the baseline (BL) and learning and memory period (A-A/B). Each neuron can theoretically adopt 1 of 15 different activity pattern ( $2^4=16$ , minus 1, for neurons never active) that we randomly named from A to O. We determined the activity pattern of every individual neuron and calculated the relative frequency ( $f_{rel}$ ) of each pattern relating to the total number of fosGFP<sup>+</sup> neurons during the respective imaging period.

For analyzing the activity history of the recall network (RN), i.e. neurons active upon recall, we selected all activity patterns that feature fosGFP expression on d4 (A,B,C,D,F,G,I,O). The relative frequencies  $f_{d4\_BL}$  and  $f_{d4\_A-A/B}$  of the respective patterns during baseline (BL) and during the learning and memory period (A-A/B, see also Fig. 2a), respectively, were calculated by dividing the number of the observed pattern by the number of neurons fosGFP<sup>+</sup> on d4.

To compare the recall network (RN) of the experimental groups, the change of relative frequencies ( $\Delta f_{d4}$ ) of any fosGFP expression pattern among the neurons composing the RN was calculated as follows:

$$\Delta f_{d4} = 100 * \frac{f_{d4\_A-A/B} - f_{d4\_BL}}{f_{d4\_BL}},$$

$\Delta f_{d4}$  is represented by a color code in Fig. 2b. To measure the similarity of recall networks, we correlated the  $\Delta f_{d4}$  values for fosGFP expression patterns A, B, C, D, F, G, I and O of a group with the values of every other group. The correlation coefficient matrix depicts the dimensionless Pearson correlation coefficients, individually weighted by the standard deviation of  $\Delta f_{d4}$  of every pattern<sup>54,55</sup>:

$$w_i = \frac{1}{\left( \frac{\sigma_i}{\sqrt{N_j \lambda}} \right)^2} \quad Eq. (1)$$

where  $\sigma_i$  is the standard deviation of the  $i$ -th element in the  $j$ -th group with  $i = A, B, C, D, F, G, I$  and  $O$  and  $j = 1, 2, 3$  and  $4$ .  $N_j$  is the size of the  $j$ -th group. The exponent  $\kappa$  can be used to differently weight group elements with a high standard deviation. The exponent  $\lambda$  is an additional weighting factor to take into account small group sizes. For instance with  $\kappa=\lambda=1$ , the denominator in Eq. (1) gives the default definition of the standard error of the mean (SEM). Due to the large element-by-element and group-by-group variations of the standard deviation and the varying group sizes we use the values  $\kappa=\lambda=4$ . Even higher values can be used, but they do not change the results significantly. Eq. (1) is calculated for each element of each group. We conservatively used the largest of the two errors in any pairwise group comparison. The resulting correlation coefficients of every group comparison were summarized in a color-coded matrix (Fig. 2f) and table (Supplementary Table 2). Diagonal entries represent comparisons between identical groups and have the value 1, which represents the highest correlation between two groups. Values from 0 to 1 are depicted by a color code. The range from -1 to 0 is not shown here as our analysis revealed no significant negative correlation coefficients. We computed the corresponding p-values at a 5% significance level for every correlation coefficient<sup>56</sup>, including a Holm-Bonferroni correction for multiple comparisons. The exact values of the correlation coefficients and the respective p-values of each pairwise comparison are listed in Supplementary Table 2.

### **Analysis of confocal images**

Nuclei were defined as c-Fos-positive if the measured fluorescence intensities of fosGFP or Alexa Fluor® 488 were above threshold ( $\geq 6$  SD above the mean BG fluorescence).

### **Analysis of $\text{Ca}^{2+}$ event frequency**

Motion correction of time-series data was performed using the batch registration function of the custom Fiji macro TurboReg<sup>53</sup>. To measure the fluorescence signal of principal CA1 neurons over time with the aim to determine the Ca<sup>2+</sup> event frequency, at least five minutes were recorded during epochs when the animal was not running. Regions of interest (ROIs) were drawn manually around individual cell bodies using the open source software ImageJ/Fiji. The change in mean grey value over time was extracted for each ROI and stored as text file.

The  $\Delta F/F$  was calculated by subtracting the baseline fluorescence  $F_0$  from the signal and dividing the value by  $F_0$ .  $F_0$  was defined as the mean of the smallest 20% of all values of a time series. In order to derive an approximation of the neuronal activity from the Ca<sup>2+</sup> transient frequency, we inferred the underlying spiking activity from the  $\Delta F/F$  traces. For this, we used the OASIS module for calcium deconvolution<sup>57</sup> that is implemented in the freely available CalmAn toolbox<sup>58</sup>. The algorithm we applied is using the threshold non-negative square (NNLS) method with a deconvolution kernel. The kernel is modeled as the difference between two exponential functions:

$$h(t) = \frac{(e^{-\frac{t}{\tau d}} - e^{-\frac{t}{\tau r}})}{\tau d - \tau r}$$

where  $\tau d$  and  $\tau r$  are the roots of the polynomial, which were chosen to simulate the kinetics of jRGECO1a:

$$f(x) = x^2 - 1.69x + 0.712$$

The algorithm has implemented a constraint on the minimal spike size  $s_{min}$  which we set in dependency of the calculated spectral noise (sn)

$$s_{min} = sn * 4$$

The number of inferred spikes was divided by the time of recording to give the frequency.

## Identification of UP and DOWN cells

For the correlation of changes in fosGFP expression and neuronal activity, fosGFP expression was determined on three consecutive time points (0 minutes, 60 minutes and 90 minutes). Identification of fosGFP-positive (fosGFP<sup>+</sup>) cells was performed as described before (see analysis of two-photon *in vivo* images). Cells had to show above-threshold fosGFP expression at one of the three time points in order to be classified as fosGFP<sup>+</sup>. For all cells that were identified as fosGFP<sup>+</sup> cells, the change in fosGFP expression was calculated by subtracting the baseline fosGFP value (at 0 minutes) from the values at the subsequent measurement time points (60 and 90 minutes, respectively).

Cells that at any of the time points showed an increase in either absolute or relative fosGFP expression larger than twice the standard deviation of all changes among the total population of fosGFP<sup>+</sup> cells at the respective time point, were classified as UP cells. Cells that showed a decrease larger than the standard deviation were classified as DOWN cells. Cells that did not change their fosGFP intensity above or below these thresholds were classified as "no change" cells. The automatic classification of UP, DOWN and no change cells was furthermore double checked by visual inspection. Note: we suspect the observation of DOWN cells to be independent of stimulus presentation, 90 minutes earlier, as this time interval is shorter than the kinetics of GFP degradation. DOWN cells served as a control for UP cells, as they share the feature of fosGFP transgene expression.

## Statistics and Reproducibility

Statistical analysis and preparation of graphs was performed in GraphPad Prism 8 (GraphPad Software Inc., La Jolla, USA). A summary of the study design can be found in the Life Sciences Reporting Summary. We did not apply statistical methods to



predetermine sample sizes, but our chosen sample sizes are similar to those generally employed in the field<sup>26,32</sup>. Subjects were randomly assigned to experimental groups before data acquisition. Assignment was determined by genotype and by aiming at a balanced proportion of sexes in each group. For data collection blinding was not possible. Data analysis was performed blind to the conditions of the experiment via encrypting file names. Mice were excluded from analysis if acquired data sets were incomplete. All data sets were tested for normality with the D'Agostino and Pearson normality test (if  $n > 6$ ) or the Shapiro-Wilk normality test (if  $n < 6$ ). Normally distributed data were tested by using paired and unpaired t-tests, one-way and two-way ANOVAs with Holm-Sidak's multiple comparisons tests. Not normally distributed data were tested by using the Mann-Whitney test or Kruskal-Wallis test with Dunn's correction for multiple comparisons. All statistical test applied in this study were two-sided. For detailed statistics including p-values and confidence intervals see Supplementary Table 1 and 2. Data presentation is described in the legends of the respective Figures.

All animal experiments in this manuscript, but the one in Fig.4g-k, were conducted once, in accordance with the European 3R guideline. The data shown in Fig. 4k, comprising groups APP/PS1 (+) and wild-type (+), were replicated with an independent experiment; pooled results of both experiments are shown. Figures were prepared with Illustrator CS5 Version 15.0.1 (Adobe).

#### **Data Availability**

The data that support the findings of this are available from the corresponding author upon request.

#### **Code Availability**

1097 The code used for analyzing the data in the current study is available from the  
1098 corresponding author on request.

1099

## 1100 **Materials & Correspondence**

1101 Further information and requests for resources and reagents should be directed to and  
1102 will be fulfilled by the lead contact, Martin Fuhrmann (martin.fuhrmann@dzne.de).

1103

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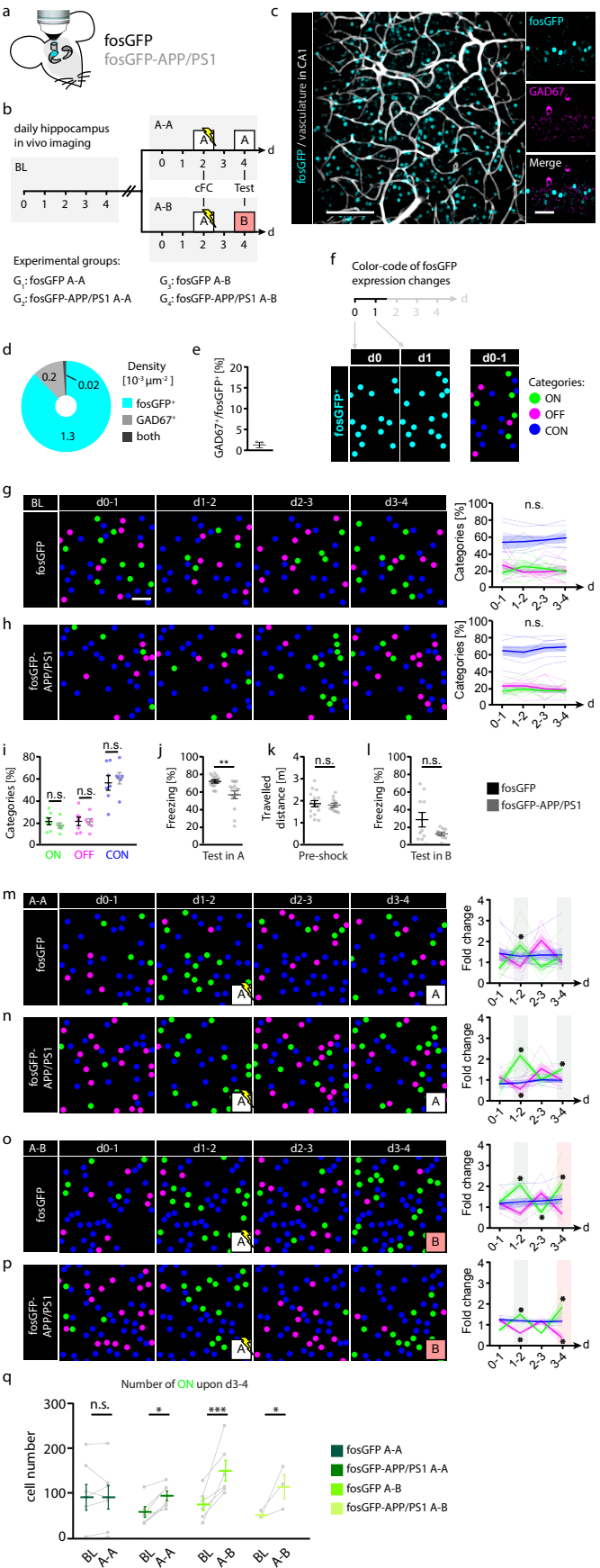
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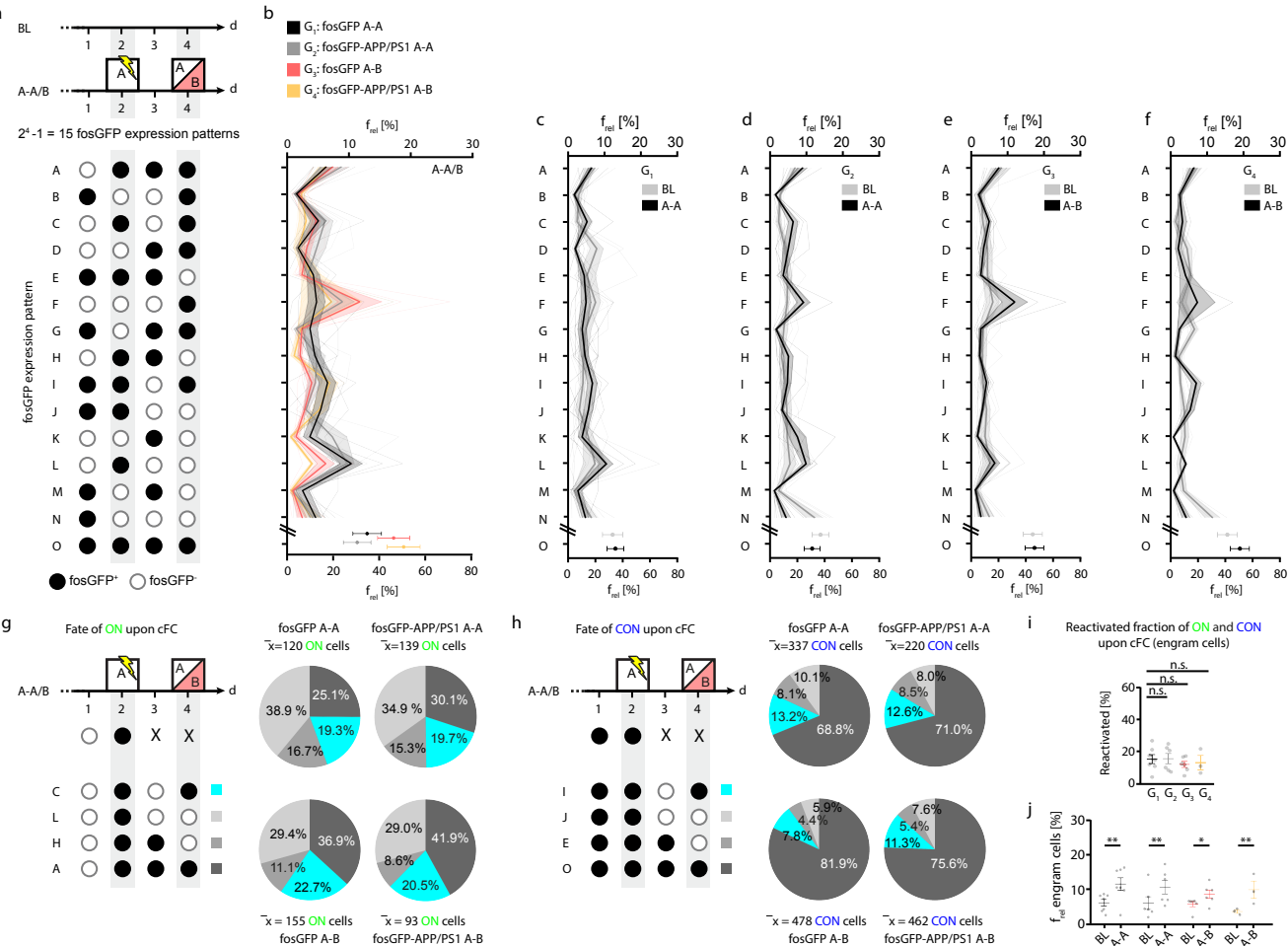
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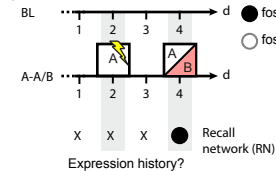
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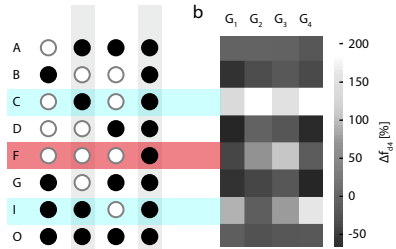




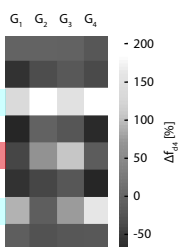
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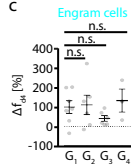
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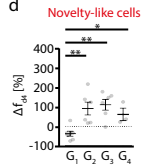
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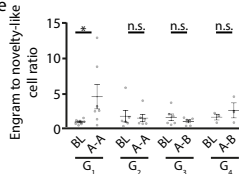
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d

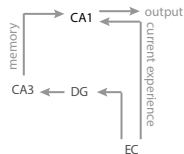


e



g

Match or mismatch?



$$\text{Engram to novelty-like cell ratio} = \frac{f(\text{pattern C}) + f(\text{pattern I})}{f(\text{pattern F})}$$

G<sub>1</sub>: fosGFP A-A

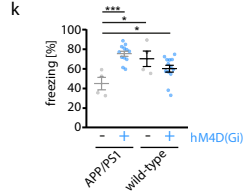
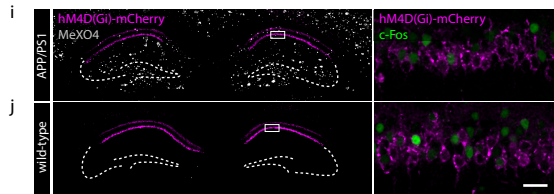
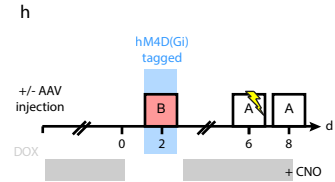
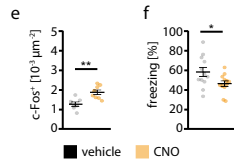
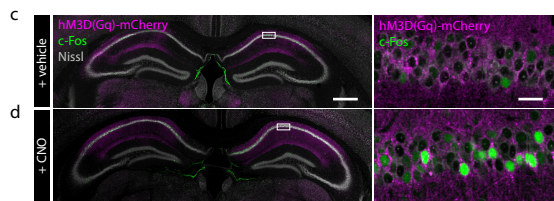
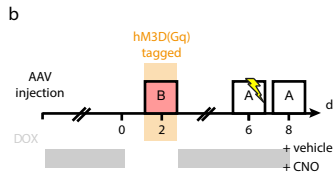
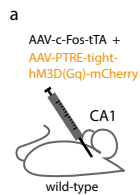
G<sub>2</sub>: fosGFP-APP/PS1 A-A

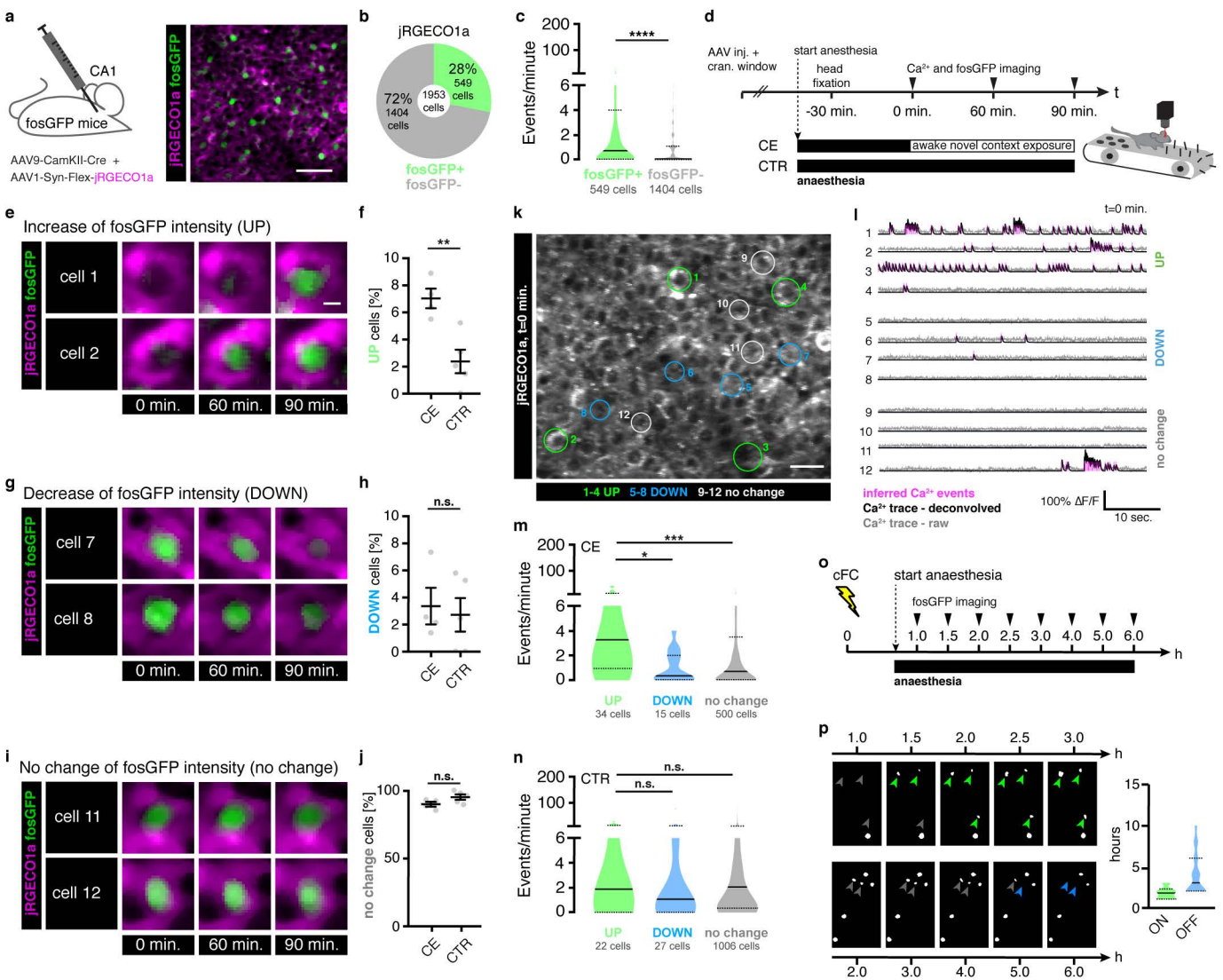
G<sub>3</sub>: fosGFP A-B

G<sub>4</sub>: fosGFP-APP/PS1 A-B

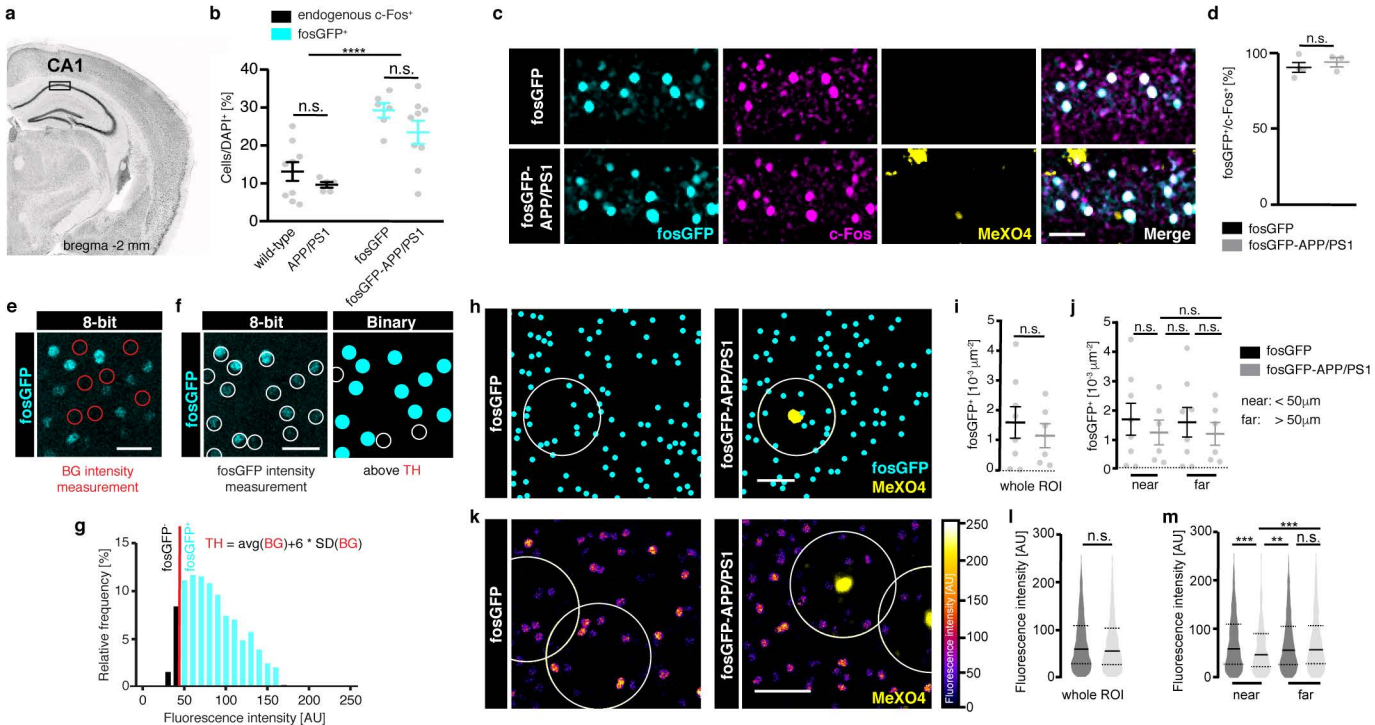
Memory trace interference during recall

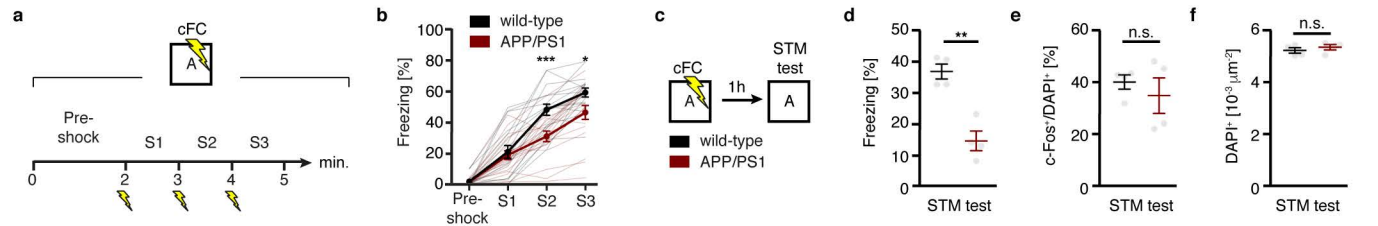
	Healthy	AD-like	Healthy/AD-like
RN in CA1			
Output	A = A Match	A ○ A Mismatch	A ○ B Mismatch
Rating	true	false	true

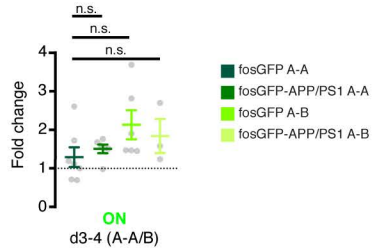


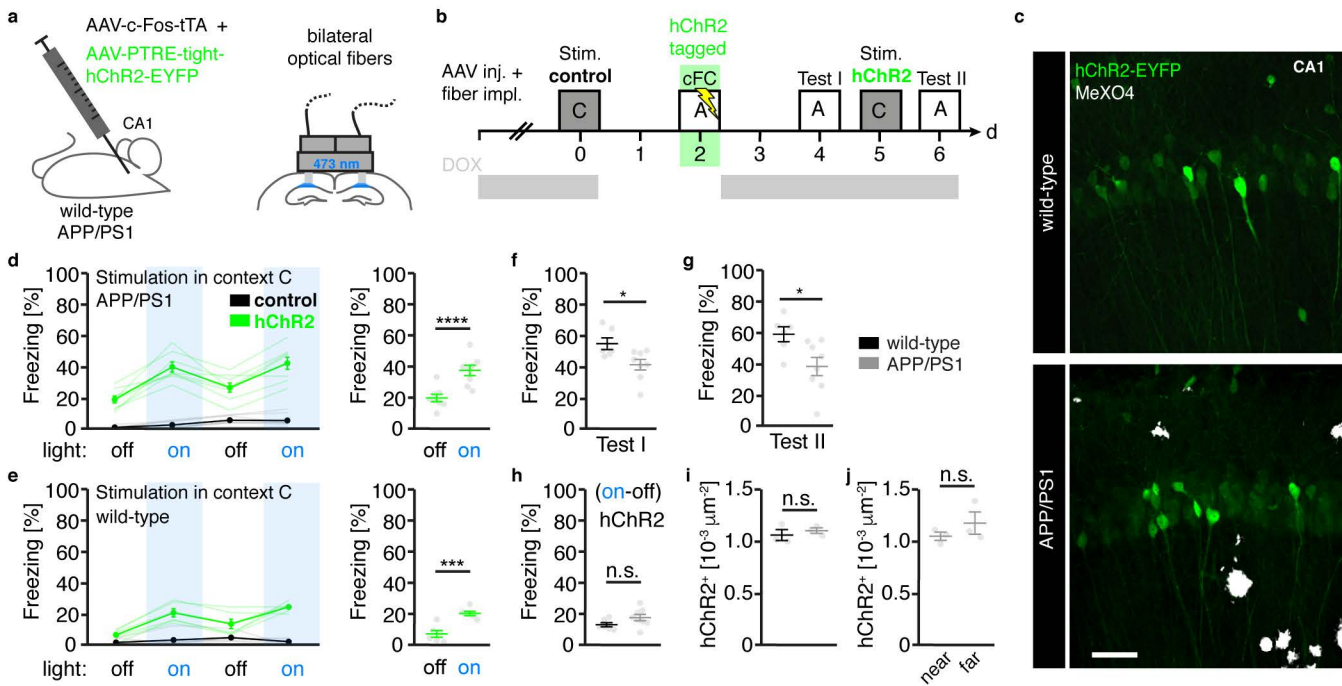


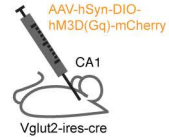
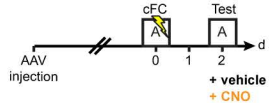
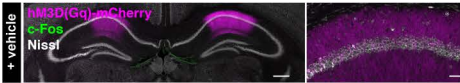
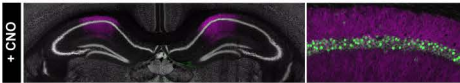
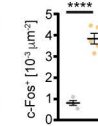
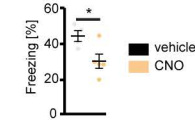


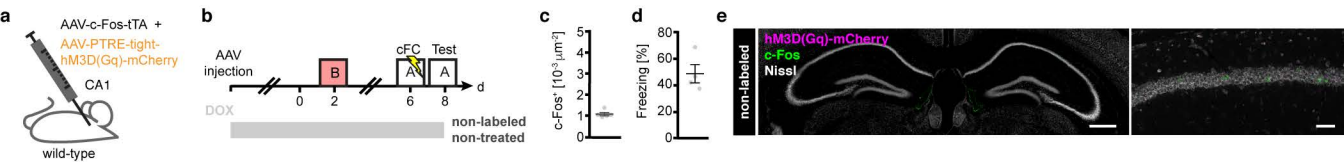


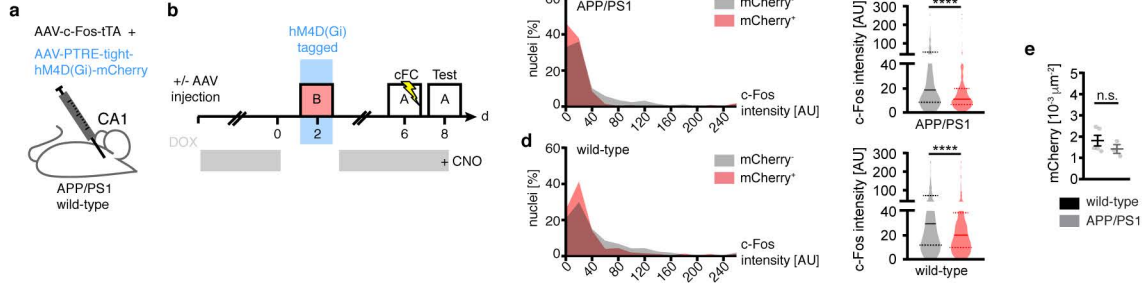


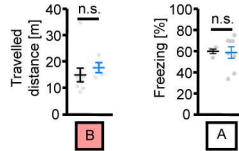
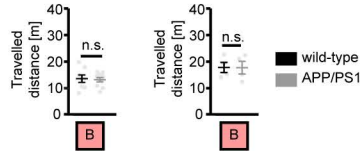
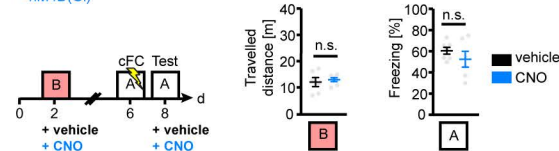




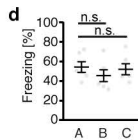
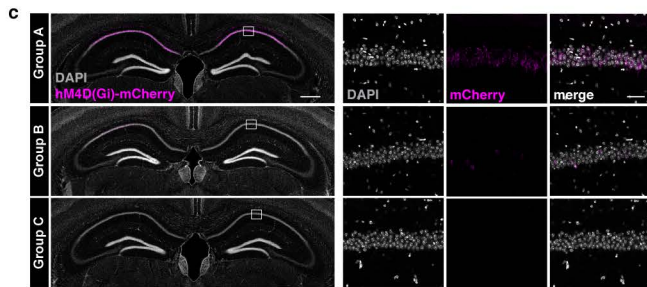
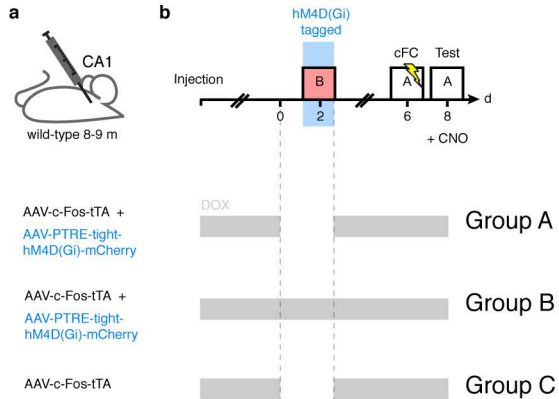
**a****b****c****d****e****f**





**a****b****c**





# Supplemental information

## **Memory trace interference impairs recall in a mouse model of Alzheimer's disease**

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<sup>2</sup>Institute of Neuropathology, University of Bonn, 53127, Germany

<sup>3</sup>Clemens-Schöpf-Institute, Technical University of Darmstadt, 64289, Germany

<sup>4</sup>Selective Vulnerability of Neurodegenerative Diseases, German Center for Neurodegenerative Diseases (DZNE), Bonn, 53127, Germany

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## Supplementary Table 1 - Details of statistical tests applied

### Notes:

- all statistical tests applied were two-sided
- n was always defined by the number of experimental mice if not stated otherwise
- All data sets were tested for normality with the D'Agostino and Pearson normality test (if  $n > 6$ ) or the Shapiro-Wilk normality test (if  $n < 6$ )

Figure	Test applied	n		Parameter [unit]	Data presentation	Test results
		exact value	def.			
1g	Two-way repeated measures ANOVA with Holm-Sidak's multiple comparison test	7 fosGFP	Mice	Neuronal categories: ON, OFF and CON [%]	Mean (continuous line) $\pm$ SEM (shaded area), fine lines show individual mice	<p>F1, Time: <math>F(3,54)=0.1309e-017</math>, <math>p&gt;0.9999</math>  F2, Categories: <math>F(2,18)=17.88</math>, <math>p&lt;0.0001</math></p> <p>F1xF2: <math>F(6,54)=0.9753</math>, <math>p=0.4509</math></p> <p>ON: d0-1 vs. d1-2, <math>p=0.4789</math>  ON: d0-1 vs. d2-3, <math>p=0.6289</math>  ON: d0-1 vs. d3-4, <math>p=0.8538</math></p> <p>OFF: d0-1 vs. d1-2, <math>p=0.4003</math>  OFF: d0-1 vs. d2-3, <math>p=0.4003</math>  OFF: d0-1 vs. d3-4, <math>p=0.4003</math></p> <p>CON: d0-d1 vs. d1-2, <math>p=0.9011</math>  CON: d0-d1 vs. d2-3, <math>p=0.8667</math>  CON: d0-d1 vs. d3-4, <math>p=0.7438</math></p> <p>Lower 95% CI of mean - Upper 95% CI;  ON:  9.62 - 27.48 (d0-1), 6.91 - 44.84 (d1-2),  16.10 - 30.69 (d2-3), 10.67 - 28.50 (d3-4)  OFF:  9.46 - 45.14 (d0-1), 14.24 - 24.31 (d1-2),  7.75 - 31.85 (d2-3), 6.52 - 35.80 (d3-4)  CON:  33.30 - 75.01 (d0-1), 36.75 - 72.96 (d1-2),  44.56 - 69.08 (d2-3), 43.87 - 74.65 (d3-4)</p>
1h	Two-way repeated measures ANOVA with Holm-Sidak's multiple comparison test	6 fosGFP-APP/PS1	Mice	Neuronal categories: ON, OFF and CON [%]	Mean (continuous line) $\pm$ SEM (shaded area), fine lines show individual mice	<p>F1, Time: <math>F(3,45)=0.007067</math>, <math>p=0.9992</math>  F2, Categories: <math>F(2,15)=45.40</math>, <math>p&lt;0.0001</math>  F1xF2: <math>F(6,45)=0.8984</math>, <math>p=0.5044</math></p> <p>ON: d0-1 vs. d1-2, <math>p=0.9089</math>  ON: d0-1 vs. d2-3, <math>p=0.9897</math>  ON: d0-1 vs. d3-4, <math>p=0.9897</math></p> <p>OFF: d0-1 vs. d1-2, <math>p=0.9684</math>  OFF: d0-1 vs. d2-3, <math>p=0.6788</math>  OFF: d0-1 vs. d3-4, <math>p=0.6191</math></p> <p>CON: d0-1 vs. d1-2, <math>p=0.6809</math>  CON: d0-1 vs. d2-3, <math>p=0.6409</math>  CON: d0-1 vs. d3-4, <math>p=0.6276</math></p> <p>Lower 95% CI of mean - Upper 95% CI;  ON:  6.29-28.21 (d0-1), 10.70-28.66 (d1-2), 9.33-26.21 (d2-3), 9.77-25.49 (d3-4)  OFF:  15.41-31.10 (d0-1), 13.41-33.42 (d1-2),  5.52-34.62 (d2-3), 9.73-27.88 (d3-4)  CON:  48.84 - 80.74 (d0-1), 44.45 - 81.79 (d1-2),  54.53 - 81.89 (d2-3), 56.04 - 82.35 (d3-4)</p>
1i	Two-way ANOVA with Holm-Sidak's multiple comparison test	7 fosGFP 6 fosGFP-APP/PS1	Mice	Average of neuronal categories ON, OFF and CON [%]	Mean $\pm$ SEM	<p>F1, Category: <math>F(2,33)=50.05</math>, <math>p&lt;0.0001</math>  F2, Genotype: <math>F(1,33)=1.803e-018</math>, <math>p&gt;0.999</math></p> <p>F1xF2: <math>F(2,33)=0.4313</math>, <math>p=0.6533</math></p> <p>fosGFP vs. fosGFP-APP/PS1,  ON: <math>p=8696</math>  OFF: <math>p=9362</math>  CON: <math>p=8696</math></p>

						Lower 95% CI of mean - Upper 95% CI; fosGFP: 14.39 - 29.30 (ON), 12.84 - 30.93 (OFF), 40.18 - 72.36 (CON) fosGFP-APP/PS1: 11.93 - 24.24 (ON), 13.77 - 29.00 (OFF), 47.20 - 73.86 (CON)
1j	Two-sided unpaired t-test	13 fosGFP 14 fosGFP-APP/PS1	Mice	Freezing [%]	Mean ± SEM	$p=0.0046$ $t=3.113$ , $df=25$ Lower 95% CI of mean - Upper 95% CI; 67.70 - 76.00 (fosGFP), 47.38 - 66.08 (fosGFP-APP/PS1)
1k	Two-sided unpaired t-test	15 fosGFP 11 fosGFP-APP/PS1	Mice	Travelled distance [m]	Mean ± SEM	$p=0.7324$ $t=0.3459$ , $df=24$ Lower 95% CI of mean - Upper 95% CI; 1.56 - 2.17 (fosGFP), 1.60 - 2.01 (fosGFP-APP/PS1)
1l	Two-sided unpaired t-test	10 fosGFP 9 fosGFP-APP/PS1	Mice	Freezing [%]	Mean ± SEM	$p=0.0933$ $t=1.1778$ , $df=17$ Lower 95% CI of mean - Upper 95% CI; 9.86 - 47.17 (fosGFP), 7.98 - 17.31 (fosGFP-APP/PS1)
1m	Two-way repeated measures ANOVA with Holm- Sidak's multiple comparison test	7 fosGFP A-A (G <sub>1</sub> )	Mice	Fold change of neuronal categories: ON, OFF and CON	Mean (continuous line) ± SEM (shaded area), fine lines show individual mice	F1, Time: $F(3,54)=0.7248$ , $p=0.5416$ F2, Categories: $F(2,18)=0.3236$ , $p=0.7277$  F1x2: $F(6,54)=7.904$ , $p<0.0001$  ON: d0-1 vs. d1-2, $p=0.0003$ ON: d0-1 vs. d2-3, $p=0.8394$ ON: d0-1 vs. d3-4, $p=0.0701$  OFF: d0-1 vs. d1-2, $p=0.0603$ OFF: d0-1 vs. d2-3, $p=0.0603$ OFF: d0-1 vs. d3-4, $p=0.1098$  CON: d0-1 vs. d1-2, $p=0.9431$ CON: d0-1 vs. d2-3, $p=0.9484$ CON: d0-1 vs. d3-4, $p=0.9484$  Lower 95% CI of mean - Upper 95% CI of mean, for ON, OFF and CON. ON: 0.26-1.20 (d0-1), 1.11-2.54 (d1-2), 0.28-1.28 (d2-3), 0.67-1.92 (d3-4) OFF: 0.58-2.29 (d0-1), 0.41-1.21 (d1-2), 1.34-2.77 (d2-3), 0.67-1.34 (d3-4) CON: 0.81-2.10 (d0-1), 0.88-1.71 (d1-2), 0.76-1.94 (d2-3), 0.51-2.20 (d3-4)
1n	Two-way repeated measures ANOVA with Holm- Sidak's multiple comparison test	6 fosGFP-APP/PS1 A-A (G <sub>2</sub> )	Mice	Fold change of neuronal categories: ON, OFF and CON	Mean (continuous line) ± SEM (shaded area), fine lines show individual mice	F1, Time: $F(3,45)=2.146$ , $p=0.1076$ F2, Categories: $F(2,15)=7.547$ , $p=0.0054$  F1x2: $F(6,45)=9.435$ , $p<0.0001$  ON: d0-1 vs. d1-2, $p<0.0001$ ON: d0-1 vs. d2-3, $p=0.2937$ ON: d0-1 vs. d3-4, $p=0.0054$  OFF: d0-1 vs. d1-2, $p=0.0452$ OFF: d0-1 vs. d2-3, $p=0.1563$ OFF: d0-1 vs. d3-4, $p=0.5204$  CON: d0-1 vs. d1-2, $p=0.8420$ CON: d0-1 vs. d2-3, $p=0.7955$ CON: d0-1 vs. d3-4, $p=0.7955$  Lower 95% CI of mean - Upper 95% CI of mean, for ON, OFF and CON. ON: 0.22-1.35 (d0-1), 1.64-2.69 (d1-2), 0.58-1.48 (d2-3), 1.21-1.80 (d3-4) OFF: 0.65-1.62 (d0-1), 0.17-0.95 (d1-2), 0.86-2.22 (d2-3), 0.56-1.14 (d3-4)

						CON: 0.66-0.98 (d0-1), 0.65-1.08 (d1-2), 0.81-1.20 (d2-3), 0.71-1.26 (d3-4)
1o	Two-way repeated measures ANOVA with Holm-Sidak's multiple comparison test	6 fosGFP A-B (G <sub>3</sub> )	Mice	Fold change of neuronal categories: ON, OFF and CON	Mean (continuous line) ± SEM (shaded area), fine lines show individual mice	<p>F1, Time: <math>F(3,45)=1.196</math>, <math>p=0.3222</math>  F2, Categories: <math>F(2,15)=2.371</math>, <math>p=0.1274</math></p> <p>F1x2: <math>F(6,45)=15.49</math>, <math>p&lt;0.0001</math></p> <p>ON: d0-1 vs. d1-2, <math>p=0.0004</math>  ON: d0-1 vs. d2-3, <math>p=0.0245</math>  ON: d0-1 vs. d3-4, <math>p=0.0002</math></p> <p>OFF: d0-1 vs. d1-2, <math>p=0.0509</math>  OFF: d0-1 vs. d2-3, <math>p=0.0509</math>  OFF: d0-1 vs. d3-4, <math>p=0.0509</math></p> <p>CON: d0-1 vs. d1-2, <math>p=0.7898</math>  CON: d0-1 vs. d2-3, <math>p=0.7898</math>  CON: d0-1 vs. d3-4, <math>p=0.6879</math></p> <p>Lower 95% CI of mean - Upper 95% CI of mean, for ON, OFF and CON.  ON:  0.80-1.66 (d0-1), 1.58-2.55 (d1-2),  0.45-1.06 (d2-3), 1.16-3.11 (d3-4)  OFF:  0.66-1.70 (d0-1), 0.35-1.06 (d1-2),  1.07-2.27 (d2-3), 0.42-0.93 (d3-4)  CON:  0.72-1.63 (d0-1), 0.80-1.72 (d1-2),  0.86-1.75 (d2-3), 0.79-1.20 (d3-4)</p>
1p	Two-way repeated measures ANOVA with Holm-Sidak's multiple comparison test	3 fosGFP-APP/PS1 (G <sub>4</sub> )	Mice	Fold change of neuronal categories: ON, OFF and CON	Mean (continuous line) ± SEM (shaded area), fine lines show individual mice	<p>F1, Time: <math>F(3,18)=1.196</math>, <math>p=0.7051</math>  F2, Categories: <math>F(2,6)=9.136</math>, <math>p=0.0151</math></p> <p>F1x2: <math>F(6,18)=9.398</math>, <math>p&lt;0.0001</math></p> <p>ON: d0-1 vs. d1-2, <math>p=0.0095</math>  ON: d0-1 vs. d2-3, <math>p=0.5589</math>  ON: d0-1 vs. d3-4, <math>p=0.0006</math></p> <p>OFF: d0-1 vs. d1-2, <math>p=0.0301</math>  OFF: d0-1 vs. d2-3, <math>p=0.8584</math>  OFF: d0-1 vs. d3-4, <math>p=0.0062</math></p> <p>CON: d0-1 vs. d1-2, <math>p=0.9625</math>  CON: d0-1 vs. d2-3, <math>p=0.9625</math>  CON: d0-1 vs. d3-4, <math>p=0.9625</math></p> <p>Lower 95% CI of mean - Upper 95% CI of mean, for ON, OFF and CON.  ON:  0.80-1.66 (d0-1), 1.58-2.55 (d1-2),  0.45-1.06 (d2-3), 1.16-3.11 (d3-4)  OFF:  0.66-1.70 (d0-1), 0.35-1.06 (d1-2),  1.07-2.27 (d2-3), 0.42-0.93 (d3-4)  CON:  0.79-1.70 (d0-1), 0.92-1.47 (d1-2),  0.83-1.46 (d2-3), 0.65-1.69 (d3-4)</p>
1q	Three-way ANOVA with Holm-Sidak's multiple comparison test	7 fosGFP A-A (G <sub>1</sub> ) 6 fosGFP-APP/PS1 A-A (G <sub>2</sub> ) 6 fosGFP A-B (G <sub>3</sub> ) 3 fosGFP-APP/PS1 A-B (G <sub>4</sub> )	Mice	ON cells	Mean ± SEM	<p>F1, Time: <math>F(1,18)=33.98</math>, <math>p&lt;0.0001</math>  F2, Treatment: <math>F(1,18)=0.3926</math>, <math>p=0.5388</math>  F3, Genotype: <math>F(1,18)=0.9842</math>, <math>p=0.3343</math></p> <p>F1x2: <math>F(1,18)=11.64</math>, <math>p=0.0031</math>  F1xF3: <math>F(1,18)=0.6249</math>, <math>p=0.4395</math>  F2xF3: <math>F(1,18)=0.1152</math>, <math>p=0.7382</math>  F1xF2xF3: <math>F(1,18)=2.586</math>, <math>p=0.1252</math></p> <p>BL vs. A-A/B,  G<sub>1</sub>: <math>p=0.9910</math>  G<sub>2</sub>: <math>p=0.0321</math>  G<sub>3</sub>: <math>p=0.0001</math>  G<sub>4</sub>: <math>p=0.0124</math></p> <p>Lower 95% CI of mean - Upper 95% CI of mean, for BL and A-A/B;  BL:</p>

						21.97-162.9 (G <sub>1</sub> ), 29.21-90.97 (G <sub>2</sub> ), 41.70-111.60 (G <sub>3</sub> ), 31.49-74.51 (G <sub>4</sub> ) A-A/B: 27.69-157.5(G <sub>1</sub> ), 68.3-123.4 (G <sub>2</sub> ), 91.42-211.6 (G <sub>3</sub> ), -1.65-233.00 (G <sub>4</sub> )
2i	Two-way ANOVA with Holm-Sidak's multiple comparison test	7 fosGFP A-A (G <sub>1</sub> ) 6 fosGFP-APP/PS1 A-A (G <sub>2</sub> ) 6 fosGFP A-B (G <sub>3</sub> ) 3 fosGFP-APP/PS1 A-B (G <sub>4</sub> )	Mice	Reactivated [%]	Mean ± SEM	F1, Treatment: $F(1,18)=0.8641$ , $p=0.3649$ F2, Genotype: $F(1,18)=0.05191$ , $p=0.8223$  F1xF2: $F(1,18)=0.01584$ , $p=0.9012$  G <sub>1</sub> vs. G <sub>2</sub> : $p=0.9352$ G <sub>1</sub> vs. G <sub>3</sub> : $p=0.7890$ G <sub>1</sub> vs. G <sub>4</sub> : $p=0.8795$  Lower 95% CI of mean - Upper 95% CI of mean, for G <sub>1</sub> , G <sub>2</sub> , G <sub>3</sub> and G <sub>4</sub> : 8.49-22.07 (G <sub>1</sub> ), 7.42-23.78 (G <sub>2</sub> ), 7.12-16.90 (G <sub>3</sub> ), -6.10-32.3 (G <sub>4</sub> )
2j	Three-way ANOVA with Holm-Sidak's multiple comparison test	7 fosGFP A-A (G <sub>1</sub> ) 6 fosGFP-APP/PS1 A-A (G <sub>2</sub> ) 6 fosGFP A-B (G <sub>3</sub> ) 3 fosGFP-APP/PS1 A-B (G <sub>4</sub> )	Mice	fos engram cells	Mean ± SEM	F1, Time: $F(1,18)=41.33$ , $p<0.0001$ F2, Treatment: $F(1,18)=0.09594$ , $p=0.7603$ F3, Genotype: $F(1,18)=1.203$ , $p=0.2873$  F1xF2: $F(1,18)=0.7675$ , $p=0.3925$ F1xF3: $F(1,18)=0.06880$ , $p=0.7961$ F2xF3: $F(1,18)=0.0006717$ , $p=0.9796$ F1xF2xF3: $F(1,18)=1.996$ , $p=0.1748$  BL vs. A-A/B (Time), G <sub>1</sub> : $p=0.0017$ G <sub>2</sub> : $p=0.0097$ G <sub>3</sub> : $p=0.0466$ G <sub>4</sub> : $p=0.0097$  Lower 95% CI of mean - Upper 95% CI of mean, for BL and A-A/B; BL: 3.95-8.56 (G <sub>1</sub> ), 1.52-10.79 (G <sub>2</sub> ), 3.92-7.75 (G <sub>3</sub> ), 1.50-5.94 (G <sub>4</sub> ) A-A/B: 7.17-16.10 (G <sub>1</sub> ), 5.65-15.78 (G <sub>2</sub> ), 5.75-11.68 (G <sub>3</sub> ), -0.60-20.67 (G <sub>4</sub> )
3c	Two-way ANOVA with Holm-Sidak's multiple comparison test	7 fosGFP A-A (G <sub>1</sub> ) 6 fosGFP-APP/PS1 A-A (G <sub>2</sub> ) 6 fosGFP A-B (G <sub>3</sub> ) 3 fosGFP-APP/PS1 A-B (G <sub>4</sub> )	Mice	Relative frequency change, $\Delta f_{d4}$ [%] of pattern C + I	Mean ± SEM	F1, Treatment: $F(1,18)=0.1969$ , $p=0.6625$ F2, Genotype: $F(1,18)=1.589$ , $p=0.2235$  F1xF2: $F(1,18)=0.9727$ , $p=0.3371$  G <sub>1</sub> vs. G <sub>2</sub> : $p=0.8407$ G <sub>1</sub> vs. G <sub>3</sub> : $p=0.5992$ G <sub>1</sub> vs. G <sub>4</sub> : $p=0.8407$  Lower 95% CI of mean - Upper 95% CI of mean, for G <sub>1</sub> , G <sub>2</sub> , G <sub>3</sub> and G <sub>4</sub> : 19.49-179.10 (G <sub>1</sub> ), -18.08-138.90 (G <sub>2</sub> ), 4.71-77.78 (G <sub>3</sub> ), -121.2-386.10 (G <sub>4</sub> )
3d	Two-way ANOVA with Holm-Sidak's multiple comparison test	7 fosGFP A-A (G <sub>1</sub> ) 6 fosGFP-APP/PS1 A-A (G <sub>2</sub> ) 6 fosGFP A-B (G <sub>3</sub> ) 3 fosGFP-APP/PS1 A-B (G <sub>4</sub> )	Mice	Relative frequency change, $\Delta f_{d4}$ [%] of pattern F	Mean ± SEM	F1, Treatment: $F(1,18)=4.525$ , $p=0.0475$ F2, Genotype: $F(1,18)=2.047$ , $p=0.1697$  F1xF2: $F(1,18)=10.27$ , $p=0.0049$  G <sub>1</sub> vs. G <sub>2</sub> : $p=0.0029$ G <sub>1</sub> vs. G <sub>3</sub> : $p=0.0013$ G <sub>1</sub> vs. G <sub>4</sub> : $p=0.0323$  Lower 95% CI of mean - Upper 95% CI of mean, for G <sub>1</sub> , G <sub>2</sub> , G <sub>3</sub> and G <sub>4</sub> : -68.43-(-5.483) (G <sub>1</sub> ), 6.86-176.90 (G <sub>2</sub> ), 40.39-182.10 (G <sub>3</sub> ), -76.08-199.90 (G <sub>4</sub> )
3e	Three-way ANOVA with Holm-Sidak's multiple comparison test	7 fosGFP A-A (G <sub>1</sub> ) 6 fosGFP-APP/PS1 A-A (G <sub>2</sub> ) 6 fosGFP A-B (G <sub>3</sub> ) 3 fosGFP-APP/PS1 A-B (G <sub>4</sub> )	Mice	Engram-to-novelty-like cell ratio	Mean ± SEM	F1, Time: $F(1,18)=2.023$ , $p=0.1720$ F2, Treatment: $F(1,18)=0.04382$ , $p=0.8365$ F3, Genotype: $F(1,18)=0.4103$ , $p=0.5299$  F1xF2: $F(1,18)=0.7903$ , $p=0.3857$ F1xF3: $F(1,18)=1.273$ , $p=0.2740$ F2xF3: $F(1,18)=1.690$ , $p=0.2101$ F1xF2xF3: $F(1,18)=4.291$ , $p=0.0530$

						<p>BL vs. A-A/B (Time),  G<sub>1</sub>: <math>p=0.0166</math>  G<sub>2</sub>: <math>p=0.9217</math>  G<sub>3</sub>: <math>p=0.9217</math>  G<sub>4</sub>: <math>p=0.9217</math></p> <p>Lower 95% CI of mean - Upper 95% CI of mean, for BL and A-A/B;  BL:  0.61-1.28 (G<sub>1</sub>), -0.35-3.88 (G<sub>2</sub>),  0.37-2.84 (G<sub>3</sub>), -0.11-3.37 (G<sub>4</sub>)  A-A/B:  0.31-8.70 (G<sub>1</sub>), 0.18-2.81 (G<sub>2</sub>),  0.50-1.56 (G<sub>3</sub>), -2.00-7.17 (G<sub>4</sub>)</p>
3f	see Supplementary Table 2					
4e	Two-sided unpaired t-test	7 vehicle-treated 8 CNO-treated	Mice	Fos+ [10 <sup>-3</sup> $\mu\text{m}^{-2}$ ]	Mean $\pm$ SEM	<p><math>p=0.0028</math>  <math>t=3.678</math>, <math>df=13</math>  Lower 95% CI of mean - Upper 95% CI of mean;  1.01-1.56 (vehicle-treated),  1.61-2.17 (CNO-treated)</p>
4f	Two-sided unpaired t-test	12 vehicle-treated 12 CNO-treated	Mice	Freezing [%]	Mean $\pm$ SEM	<p><math>p=0.0379</math>  <math>t=2.209</math>, <math>df=22</math>  Lower 95% CI of mean - Upper 95% CI of mean;  48.44-68.71 (vehicle-treated),  40.38-52.87 (CNO-treated)</p>
4k	Two-way ANOVA with Holm-Sidak's multiple comparison test	4 APP/PS1 (-) 12 APP/PS1 (+) 4 wild-type (-) 13 wild-type (+)	Mice	Freezing [%]	Mean $\pm$ SEM	<p>F1, Treatment: <math>F(1,29)=4.504</math>, <math>p=0.0425</math>  F2, Genotype: <math>F(1,29)=1.094</math>, <math>p=0.3042</math></p> <p>F1x F2: <math>F(1,29)=17.86</math>, <math>p=0.0001</math></p> <p>APP/PS1(-) vs. wild-type(-): <math>p=0.0102</math>  APP/PS1(-) vs. APP/PS1(+): <math>p=0.0003</math>  APP/PS1(-) vs. wild-type(+): <math>p=0.0321</math></p> <p>Lower 95% CI of mean - Upper 95% CI of mean;  24.58-65.35 APP/PS1 (-),  69.64-80.99 APP/PS1 (+),  45.02-95.31 wild-type (-),  52.51-67.70 wild-type(+)</p>

Ext. Data Figure	Test applied	n		Parameter [unit]	Data presentation	Test results
		Exact value	def.			
1c	Two-sided Mann-Whitney test	549 fosGFP-positive (fosGFP+) and 1404 fosGFP-negative (fosGFP-) cells	pooled cells of 4 fosGFP mice	Events/minute	Median, 25%- and 75%-quartiles, borders of plot show minimum and maximum	$p < 0.0001$ Mann Whitney U = 296409  Lower 95% CI of mean - Upper 95% CI of mean; 4.39-6.52 (fosGFP+), 1.49-2.04 (fosGFP-)
1f	Two-sided unpaired t-test	5 CTR 4 CE	Mice	Proportion of UP [%]	Mean $\pm$ SEM	$p = 0.0050$ $t = 4.034$ , $df = 7$  Lower 95% CI of mean - Upper 95% CI of mean 0.09 4.39 (CTR), 4.22 9.37 (CE)
1h	Two-sided unpaired t-test	5 CTR 4 CE	Mice	Proportion of DOWN [%]	Mean $\pm$ SEM	$p = 0.7358$ $t = 0.3512$ , $df = 7$ Lower 95% CI of mean - Upper 95% CI of mean; -0.74-6.13 (CTR), -0.96-7.63 (CE)
1j	Two-sided unpaired t-test	5 CTR 4 CE	Mice	Proportion of no change [%]	Mean $\pm$ SEM	$p = 0.0957$ $t = 1.925$ , $df = 7$ Lower 95% CI of mean - Upper 95% CI of mean; 89.69-100.40 (CTR), 84.15-95.58 (CE)
1m	Kruskal-Wallis test with Dunn's multiple comparison test	34 UP 15 DOWN 500 no change	pooled cells of 4 fosGFP CE mice	Events/minute	Median, 25%- and 75%-quartiles, borders of plot show minimum and maximum	Kruskal-Wallis statistic 13.42, $p = 0.0012$  UP vs. DOWN: $p = 0.0189$ UP vs. no change: $p = 0.0007$ Lower 95% CI of mean - Upper 95% CI of mean; 4.54-12.48 (UP), 0.35-1.76 (DOWN), 4.24-6.51 (no change)
1n	Kruskal-Wallis test with Dunn's multiple comparison test	22 UP 27 DOWN 1006 no change	pooled cells of 6 fosGFP CTR mice	Events/minute	see above (1m)	Kruskal-Wallis statistic 1.973, $p = 0.3728$  UP vs. DOWN: $p = 0.9384$ UP vs. no change: $p > 0.9999$ Lower 95% CI of mean - Upper 95% CI of mean; 2.31-9.59 (UP), 0.15-12.16 (DOWN), 8.97-11.61 (no change)
2b	Two-way ANOVA with Holm-Sidak's multiple comparison test	9 wild-type 5 APP/PS1 6 fosGFP 9 fosGFP-APP/PS1	Mice	Neurons/DAPI+ [%]	Mean $\pm$ SEM	$F_{1, 25} = 30.86$ , $p < 0.0001$ $F_{2, \text{Genotype}} = 2.988$ , $p = 0.0962$ $F_{1 \times F2} = 0.1809$ , $p = 0.6743$  cFos wild-type vs. cFos APP/PS1: $p = 0.3785$ cFos wild-type vs. fosGFP: $p = 0.0010$ cFos wild-type vs. fosGFP-APP/PS1: $p = 0.0137$ cFos APP/PS1 vs. fosGFP: $p = 0.0006$ cFos APP/PS1 vs. fosGFP-APP/PS1: $p = 0.0066$ fosGFP vs. fosGFP-APP/PS1: $p = 0.2421$  Lower 95% CI of mean - Upper 95% CI of mean; 7.54-19.05 (wild-type), 7.65-11.89 (APP/PS1), 24.50-34.46 (fosGFP) 16.61-30.69 (fosGFP-APP/PS1)
2d	Two-sided unpaired t-test	4 fosGFP 3 fosGFP-APP/PS1	Mice	fosGFP+/cFos+ [%]	Mean $\pm$ SEM	$p = 0.4882$ $t = 0.7478$ , $df = 5$



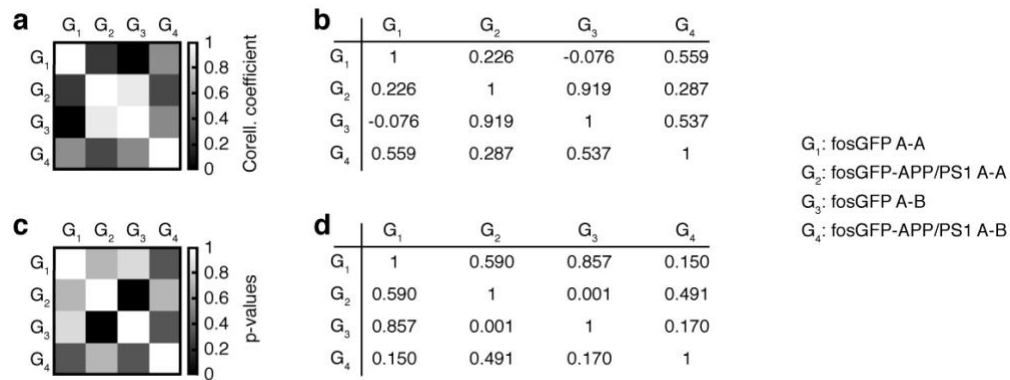
						Lower 95% CI of mean - Upper 95% CI of mean; 80.71-101.50 (fosGFP), 81.15-108.10 (fosGFP-APP/PS1)
2i	Two-sided unpaired t-test	8 fosGFP 6 fosGFP-APP/PS1	Mice	fosGFP+ [10-3 μm-2]	Mean ± SEM	$p=0.5476$ $t=0.6188$ , $df=12$  Lower 95% CI of mean - Upper 95% CI of mean; 0.38-2.87 (fosGFP), 0.14-2.23 (fosGFP-APP/PS1)
2j	Two-way ANOVA with Holm-Sidak's multiple comparison test	8 fosGFP 6 fosGFP-APP/PS1	Mice	fosGFP+ [10-3 μm-2]	Mean ± SEM	F1, Distance: $F(1,24)=0.02032$ , $p=0.8878$ F2, Genotype: $F(1,24)=0.7159$ , $p=0.4059$  $F1 \times F2$ : $F(1,24)=0.002741$ , $p=0.9587$  fosGFP near vs. fosGFP-APP/PS1 near: $p=0.9827$ fosGFP far vs. fosGFP-APP/PS1 far $p=0.9827$ fosGFP far vs. fosGFP-APP/PS1 near $p=0.9827$ fosGFP-APP/PS1 near vs. fosGFP-APP/PS1 far $p=0.9863$  Lower 95% CI of mean - Upper 95% CI of mean; 0.37-2.98 (fosGFP - near), 0.12-2.32 (fosGFP-APP/PS1 - near), 0.36-2.79 (fosGFP - far), 0.16-2.19 (fosGFP-APP/PS1 - far)
2l	Two-sided Mann-Whitney test	8 fosGFP 6 fosGFP-APP/PS1	Mice	Intensity [AU]	Median, 25%- and 75%-quartiles, borders of plot show minimum and maximum	$p=0.0937$ Mann-Whitney U = 2921553  Lower 95% CI of mean - Upper 95% CI of mean; 71.16-75.33 (fosGFP), 69.01-74.05 (fosGFP-APP/PS1)
2m	Kruskal-Wallis test with Dunn's correction for multiple comparisons	8 fosGFP 6 fosGFP-APP/PS1	Mice	Intensity [AU]	see above (2l)	Kruskal-Wallis statistic 19.57, $p=0.0002$  fosGFP near vs. fosGFP-APP/PS1 near: $p=0.0003$ fosGFP far vs. fosGFP-APP/PS1 far $p>0.9999$ fosGFP far vs. fosGFP-APP/PS1 near $p=0.0032$ fosGFP-APP/PS1 near vs. fosGFP-APP/PS1 far $p=0.0004$  Lower 95% CI of mean - Upper 95% CI of mean; 71.08-77.12 (fosGFP - near), 60.23-68.68 (fosGFP-APP/PS1 - near), 69.58-75.35 (fosGFP - far), 71.78-78.01 (fosGFP-APP/PS1 - far)
3b	Two-way ANOVA with Holm-Sidak's multiple comparison test	17 wild-type 18 APP/PS1	Mice	Freezing[%]	Mean ± SEM	F1, Time: $F(3,99)=185.6$ , $p<0.0001$ F2, Genotype: $F(1,99)=5.443$ , $p=0.0259$  $F1 \times F2$ : $F(3,99)=6.317$ , $p=0.0006$  wild-type vs. APP/PS1, BL: $p=0.9868$ S1: $p=0.8692$ S2: $p=0.0008$ S3: $p=0.0142$  Lower 95% CI of mean - Upper 95% CI of mean; wild-type: 0.69-4.17 (Pre-Shock), 12.90-30.4 (S1), 41.13-56.48 (S2), 53.76-66.02 (S3) APP/PS1: 1.01-3.66 (Pre-Shock), 13.57-25.51 (S1), 24.34-38.95 (S2), 37.58-56.53 (S3)

3d	Two-sided unpaired t-test	4 wild-type 4 APP/PS1	Mice	Freezing[%]	Mean ± SEM	$p=0.0013$ $t=5.642$ , $df=6$  Lower 95% CI of mean - Upper 95% CI of mean; 29.32-44.53 (wild-type), 4.74-24.67 (APP/PS1)
3e	Two-sided Mann-Whitney test	4 wild-type 4 APP/PS1	Mice	cFos+/DAPI + [%]	Mean ± SEM	$p>0.9999$ Mann-Whitney U = 8  Lower 95% CI of mean - Upper 95% CI of mean; 31.56-49.01 (wild-type), 13.33-56.80 (APP/PS1)
3f	Two-sided unpaired t-test	4 wild-type 4 APP/PS1	Mice	DAPI+ [10-3 μm-2]	Mean ± SEM	$p=0.4325$ $t=0.8411$ , $df=6$  Lower 95% CI of mean - Upper 95% CI of mean; 4.88-5.53 (wild-type), 5.00-5.65 (APP/PS1),
4	Two-way ANOVA with Holm-Sidak's multiple comparison test	7 fosGFP A-A (G <sub>1</sub> ) 6 fosGFP-APP/PS1 A-A (G <sub>2</sub> ) 6 fosGFP A-B (G <sub>3</sub> ) 3 fosGFP-APP/PS1 A-B (G <sub>4</sub> )	Mice	fold change	Mean ± SEM	F1, Treatment: $F(1,18)=3.581$ , $p=0.0747$ F2, Genotype: $F(1,18)=0.01632$ , $p=0.8998$  $F1 \times F2$ : $F(1,18)=0.67387$ , $p=0.4225$  G <sub>1</sub> vs. G <sub>2</sub> : $p=0.5821$ G <sub>1</sub> vs. G <sub>3</sub> : $p=0.1197$ G <sub>1</sub> vs. G <sub>4</sub> : $p=0.4599$  Lower 95% CI of mean - Upper 95% CI of mean for G <sub>1</sub> , G <sub>2</sub> , G <sub>3</sub> and G <sub>4</sub> ; 0.67-1.92 (G <sub>1</sub> ), 1.22-1.80 (G <sub>2</sub> ), 1.16-3.11 (G <sub>3</sub> ), -0.06-3.74 (G <sub>4</sub> )
5d, right	Two-sided paired t-test	8 APP/PS1	Mice	Freezing[%]	Mean ± SEM	$p<0.0001$ $t=8.793$ , $df=7$  Lower 95% CI of mean - Upper 95% CI of mean; 13.98-25.43 (off), 29.54-45.47 (on),
5e, right	Two-sided paired t-test	6 wild-type	Mice	Freezing %]	Mean ± SEM	$p=0.0002$ $t=9.811$ , $df=5$ Lower 95% CI of mean - Upper 95% CI of mean; 1.31-12.60 (off), 16.62-23.73 (on)
5f	Two-sided unpaired t-test	6 wild-type 8 APP/PS1	Mice	Freezing[%]	Mean ± SEM	$p=0.0223$ $t=2.622$ , $df=12$  Lower 95% CI of mean - Upper 95% CI of mean; 44.82-64.39 (wild-type), 32.90-49.20 (APP/PS1)
5g	Two-sided unpaired t-test	6 wild-type 8 APP/PS1	Mice	Freezing %]	Mean ± SEM	$p=0.0226$ $t=2.614$ , $df=12$  Lower 95% CI of mean - Upper 95% CI of mean;  46.44-71.71 (wild-type), 24.07-51.81 (APP/PS1)
5h	Two-sided unpaired t-test	6 wild-type 8 APP/PS1	Mice	Freezing [%]	Mean ± SEM	$p=0.1066$ $t=1.745$ , $df=12$  Lower 95% CI of mean - Upper 95% CI of mean; 9.75-16.68 (wild-type), 13.01-22.59 (APP/PS1)
5i	Two-sided	3 wild-type	Mice	hChR2+	Mean ± SEM	$p=0.5060$

	unpaired t-test	3 APP/PS1		[10 <sup>-3</sup> μm <sup>-2</sup> ]		$t=0.7298$ , $df=4$  Lower 95% CI of mean - Upper 95% CI of mean; 0.84-1.29 (wild-type), 0.99-1.22 (APP/PS1)
5j	Two-sided unpaired t-test	3 wild-type 3 APP/PS1	Mice	hChR2+ [10 <sup>-3</sup> μm <sup>-2</sup> ]	Mean ± SEM	$p=0.3339$ $t=1.098$ , $df=4$  Lower 95% CI of mean - Upper 95% CI of mean; 0.88-1.22 (wild-type), 0.72-1.65 (APP/PS1)
6e	Two-sided unpaired t-test	5 CNO-treated 4 vehicle-treated	Mice	Fos+ [10 <sup>-3</sup> μm <sup>-2</sup> ]	Mean ± SEM	$p<0.0001$ $t=10.06$ , $df=7$  Lower 95% CI of mean - Upper 95% CI of mean; 0.43-1.16 (vehicle-treated), 3.12-4.53 (CNO-treated)
6f	Two-sided unpaired t-test	5 CNO-treated 4 vehicle-treated	Mice	Freezing[%]	Mean ± SEM	$p=0.0316$ $t=2.678$ , $df=7$  Lower 95% CI of mean - Upper 95% CI of mean; 34.26-54.17 (vehicle-treated), 18.57-41.13 (CNO-treated)
8c, right	Two-sided Mann-Whitney test	1062 mCherry-negative (mCherry-) 201 mCherry-positive (mCherry+)	pooled cells of 3 APP/PS1 mice	Intensity [AU]	Median, 25%- and 75%-quartiles, borders of plot show minimum and maximum	$p<0.0001$ Mann Whitney U 78979  Lower 95% CI of mean - Upper 95% CI of mean; 38.53-44.84 (mCherry-), 18.06-31.16 (mCherry+),
8d, right	Two-sided Mann-Whitney test	1062 mCherry- negative (mCherry-) 494 mCherry-positive (mCherry+)	pooled cells of 5 wild-type mice	Intensity [AU]	see above (9c, right)	$p<0.0001$ Mann Whitney U 218609  Lower 95% CI of mean - Upper 95% CI of mean; 47.81-54.67 (mCherry-), 33.16-42.16 (mCherry+)
8e	Two-sided unpaired t-test	5 wild-type 3 APP/PS1	Mice	mCherry+ [10 <sup>-3</sup> μm <sup>-2</sup> ]	Mean ± SEM	$p=0.3251$ $t=1.072$ , $df=6$  Lower 95% CI of mean - Upper 95% CI of mean; 1.094-2.48 (wild-type), 0.51-2.28 (APP/PS1)
9a, left	Two-sided Mann-Whitney test	4 vehicle-treated 8 CNO-treated	Mice	Travelled distance [m]	Mean ± SEM	$p=0.1483$ Mann Whitney U 8  Lower 95% CI of mean - Upper 95% CI of mean; 8.98-1.03 (vehicle-treated), 11.60-23.96 (CNO-treated)
9a, right	Two-sided unpaired t-test	4 vehicle-treated 8 CNO-treated	Mice	Freezing [%]	Mean ± SEM	$p=0.8926$ $t=0.1385$ , $df=10$  Lower 95% CI of mean - Upper 95% CI of mean; 52.37-66.25 (vehicle-treated), 45.25-71.14 (CNO-treated)
9b, left	Two-sided unpaired t-test	8 wild-type 10 APP/PS1	Mice	Travelled distance [m]	Mean ± SEM	$p=0.8043$ $t=0.2519$ , $df=16$  Lower 95% CI of mean - Upper 95% CI of mean; 11.26-15.02 (wild-type), 10.21-16.86 (APP/PS1)

9b, right	Two-sided unpaired t-test	4 wild-type 4 APP/PS1	Mice	Travelled distance [m]	Mean ± SEM	<p><math>p=0.9871</math>  <math>t=0.01682</math>, <math>df=6</math></p> <p>Lower 95% CI of mean - Upper 95% CI of mean;  11.60-23.96 (wild-type),  10.11-25.35 (APP/PS1)</p>
9c, left	Two-sided unpaired t-test	6 vehicle-treated 7 CNO-treated	Mice	Travelled distance [m]	Mean ± SEM	<p><math>p=0.6253</math>  <math>t=0.5023</math>, <math>df=11</math></p> <p>Lower 95% CI of mean - Upper 95% CI of mean;  7.79-16.59 (vehicle-treated),  11.35-14.78 (CNO-treated)</p>
9c, right	Two-sided unpaired t-test	6 vehicle-treated 7 CNO-treated	Mice	Freezing [%]	Mean ± SEM	<p><math>p=0.3159</math>  <math>t=1.051</math>, <math>df=11</math></p> <p>Lower 95% CI of mean - Upper 95% CI of mean;  52.39-67.66 (vehicle-treated),  33.13-71.01 (CNO-treated)</p>
10d	One-way ANOVA with Holm-Sidak's multiple comparison test	n=6 group A n=6 group B n=6 group C	Mice	Freezing [%]	Mean ± SEM	<p>F1, Treatment: <math>F(2,15)=0.6322</math>, <math>p=0.5450</math></p> <p>A vs. B: <math>p=0.5053</math>  A vs. C: <math>p=0.7885</math></p> <p>Lower 95% CI of mean - Upper 95% CI of mean;  39.95-68.01 (group A),  29.76-60.75 (group B),  37.34-66.22 (group C)</p>

**Supplementary Table 2 Correlation coefficients and p-values of the analysis in Figure 2e**



**Recall network of APP/PS1 mice shows high similarity to mice encountering a novel context. a,b**, Two-sided Pearson correlation coefficients displayed color-coded in a matrix of Fig. 3f (**a**) and numerically in a table (**b**). **c,d**, P-values of the correlations in **a** and **b** depicted in a color-code (**c**) and numerically (**d**). Data of Fig. 2, according to Fig. 1 from n=7 fosGFP A-A (G<sub>1</sub>), n=6 fosGFP-APP/PS1 A-A (G<sub>2</sub>), n=6 fosGFP A-B (G<sub>3</sub>) and n=3 fosGFP-APP/PS1 A-B (G<sub>4</sub>) mice; data include BL and A-A/B imaging, comprising measurements from 4322 (G<sub>1</sub>), 5099 (G<sub>2</sub>), 3755 (G<sub>3</sub>) and 2195 (G<sub>4</sub>) cells, respectively; statistics were done over mice; **a-d**, two-sided t-test corrected for multiple comparisons with Holm-Bonferroni.