Natural forgetting is modulated by experience

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Summary

Memories are stored as ensembles of engram neurons and their successful recall involves the reactivation of these cellular networks. While progress has been made in understanding the biology of engrams, significant gaps remain in connecting these cell ensembles with the process of forgetting. Here, we examine whether forgetting is governed by changes in engram plasticity and suggest that it helps animals prioritize relevant memory representations for adaptive behavior. We utilized a mouse model of object memory and investigated the conditions in which a memory could be preserved, retrieved, or forgotten. The results indicate that engram activity correlated with the rate of forgetting. Direct modulation of engram activity via optogenetic stimulation or inhibition either facilitated or prevented the recall of an object memory. In addition, the modulation of engram activity was able to prevent forgetting itself. Moreover, through pharmacological and behavioral interventions, we successfully prevented or accelerated forgetting of an object memory. Finally, we show that these results can be explained by a computational model in which engrams that are subjectively less relevant for adaptive behavior are more likely to be forgotten. Together, these findings suggest that forgetting is an adaptive form of engram plasticity that involves circuit remodeling, which allows engrams to switch from an accessible state to an inaccessible state.

Introduction

How does the environment prime the brain for optimum learning? For learning to be efficient, information must be encoded in a discrete, non-overlapping fashion. Without such a process, memory recall would suffer high interference from similar experiences (1). Furthermore, for an organism to adapt to its environment there must be processes by which learned information is updated or replaced with new more relevant information (2). For example, learning that a specific food source is no longer present in a previous location, or that a given action stops yielding the same outcome (2–4). Without a process to update or forget irrelevant information, adaptability would be impaired, with frequent instances of correct but outdated memory recall, and the subsequent biased behavioral response (4–7).

Memories are stored as ensembles of engram cells that undergo specific forms of plasticity during learning, and their successful recall involves the reactivation of these cellular networks (8–12). However, despite advancements in understanding the biology of engram cells, work is still needed to fully elucidate how these cell ensembles contribute to the process of forgetting (12–14). We argue that a new conceptualization is necessitated by recent behavioral and physiological findings that emphasize retrieval deficits as a key characteristic of memory impairment, supporting the idea that memory accessibility may be driven by learning feedback from the environment (7, 13–18). Little is understood about the role experience plays in altering forgetting rates. Recently it has been suggested that different forms of forgetting may exist along a gradient of engram expression (13). Indeed, numerous studies have suggested that memory recall (15, 17, 19–22), and most recently forgetting, require engram activity (18, 23). Long-term memories of salient experiences can last a lifetime and must

involve significant and specific changes to brain structure, while the successful recall of learned information requires the reactivation of these cell ensembles (10). In contrast, forgetting occurs when engrams are not or cannot be reactivated (13, 24). In severe amnesic states, this may be due to the destruction of the engram itself (13, 25–28). However, in other pathological and non-pathological states, forgetting may be due to reduced accessibility of engrams that otherwise endure (28).

Altered engram accessibility may be caused by structural plasticity in the engram or indeed by competing engrams of similar or recent experiences (13, 29–33). We hypothesize that natural forgetting represents a reversible suppression of engram ensembles due to experience and perceptual feedback, prompting cellular plasticity processes that modulate memory access adaptively. In pathological cases of memory loss these forgetting triggers are aberrantly initiated causing maladaptive forgetting (13). To investigate this idea, we developed a forgetting paradigm based on an object recognition task and interrogated the conditions in which a memory could be preserved, retrieved, or forgotten to elucidate the mechanisms that govern engram accessibly or “memory expression”. Based on our experimental results, we developed a computational model that dynamically updates engram accessibility in response to perceptual feedback, where engrams that are subjectively less relevant for adaptive behavior are more likely to be forgotten.

Results

Natural forgetting of an object memory

We developed a behavioral task in which mice displayed natural forgetting across time. We based this task on an object recognition paradigm which utilizes the natural tendency of mice to explore novel stimuli to assess recognition memory (Figure 1a) (34, 35). During the acquisition phase all mice spent equivalent times exploring the two sample objects (Supplementary Figure S1a). During object memory recall, mice tested 24 hours (h) after training spent significantly more time exploring the novel object compared to mice tested 2 weeks (wk) after training (Figure 1b). This was further demonstrated by a significant difference between the discrimination index of the 24 h and 2 wk group (Figure 1c). Together, these data indicate that mice successfully recall an object memory 24 hours after acquisition, while at 2 weeks there was no preference for the novel object suggesting the original familiar object had been forgotten.

Engram reactivation and object memory

In order to assess engram reactivation on object memory retrievability an AAV_9-TRE-ChR2-EYFP virus was injected into the dentate gyrus of c-fos-tTA mice (36, 37). The immediate early gene *c-fos* becomes upregulated following neural activity and as such the c-fos-tTA transgene selectively expresses tTA in active cells (37, 38). The activity-dependent tTA induces expression of ChR2-EYFP following a learning event (Figure 1d, e). To restrict activity-dependent labelling to a single learning experience, mice were maintained on diet containing doxycycline (DOX) which prevents binding of tTA

to the TRE elements of the TetO promoter, thus controlling the activity-dependent labelling to a specific time window of the object acquisition training (Figure 1d-f). Prior to training (36 h) doxycycline was removed from the animal's diet, thereby allowing for activity-dependent labelling during the subsequent acquisition training (Figure 1f). During the acquisition phase all c-fos-tTA mice spent equivalent times exploring the two sample objects (Supplementary Fig S1b). Again, during the object recall test, c-fos-tTA mice tested 24 hours after acquisition training spent significantly more time exploring the novel object compared to mice tested 2 weeks later (Figure 1g). This memory impairment was further demonstrated by a significant difference between the discrimination index of the 24 h tested and 2 wk tested mice (Figure 1h). When comparing the engram ensemble between the two cohorts, we observed no significant difference in the number of engram cells between the 24 h and 2 wk tested groups ($EYFP^+$) (Figure 1j, k). Similarly, there was no significant difference in c-Fos activity within the dentate gyrus between the 24 h tested or 2 wk tested mice (Figure 1l). However, when comparing engram reactivation, that is the number of engram cells ($EYFP^+$) that also express c-Fos during the recall test, which indicates these cells were active during encoding and then became active again during the memory recall (Figure 1j), there was a significant decrease in engram reactivation in mice that were tested 2 weeks after training and that had forgotten the object memory (Figure 1m). Furthermore, the level of engram reactivation correlated with the performance in object discrimination (Figure 1i). Together these data suggest that natural forgetting may be driven by a reduction in engram ensemble activity.

Engram spine density decreased following natural forgetting

In order to gain further insight into the changes that occur within the memory engram following natural forgetting we performed morphological analysis of engram dendritic spines following successful memory recall at 24 hours or forgetting at 2 weeks. Following natural forgetting mice displayed a significant reduction in spine density (Figure 1n). Moreover, there was also a significant decrease in spine volume (Figure 1o, p). Together, these findings suggest that in addition to the reduced engram activity, forgetting may also induce a level of architectural change within the ensemble itself.

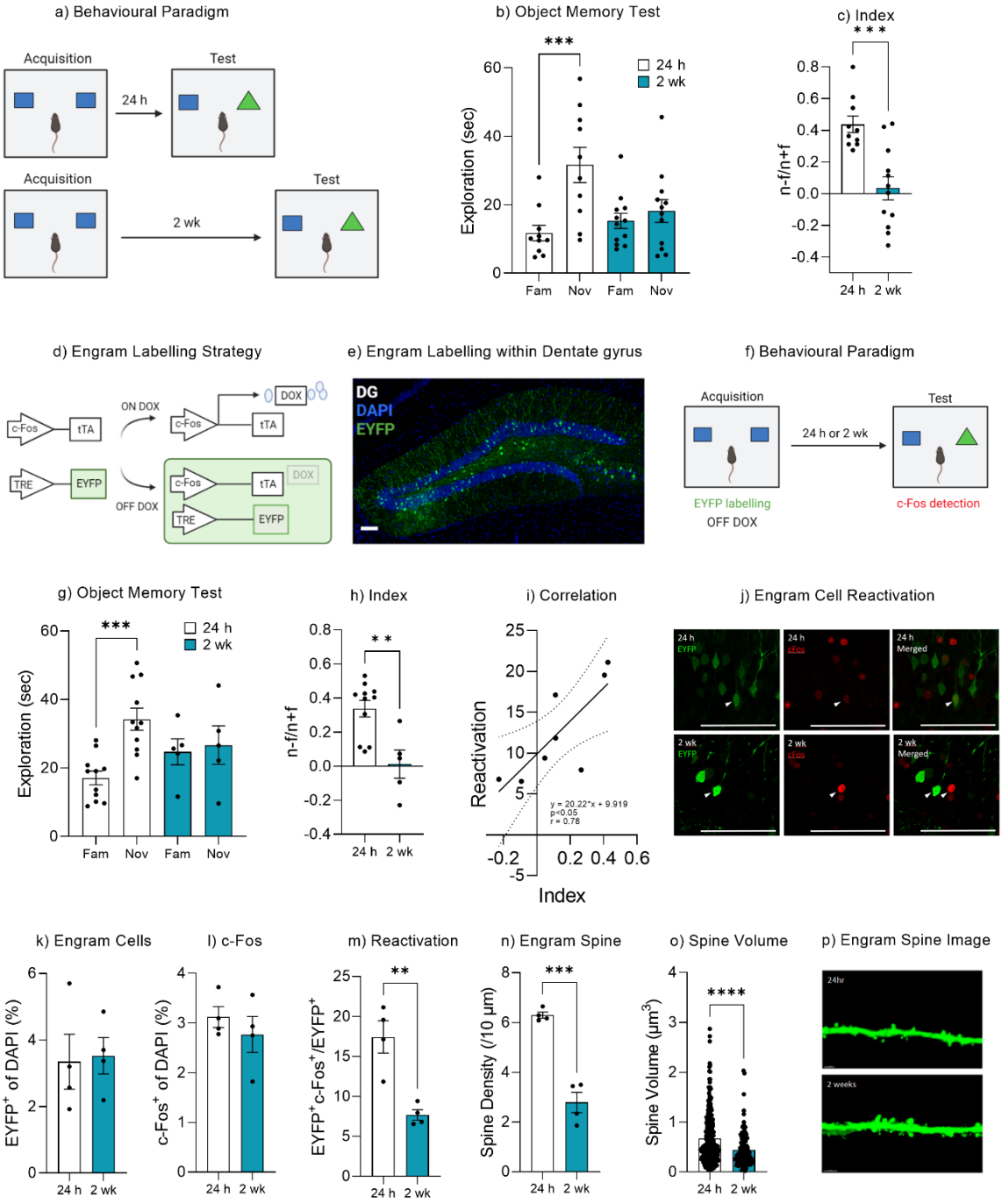

Figure 1: ***(a)*** *Object recognition paradigm* ***(b)*** *Object memory test for recall at 24 h or 2 wk* ***(c)*** *Discrimination index* ***(d)*** *Schematic of c-Fos-tTA engram labelling system* ***(e)*** *Representative image of engram labeling within the dentate gyrus* ***(f)*** *Engram labeling of object memory and c-Fos detection following recall* ***(g)*** *Object memory test for recall at 24 h or 2 wk* ***(h)*** *Discrimination index* ***(i)*** *Correlation between Discrimination Index and Engram reactivation* ***(j)*** *Representative image EYFP⁺ cells, c-Fos⁺ cells and Merged EYFP⁺ and c-Fos⁺ for both 24 h and 2 wk test* ***(k)*** *Engram cells (****l****) c-Fos⁺ cells* ***(m)*** *Engram reactivation* ***(n)*** *Engram spine density average per mouse,* ***(o)*** *Engram spine volume average per dendrite* ***(p)*** *Representative image of engram dendrite for morphological analysis. Bar graphs indicates average values in n = 4-11 per group (**p<0.01, ***p<0.001). Data graphed as means ± SEM. Scale bar 100um.*

Dentate gyrus engrams are necessary and sufficient for recall of an object memory

To test the hypothesis that the retrieval of an object memory requires the activity of engram cells within the dentate gyrus, we labelled engrams with the virus AAV_9-TRE-ArchT-GFP and inhibited engram activity during memory retrieval 24 hours following acquisition training (Figure 2a). During the acquisition phase all mice spent equivalent times exploring the two sample objects (Supplementary Figure S2a). During the object recall test the No Light control mice spent significantly more time with the novel object, indicating successful memory retrieval (Figure 2b). Whereas mice in which the engram was inhibited spent equal time exploring both objects, indicating mice failed to distinguish the novel from the familiar object (Figure 2b). This was further demonstrated by a significant difference between the discrimination index of the No Light control and Light-induced inhibition groups (Figure 2c). Together, these data demonstrate that the engram cells within the dentate gyrus are required for successful retrieval of an object memory.

Since the data suggested that forgetting correlated with a reduction in engram activity (Figure 1) and that inhibition of the ensemble was sufficient to block successful recall of an object memory (Figure 2b, c), we next asked if the retrieval of an object memory could be induced via activation of the engram after natural forgetting. We labelled engrams cells within the dentate gyrus with the virus AAV_9-TRE-ChR2-EYFP and optogenetically activated the ensemble after natural forgetting. Specifically, optogenetic stimulation was induced just prior to memory recall (3min), but not during the recall test itself (Figure 2d). During the acquisition phase all mice spent equivalent times exploring the two sample objects (Supplementary Figure S2b). During the object recall test the No Light control mice displayed equal exploration of both the novel and familiar objects suggesting mice had forgotten the familiar object (Figure 2e). Whereas

mice that underwent optogenetic activation spent more time exploring the novel object, suggesting the original object memory had been retrieved (Figure 2e). This was further demonstrated by a significant difference between the discrimination index of the No Light control and Light-induced activation group (Figure 2f). These data suggest that artificial activation of the original engram was sufficient to induce recall of the forgotten object memory. Together, the results of Figure 2 suggest that engram activity can modulate memory retrieval, where activation of the engram ensemble is both necessary for successful memory retrieval as well as sufficient to induced recall despite natural forgetting.

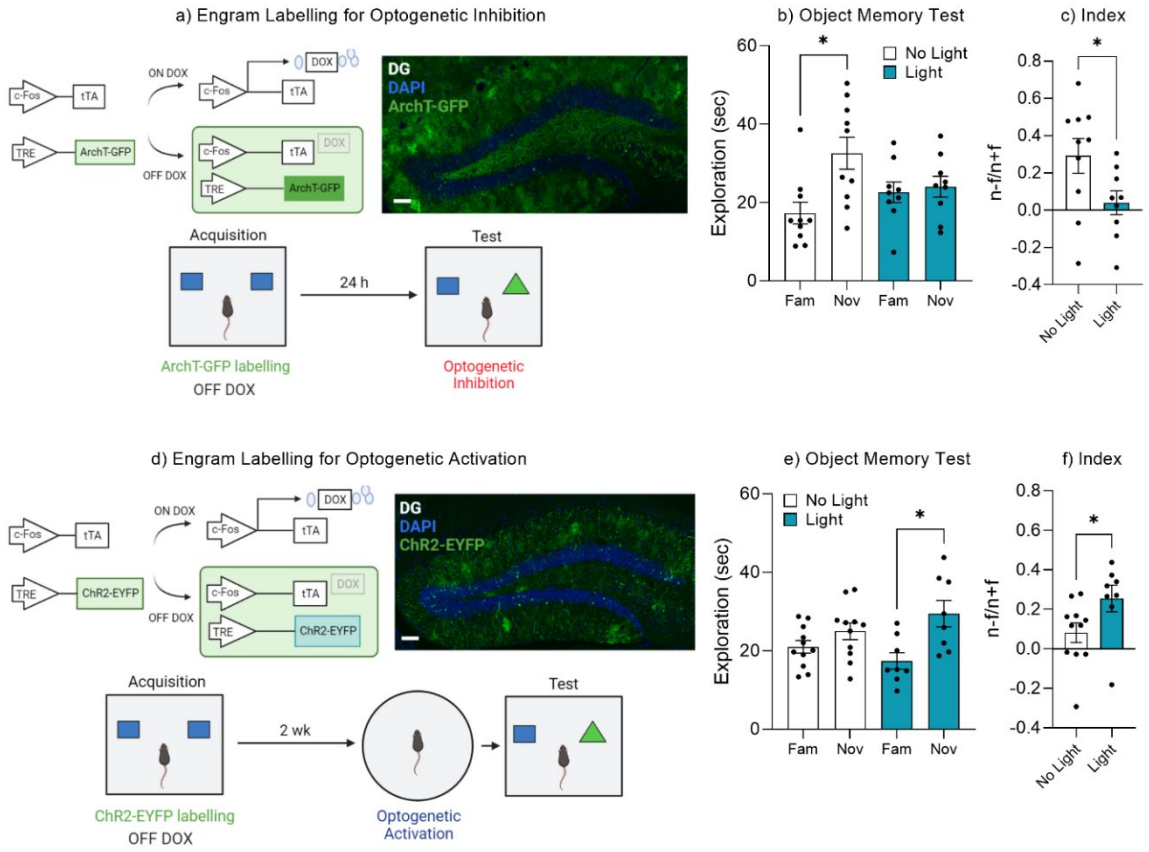

Figure 2: ***(a)*** *Engram labelling for optogenetic inhibition and behavioral timeline* ***(b)*** *Object memory test* ***(c)*** *Discrimination index* ***(d)*** *Engram labelling for optogenetic activation and behavioral timeline* ***(e)*** *Object memory test* ***(f)*** *Discrimination index Bar graphs indicate average values in n = 9-12 per group (*p<0.05). Data graphed as means ± SEM. Scale bar 100um.*

Environmental enrichment reduces the rate of forgetting by increasing engram activation and hippocampal neurogenesis

Environmental enrichment has been shown to enhance cognitive function and improve memory retention (39–43). We, therefore sought to investigate how enrichment might alter the rate of forgetting through changes in the engram. First, we characterized the forgetting curve of mice housed under either standard or enriched conditions (Figure 3a). During the acquisition phase all mice spent equivalent times exploring the two sample objects (Supplementary Figure S3a, c, e, g). During object recall test both standard housed (Std) and Environmental Enrichment housed (EE) mice spent significantly more time with the novel object when tested 24 hours or 1 week after acquisition training, indicating intact memory recall (Figure 3b, S3b &d). Crucially though, when tested 2 or even 3 weeks after training the enriched housed mice were still able to recall the familiar object, whereas the standard mice displayed chance level performance (Figure 3b, S3e &h).

In order to assess the impact of environmental enrichment on engram activity we again injected an AAV_9-TRE-ChR2-EYFP virus into c-fos-tTA mice with to label engram cells within the dentate gyrus. Following surgery, mice were housed in either standard or enriched housing for 3 weeks before behavioral testing and remained in the housing condition for the duration of the experiment. During the acquisition phase all mice spent equivalent times exploring the two sample objects (Supplementary Figure S3i). Again, we observed that mice housed in enrichment maintained the object memory 2 weeks after training (Figure 3c). This was further demonstrated by a significant difference between the discrimination index of standard housed (Std) and enriched (EE) mice (Figure 3d). When comparing the engram size between standard and enriched mice, we observed no significant difference in the number of engram cells

following enrichment (Figure 3e &f). Similarly, there was also no significant difference in c-Fos activity (Figure 3g). However, there was a significant increase in engram reactivation in mice that underwent enrichment (Figure 3h). These data suggest that environmental enrichment reduces the rate of forgetting, by increasing or maintaining engram reactivation. Furthermore, environmental enrichment increased hippocampal neurogenesis, with both the number of immature neurons (doublecortin^{+} cells) (Figure 3i &j) as well as neuronal survival (BrdU-NeuN positive cells) (Figure 3k &l). Together the data in Figure 3 suggest environmental enrichment increases the accessibility of the original engram, through maintaining the engram reactivation to retrieval cues. Moreover, this enhanced memory expression may be achieved through the up regulation of hippocampal neurogenesis.

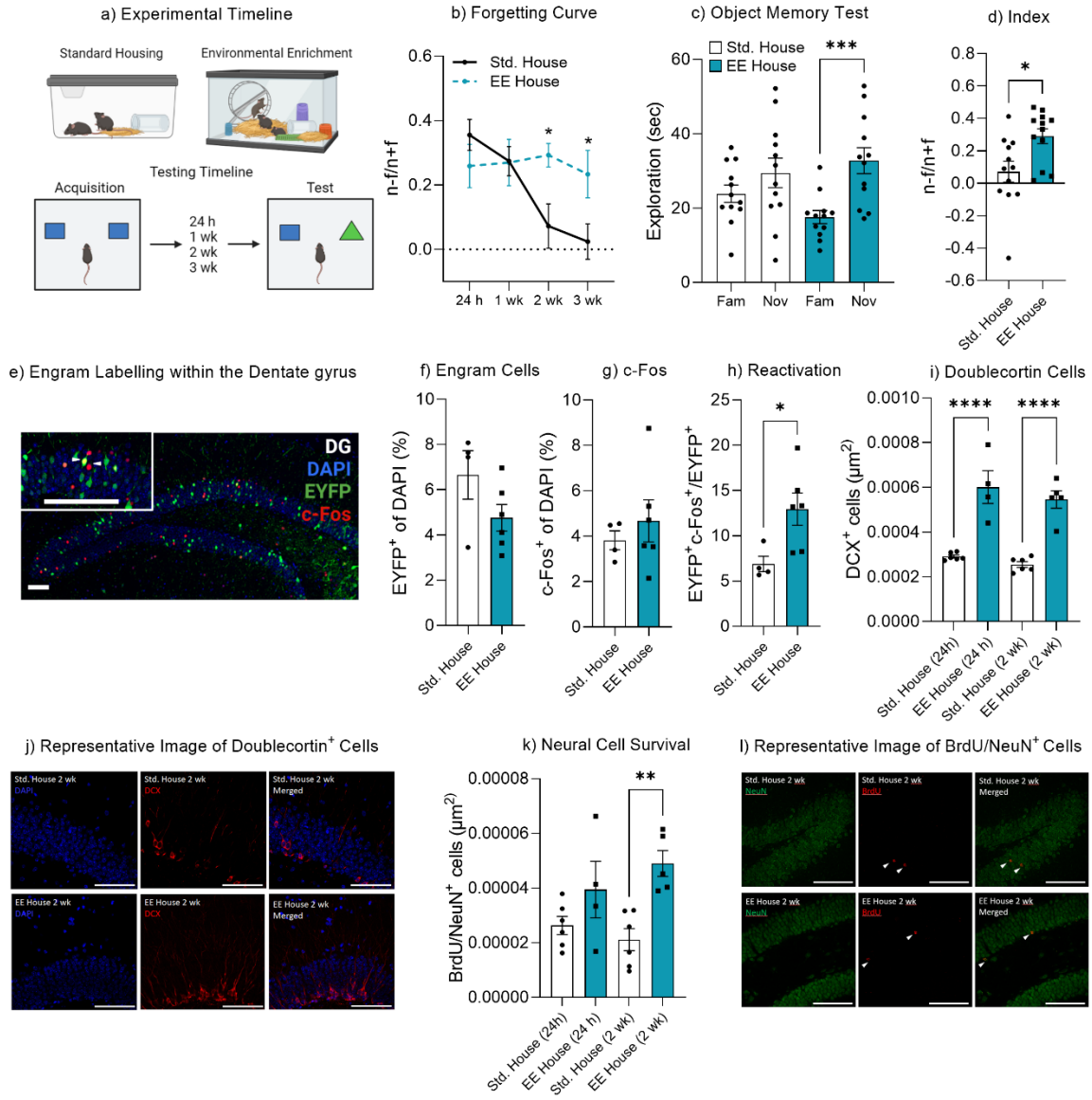

Figure 3: ***(a)*** *Enrichment behavioral paradigm* ***(b)*** *Natural forgetting curve of an object memory at 24 h, 1 wk, 2 wk and 3 wk* ***(c)*** *Object memory test* ***(d)*** *Discrimination index* ***(e)*** *Engram labelling within the dentate gyrus* ***(f)*** *Engram cells* ***(g)*** *c-Fos⁺ cells* ***(h)*** *Engram reactivation* ***(I)*** *Doublecortin⁺ cells* ***(j)*** *Representative image of Doublecortin⁺ cells* ***(k)*** *Neural cell survival* ***(l)*** *Representative image of BrdU/NeuN⁺ cells. Bar graphs indicate average values in n = 4-12 per group (*p<0.05, **p<0.01, ***p<0.001). Data graphed as means ± SEM. Scale Bar 100um.*

Exposure to the original stimuli facilities memory recall

We next sought to investigate conditions in which learning may alter engram activity and subsequently switch a forgotten memory from an inaccessible state to an

accessible state. Previous work from both human experimental psychology studies and rodent behavioral paradigms have shown that a brief exposure to reminder cues can aid memory recall (44–46). Here we modified our object-based paradigm to include a brief exposure to the original encoding environment and objects (Figure 4a). We first demonstrated that a brief reminder exposure of 5 mins was insufficient to induce new learning or form a lasting memory (Supplementary Figure S4a). Mice given only a brief acquisition period of 5 mins, exhibited no preference for the novel object when tested 1 hour after training, suggesting the absence of a lasting object memory (Supplementary Figure S4b & c). Next, an AAV_9-TRE-ChR2-EYFP virus was injected into c-fos-tTA mice to label engram cells in the dentate gyrus. During the acquisition phase all mice spent equivalent times exploring the two sample objects (Supplementary Figure 4d). During the object recall test, mice within the control group spent a similar amount of time exploring both the novel and familiar object, again suggesting the original object memory was forgotten (Figure 4b). However, mice given a brief reminder session 1 hour prior to the recall test spent significantly more time exploring the novel object (Figure 4b). This was further demonstrated by a significant difference between the discrimination index of the Control and Reminder group (Figure 4c). Furthermore, the mice that underwent the reminder session and displayed intact memory recall also exhibited an increase in original engram reactivation (Figure g), while there was no difference in the engram size (Figure 4e) or the level of neuronal activity within the dentate gyrus (Figure 4f). Together, these data indicate that a brief reminder of the original stimuli facilitates the transition of a forgotten memory to an accessible memory via modulating engram activity.

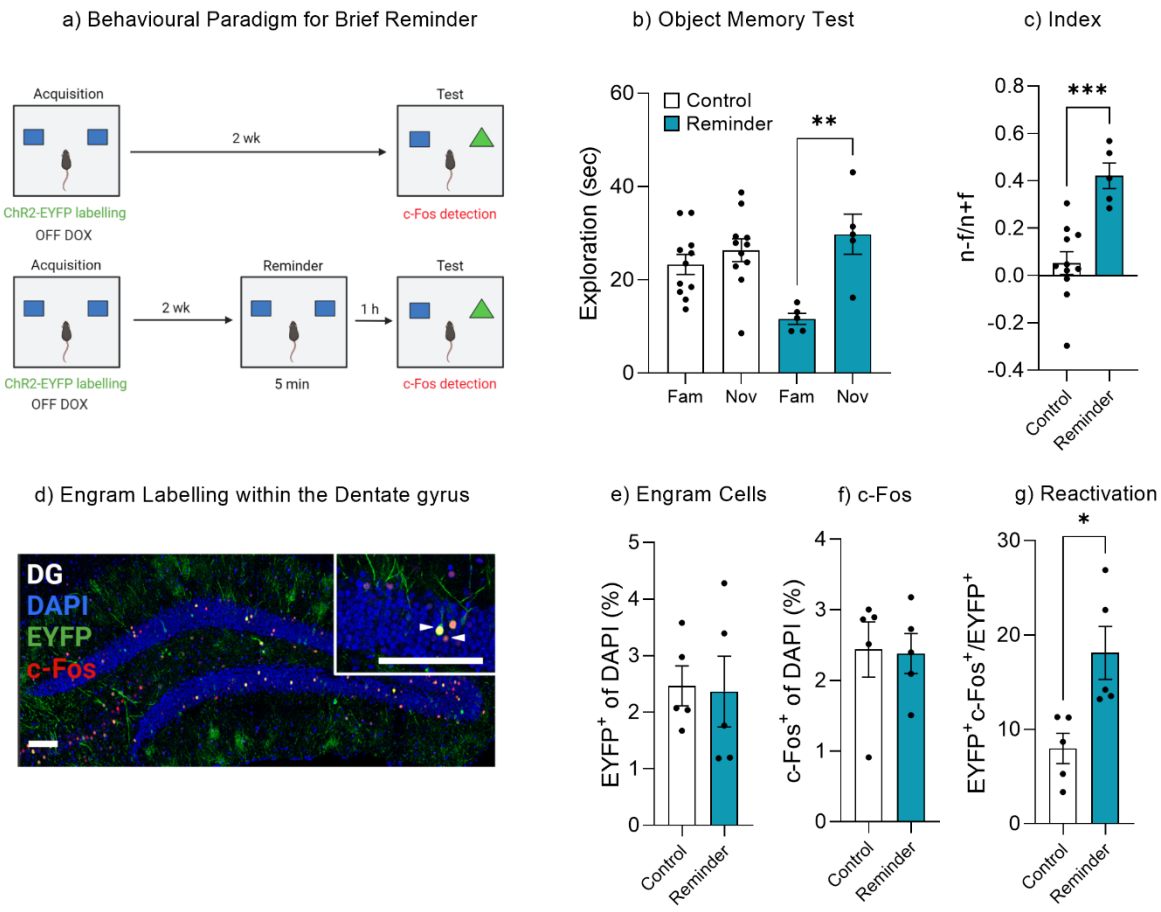

Figure 4: *(**a**) Behavioral timeline for a brief reminder exposure (**b**) Object memory test (**c**) Discrimination index (**d**) Engram labelling within the dentate gyrus (**e**) Engram cells (**f**) c-Fos⁺ cells (**g**) Engram reactivation. Bar graphs indicates average values in n = 5-11 per group (*p<0.05, **p<0.01, ***p<0.001). Data graphed as means ± SEM. Scale Bars 100um.*

Repeated exposure to the training environment facilitates forgetting

Given that memory recall could be facilitated by optogenetic activation (Figure 2), maintained by enrichment (Figure 3), or even retrieved after forgetting by a brief exposure to natural cues “reminder” (Figure 4), we wanted to test the hypothesis that forgetting was driven by an adaptive process which updates memory engrams according to environmental feedback. We therefore developed an altered version of our object memory task where mice were repeatedly reintroduced to the training context in the absence of objects (Figure 5a). We hypothesized that the repeated

exposure to the training context without objects would update the original memory engram signaling that the objects were no longer relevant to the environment. During the acquisition phase all mice spent equivalent times exploring the two sample objects (Supplementary Figure S5a). During the recall test 1 week after training control mice spent significantly more time exploring the novel object, indicating a retrievable object memory (Figure 5b). Whereas mice that had been repeatedly exposed to the training environment devoid of objects spent a similar amount of time exploring both objects, suggesting an updated and now inaccessible engram. This was further demonstrated by a significant difference in the discrimination index between the control and empty box exposed mice (Figure 5c).

In order to confirm that the experience during the repeated exposure was indeed updating the original engram we ran a second cohort of c-fos-tTA mice which had been implanted with an optogenetic fiber and expressed the inhibitory opsin ArchT (Figure 5d). Following acquisition training the engram was optogenetically silenced during the repeated context-only exposures (Figure 5d). Again, during the recall test, the No Light control group spent a similar amount of time exploring both objects (Figure 5e), while the Light-induced inhibition mice, in which the engram was silenced, spent significantly more time exploring the novel object (Figure 5e). This effect was further demonstrated by a significant difference in discrimination index (Figure 5f). Combined these data suggest that experience following the encoding of an object memory can update the original memory engram accelerating the rate at which the information is forgotten. Furthermore, this is an adaptive process which requires the activity of the engram ensemble.

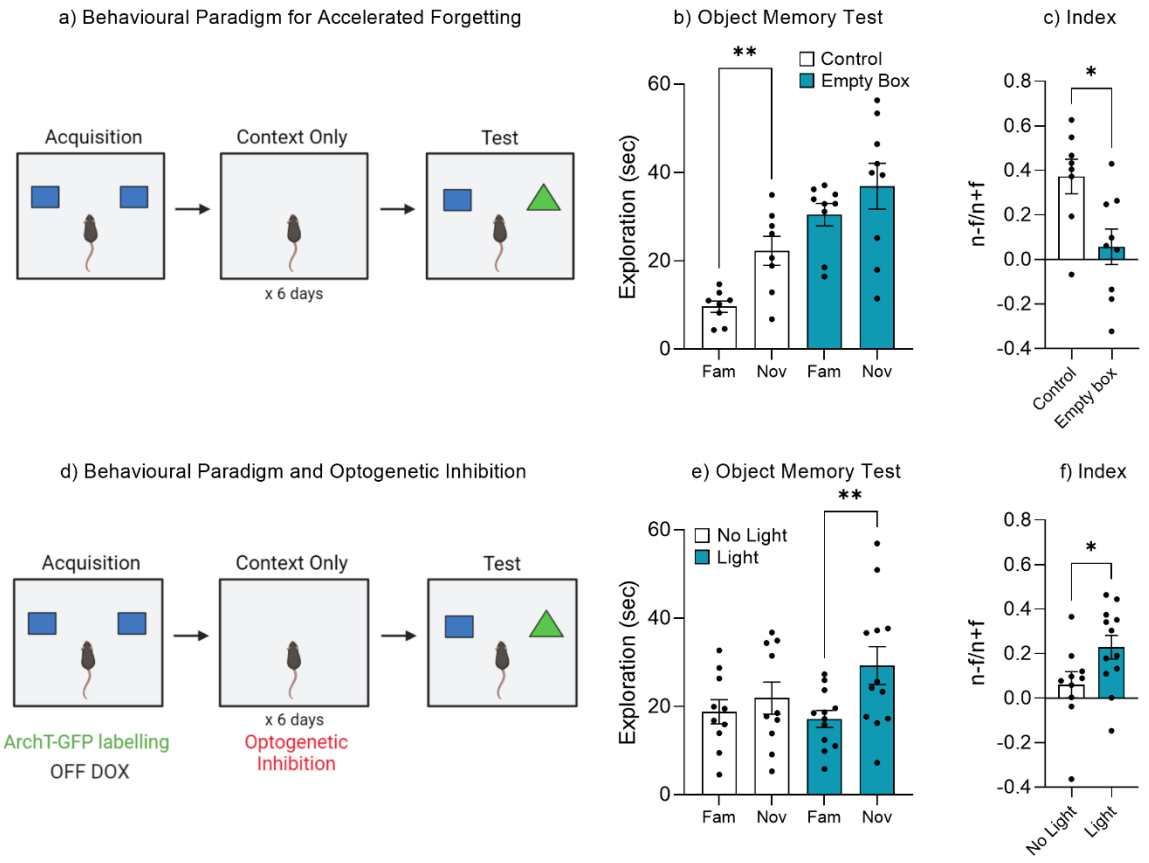

Figure 5: ***(a)*** *Behavioral paradigm* ***(b)*** *Object memory test* ***(c)*** *Discrimination index* ***(d)*** *Behavioral paradigm for optogenetic inhibition during repeated context exposure* ***(e)*** *Object memory test* ***(f)*** *Discrimination index. Bar graphs indicates average values in n = 8-12 per group (*p<0.05, **p<0.01). Data graphed as means ± SEM.*

Rac1 mediates forgetting of an object memory

Rac1 has previously been shown to mediate forgetting of both contextual as well as social memory (47–50). We therefore investigated the role of Rac1 signaling in mediating natural forgetting of an object memory. We first injected a Rac1 inhibitor into wild-type mice with following object memory encoding and tested recall after 2 weeks, once the object memory was forgotten (Figure 6a). Mice that were administered saline displayed typical forgetting at 2 weeks, as indicated by similar object exploration (Figure 6b), while mice treated with a Rac1 inhibitor displayed intact memory recall, spending significantly more time with the novel object (Figure 6b). This was further

demonstrated by a significant difference in discrimination index (Figure 6c). Together these data demonstrate that the inhibition of Rac1 following memory encoding is sufficient to enhance the retrievability of an object memory. Given Rac1-mediated retrieval, it stands to reason that by activating Rac1 forgetting maybe accelerated. We next injected a a Rac1 activator into a second cohort of wild-type mice following training (Figure 6d). This time, the saline control showed intact memory retrievability, with mice spending more time with the novel object (Figure 6e). Whereas the mice administered with the Rac1 activator displayed a memory impairment, spending a similar amount of time with both objects. This was further demonstrated by a significant difference in discrimination index (Figure 6f). Combined these data demonstrate the Rac1 signaling is involved in memory retrievability and may therefore be a signaling mechanism involved in adaptive forgetting.

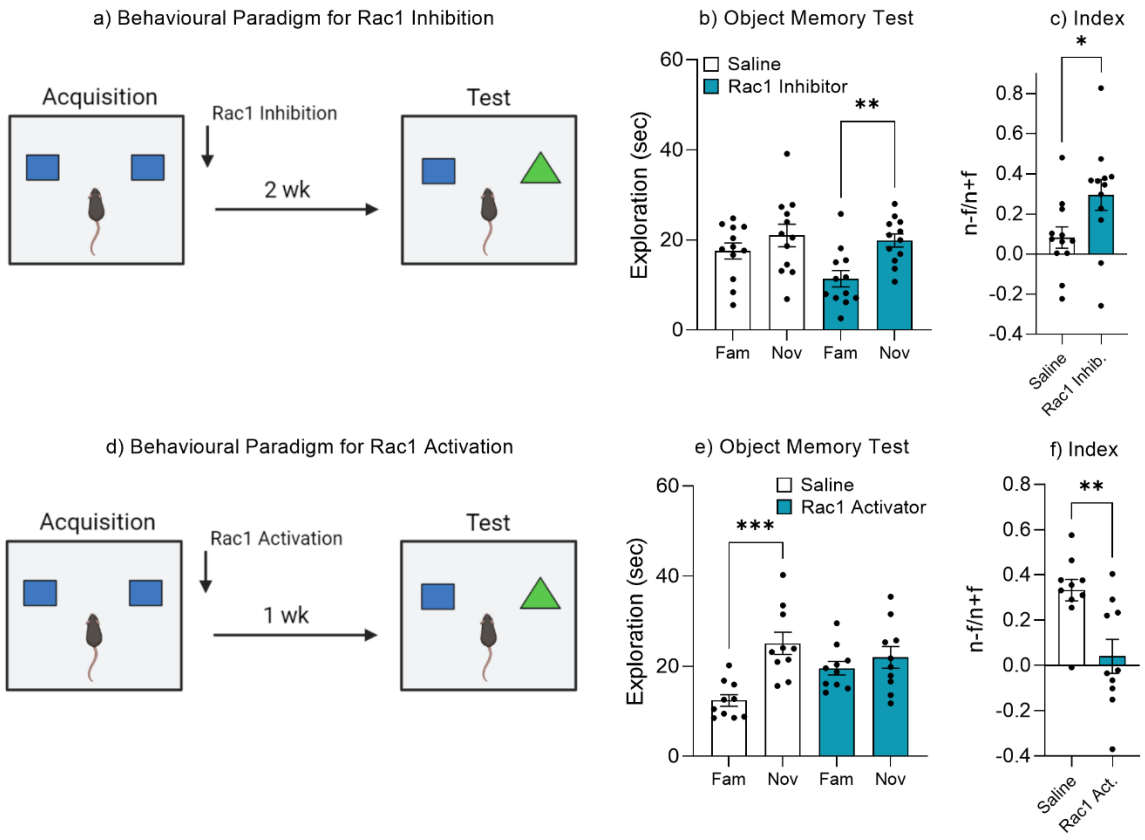

Figure 6: ***(a)*** *Experimental timeline and drug administration for Rac1 inhibition* ***(b)*** *Object memory test* ***(c)*** *Discrimination index* ***(d)*** *Experimental timeline and drug administration for Rac1 activation* ***(e)*** *Object memory test* ***(f)*** *Discrimination index. Bar graphs indicates average values in n = 10-12 per group (*p<0.05, **p<0.01, ***p<0.001). Data graphed as means ± SEM.*

Learning Model Explains Forgetting Dynamics

Utilizing a mouse model of object memory, we have investigated the conditions in which a memory could be preserved, retrieved, or forgotten. The data indicate that forgetting is a dynamic process that can be explained as a gradient of engram activity, where the level of memory expression is experience dependent and influenced by the animal's rate at which it learns from new experiences (Figure 7a-c). Based on these findings, we sought to develop a computational model that formalizes the idea that forgetting is a form of learning and to integrate the empirical findings into a coherent, mechanistic, and quantitative framework. This model updates engram accessibility in

response to perceptual feedback, so that engrams that are subjectively less relevant for adaptive behavior are more likely to be forgotten. Our modeling analyses suggest that the "learning rate", which is a key parameter of adaptive learning models, could also govern the speed of forgetting. Accordingly, faster forgetting is driven by higher rates at which mice learn from perceptual feedback.

We interpret forgetting as an adaptive learning process that helps the animal to prioritize relevant information in memory (Figure 7). In particular, we applied an error-driven learning model (51, 52), where the learning rate and expectation of one's environment can alter the efficiency of recall/forgetting plasticity (13). Based on the current memory representations stored in engrams, the animal predicts what it will encounter in its environment (e.g., what objects) and adjusts these predictions as a function of its experiences (e.g., the presence or absence of objects). Within this conceptual framework, the animal learns what features of the environment are currently relevant and thus important for remembering.

Supporting the idea that forgetting can be cast as an adaptive learning process, model simulations showed similar forgetting curves as mice in our experiment. We first applied the model to exploration behavior of mice in the standard housing condition that included data across four test time points (Figure 3). To obtain an empirical estimate of the subjects' learning rate governing the speed of forgetting, we fitted the model to the data using a maximum-likelihood approach (see Methods). Here, the learning rate indicating the weight given to negative prediction errors was $\alpha^{-} = 0.07$, explaining the increase in forgetting across time (see Supplementary Table 1 for the other parameter estimates). Simulated forgetting curves that were based on the

estimated parameters showed a close similarity to mice in the standard housing condition (Figure 7d; see Supplementary Figure S7 and S8 for a direct comparison of model predictions and empirical data).

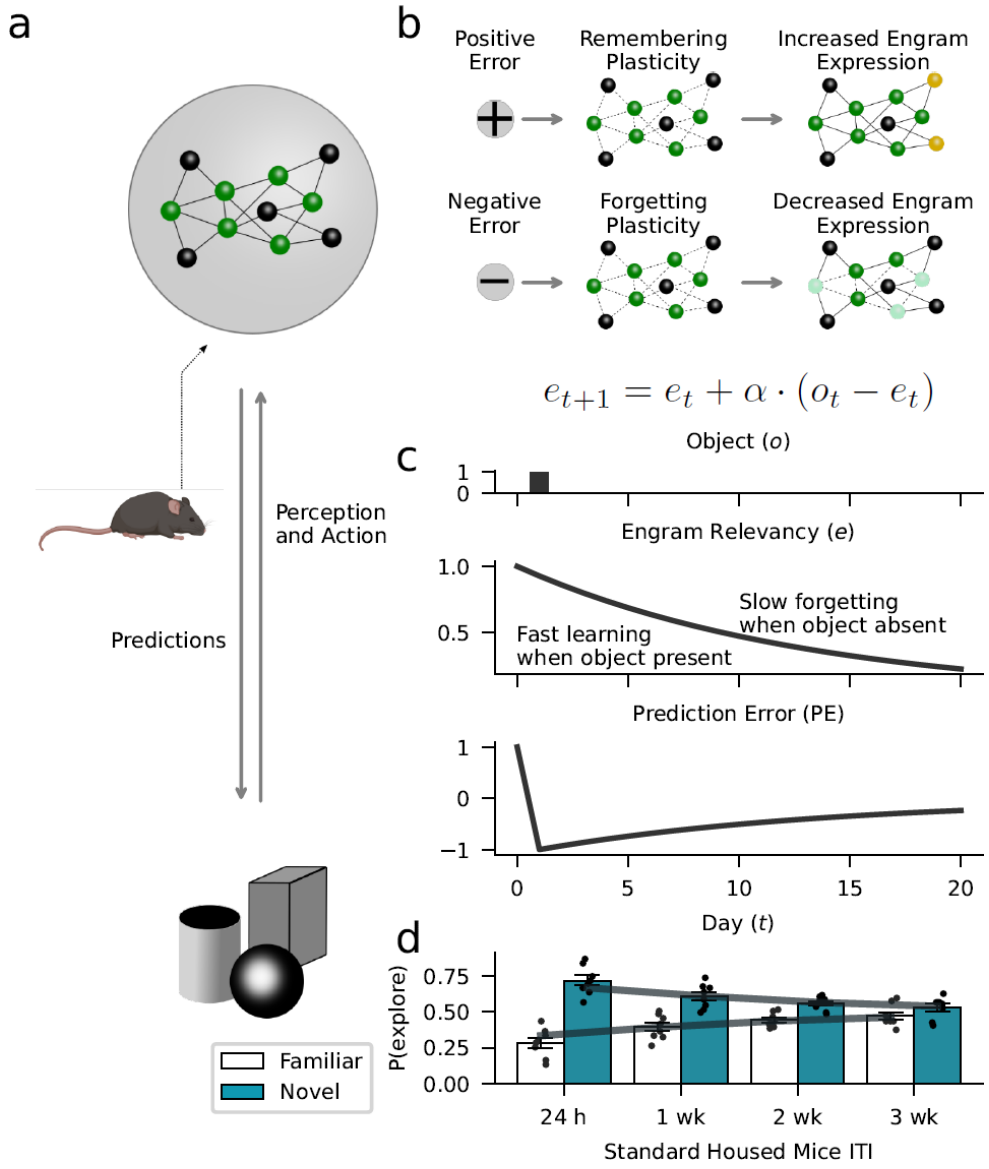

Figure 7: *Forgetting as adaptive learning. Our model assumes that animals create and update memory engrams to flexibly adjust their behavior to their environment.* ***(a)*** *Based on learned representations, animals constantly predict what happens in the environment (e.g., the occurrence of objects), and if predictions are violated (prediction errors), engrams are updated to improve the accuracy of future predictions. Here, established engram cells are shown in green; non-engram cells in gray.* ***(b)*** *Positive prediction errors signaling the occurrence of an unexpected event (e.g., new object) induce a learning process that increases the probability of remembering. This might rely on the recruitment of new engram cells (shown in yellow). In contrast, negative prediction errors signaling the absence of an expected event (e.g., predicted object*

did not appear) induce forgetting. This might rely on "forgetting" plasticity reducing access to engrams (light green cells). ***(c)*** *Our model formalizes this perspective based on the notion of "engram relevancy". Higher engram relevancy makes it more likely that an engram is behaviorally expressed, e.g., through exploration behavior. The presentation of a novel object (upper panel) leads to a high engram relevancy (middle panel) in response to a positive prediction error (lower panel). The absence of an expected object decreases engram relevancy through negative prediction errors.* ***(d)*** *Model simulations corroborate the behavioral effects of our data (Figure 3a). Gray lines and bars show the average exploration probability for the familiar and novel object according to the model; markers show simulated mice.*

The model also offers an explanation for the key results of the different experimental interventions. Experiencing objects during environmental enrichment that resemble the familiar objects in the memory test might nudge subjects to infer a higher engram relevancy that is more robust against prediction errors leading to higher engram expression (Figure 8a & 8b, d, f). Supporting the perspective of forgetting as learning, the estimated learning rate in response to negative errors driving forgetting $\alpha^- = 0$. Thereby, negative prediction errors did not induce forgetting so that memory performance was constant across 4 weeks (see also Supplementary Figure S7). From a computational perspective, a low learning rate may also explain the high memory performance of the Rac1-inhibition group. For this group, model fitting revealed a similar learning rate to the enrichment group (learning rate for negative prediction errors $\alpha^- = 0.01$), suggesting that the inhibition of Rac1 made subjects largely ignore negative prediction errors that would normally drive forgetting. In contrast to reduced learning preventing forgetting due to Rac1 inhibition, Rac1 activation might speed forgetting through a higher learning rate. Similarly, we found accelerated forgetting following repeated exposure to the context-only training environment. Model simulations (Figure 8c) assuming a higher learning rate and, therefore, reduced engram expression, reproduced these findings (Figure 5 & 6).

A crucial assumption of our model is that seemingly forgotten engrams are not necessarily lost but rather inaccessible when the inferred object relevance is low. This is consistent with the experimental results above, where we presented evidence for the reinstatement of memory representations based on real or artificial reminder cues (Figure 2 and 4). Here, we modeled memory reinstatement through reminder cues by inducing an artificial, positive prediction error in response to the reminder that boosted the inferred object relevance. Similar to the experimental data, the modeled reminder cues increased engram expression and yielded reduced forgetting rates (Figure 8e).

Finally, according to our model comparison, the model fits the data better than a baseline model predicting random forgetting rates, suggesting that the model describes the data accurately and above chance-level (Supplementary Figure S9). We also performed a parameter-recovery analysis suggesting that parameters can sufficiently be estimated despite the limited data for model fitting (Supplementary Figure S10). Our modeling results support the perspective that natural forgetting is a form of adaptive learning that alters engram accessibility in response to environmental feedback. Accordingly, environmental, optogenetic, and pharmacological manipulations might change the learning rate, suggesting that miscalibrated learning rates could indeed give rise to pathological forgetting. In summary, our model provides a mechanistic interpretation of our own data, a parsimonious explanation for the dynamics of natural forgetting, and could inform future studies on pathological forgetting.

Figure 8

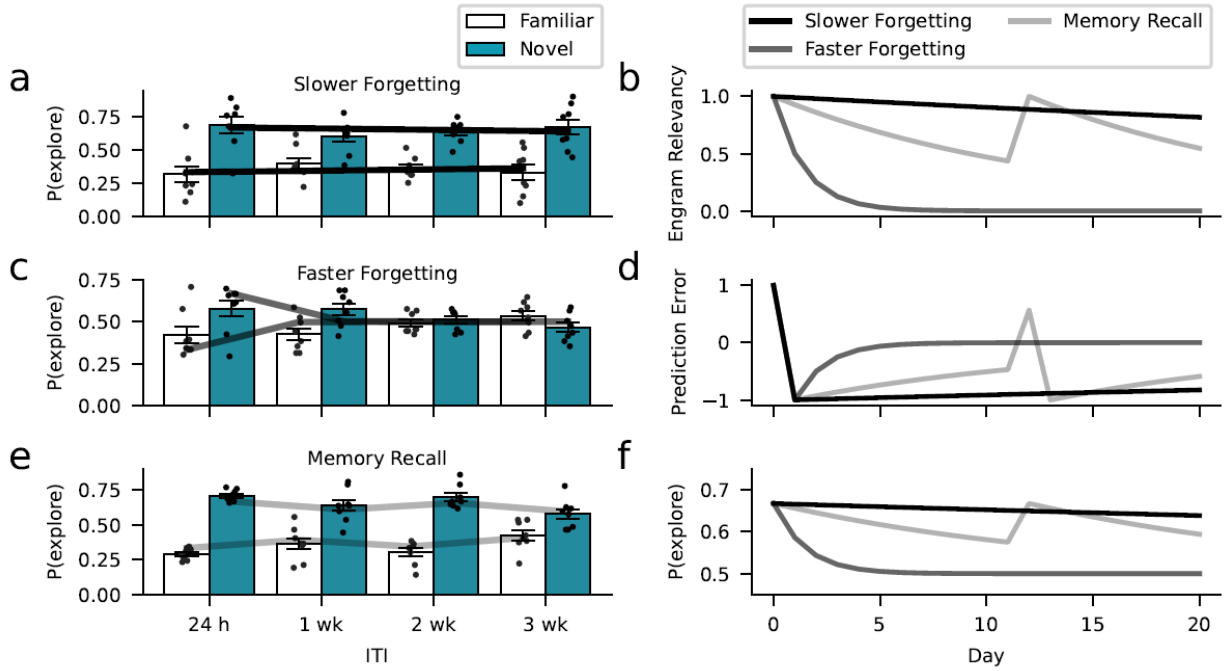

Figure 8: *Learning model captures the dynamics of forgetting. Environmental, optogenetic, and pharmacological manipulations might modulate the speed of forgetting by altering key parameters of our model. Simulations with different learning-rate parameters explain the forgetting dynamics of the different experimental conditions.* ***(a)*** *The enrichment and Rac1-inhibition conditions were successfully captured using a low learning rate (0.01, similar to the empirical estimates).* ***(c)*** *In contrast, assuming a larger learning rate (0.5), we could capture faster forgetting as observed in the Rac1-activator and context-only conditions.* ***(e)*** *Moreover, improved memory performance after reminder cues can be explained by assuming that these interventions induce a positive prediction error boosting object relevancy. Here, we assumed a learning rate of 0.07 (based on the empirical estimate).* ***(b)*** *Development of engram relevancy and* ***(d)*** *prediction errors across conditions.* ***(f)*** *Probability of exploring the novel object plotted separately for each condition.*

Discussion

For an organism to adapt to its environment, there must be processes by which learned information is updated or replaced with new, more relevant information (1, 2, 13). Forgetting may therefore be an adaptive process that is driven by feedback from the environment (13). This experience-dependent process helps an animal to prioritize relevant information in memory. Here, we investigated the conditions in which an object memory could be preserved, retrieved, or forgotten. Under baseline conditions mice were able to recall an object memory 24 hour post-training but exhibited impaired recall after two weeks, indicating the initial memory was forgotten. The results demonstrated that engram activity correlated with the rate of forgetting. Moreover, these reactivated cells were shown to be a functional component of the initially encoded memory as the direct modulation of engram activity via optogenetic stimulation or inhibition either facilitated or prevented the recall of an object memory. These findings are in support of previous work which has shown that engram activation is associated with the level of memory recall (2, 46, 53, 54). In addition, they support a role for the hippocampus in object memory retrieval (55, 56). This two-way memory retrieval/blocking modulation opens new lines of investigation into the treatment of memory disorders (e.g., non-drug and/or combined treatments involving learning/experience-based therapies such as environmental enrichment). Indeed, previous literature has shown the benefits of environmental enrichment in alleviating memory impairment in Alzheimer's disease (57–59). One potential mechanism by which enrichment or more generally, experience, may enhance memory is through the modification of memory engram activity. We showed that the rate of forgetting, and the level of engram activation could also be modulated through learned experience, where memory recall was extended following environmental enrichment, up to 3 weeks post

training. Furthermore, enrichment increased engram reactivation and facilitated memory recall. This finding is supported by earlier work, which showed exposure to an enriched environment prior to learning improved engram reactivation and rescued memory recall in a mouse model of Fragile X syndrome (60).

The hippocampus has been identified as one of the only regions where postnatal neurogenesis continues throughout life (61). However, the specific contribution of these new neurons to the memory engram is not fully understood (62, 63). Levels of adult hippocampal neurogenesis do not remain constant throughout life and can be altered by experience (41–43, 64). Moreover, these adult born neurons have been suggested to contribute to forgetting (65, 66). Here, we showed that environmental enrichment increased the level of doublecortin cells, a measure of immature neurons as well as neuronal survival which coincided with memory recall up to 2-3 weeks after training. Our findings support the role of neurogenesis in modulating memory as the key effect occurs when the increase in neurogenesis occurs, either before encoding resulting in enhanced memory, or after encoding, resulting in memory decay (63).

Our results also showed that forgetting could be reversed following a brief reminder experience, which was associated with a corresponding increase in engram activity. Previous work from both human and rodent behavioral studies have shown that a brief exposure to reminder cues can aid memory recall (44–46). We further demonstrated that forgetting could be accelerated following repeated exposure to the training environment, therefore signaling the updated irrelevance of the object memory. This updating through experience was blocked when the original memory was inhibited. Our findings indicate that the degree of learning and memory specificity corresponded with engram activity (67). The learning rate may therefore offer insight into the mechanism of forgetting. Here the learning rate was altered following environmental

enrichment as well following repeated context exposure, which in turn affected the rate of forgetting. Together, this suggests the possibility of an "optimal" learning rate that yields environmentally adaptive, natural forgetting and that learning rates that are too high are linked to amnesia and learning rates that are too low to some sort of hypermnesia.

We proposed a parsimonious computational model that integrates our findings into a cognitively plausible framework in which memories more relevant for adaptive behavior are more likely to be accessible than memories representing irrelevant, outdated information. Our model formalizes forgetting as a learning mechanism in which perceptual feedback changes how accessible an engram is. As such, our modeling analyses suggest that the different experimental manipulations altered the learning rate determining how rapidly engrams switch from accessible to inaccessible states in response to environmental feedback, such as the presence or absence of objects. This model suggests that an emergent property underlying forgetting is the prediction error. Within this framework, forgetting is a form of learning that is a predictive process whereby experience drives changes in the learning rate of an organism and the expectation of one's environment can alter the efficiency of recall/forgetting plasticity (13). It therefore follows that prediction errors may determine whether an engram is strengthened, leading to higher engram expression, or weakened, leading to lower engram expression (Figure 7b). Positive prediction errors indicate that an unexpected event has taken place (e.g., some unexpected object is present). We assume that positive errors induce plasticity processes that alter the engram and increase the likelihood of engram expression. In contrast, negative errors indicate that something that was expected has not taken place (e.g., object absent). Consequently, negative errors induce forgetting plasticity and yield decreased engram

expression. Indeed, dopamine has is known to play a role in prediction error and learning rates (68, 69). Moreover, dopamine has been shown to differently regulate Rac1 to modulate behavioral plasticity (29, 70). Here, we showed that the inhibition of Rac1 prevented forgetting, while its activation following memory encoding accelerated the rate of forgetting. This finding is in agreement with previous work where Rac1 impaired memory recall by driving forgetting-induced plasticity (29, 47–49, 71). Future work should investigate the link between learning rates and Rac1 signaling on engram reactivation and morphology.

Our model could be a starting point for more comprehensive models that account for forgetting across different experimental paradigms. Recent work suggests that retroactive interference could emerge from the interplay of multiple engrams competing for accessibility (18). Future models could explicitly incorporate multiple engrams and their competition, explaining a broader range of forgetting effects. Several approaches to modeling extinction (72, 73), memory interference (74), or the creation and updating of motor memories (75) that more explicitly assumed multiple memory representations could inform such a generalized model of natural forgetting.

Learning and memory allow humans and animals to maintain and update predictions about future outcomes. While having access to a large number of memories is adaptive in vast environments, it is equally important to prioritize the accessibility of the most relevant memories and to forget outdated information. Suppressing stored information through natural forgetting might therefore promote adaptive behavior. However, forgetting too much (e.g., amnesia) or too little (hypermnesia) under pathological conditions is maladaptive. Here, we utilized a mouse model of object memory and investigated the conditions in which a memory could be preserved, retrieved, or forgotten. Moreover, through pharmacological and behavioral

interventions, we successfully prevented or accelerated forgetting. Based on these findings and our computational model in which memories subjectively less relevant to adaptive behavior are more likely to be forgotten we conclude that natural forgetting may, therefore be considered a form of adaptive learning and that miscalibrated learning rates governing memory accessibility could give rise to pathological forgetting.

Methods

Animals

All wild-type behavior was conducted with male C57BL/6J mice aged 7-12 weeks, bred in-house from Charles River breeding pairs. c-fos-tTA mice were generated in-house by breeding TetTag mice with C57BL/6J mice and selecting offspring carrying the c-fos-tTA (38). All mice used for the experiments were male between 8–12 weeks old at the time of surgery and had been raised on food containing 40 mg kg-1 doxycycline for at least five days prior to surgery. c-fos-tTA mice remained on doxycycline food for the duration of the experiments. Doxycycline was removed from the diet for 36 hours prior to object acquisition to allow for engram labelling, following completion of the object acquisition period c-fos-tTA mice were placed back on the doxycycline diet.

All mice were grouped housed in standard housing conditions, with temperature 22 ± 1°C, relative humidity 50% and a 12:12 hour light-dark cycle (lights on 0730h) and had ad libitum access to food and water. All experiments were conducted in accordance with the European Directive 2010/63/EU and under an authorization issued by the Health Products Regulatory Authority Ireland and approved by the Animal Ethics Committee of Trinity College Dublin

Virus-mediated gene expression

The recombinant AAV vectors used for viral production were AAV-TRE-ChR2-EYFP and AAV-TRE-ArchT-GFP. Plasmids were serotyped with AAV9 coat proteins and packaged commercially by Vigene. The recombinant AAV vectors were injected with viral titers were 1 X 10^{13} genome copy (GC) ml-1 for AAV_9-TRE-ChR2-EYFP and AAV_9-TRE-ArchT-GFP.

Stereotaxic viral injection and Optic Fiber implantation

Mice were anaesthetized with Avertin 500 mg kg^{-1} and placed into a stereotaxic frame (World Precision Instruments). Bilateral craniotomies were performed using 0.5 mm diameter drill and the virus was injected using a 10 ml Hamilton microsyringe filled with mineral oil. A microsyringe pump and its controller were used to maintain the speed of the injection. The needle was slowly lowered to the target site and remained for 5 min before the beginning of the injection. For engram labeling and activation studies (AAV_9-TRE-ChR2-EYFP) or inhibition studies (AAV_9-TRE-ArchT-GFP) (Volume; 300nl) virus was injected bilaterally into the DG using the coordinates AP: -2 mm, ML: ± 1.35 mm, DV: -2 mm relative to Bregma at a rate of 60nL/min. Followed by a 10 min diffusion period. For optogenetic experiments, a Doric optical fiber (200 µm core diameter; Doric Lenses) was implanted above the injection site (–2 mm AP; ± 1.35 mm ML; –1.85 mm DV). A layer of adhesive cement (C&B Metabond) was applied followed by dental cement to secure the optic fiber implant to the skull. Mice were given 1.5 mg kg^{-1} meloxicam as analgesic. Animals were allowed two weeks to recover before behavioral testing.

Engram labelling strategy

The AAV_9-TRE-ChR2-EYFP virus was injected into the dentate gyrus of c-fos-tTA mice, under the control of a *c-fos* promoter (37, 38). The immediate early gene *c-fos* becomes upregulated following neural activity and as such the c-fos-tTA transgene selectively expresses tTA in active cells (36, 76). The activity-dependent tTA induces expression of ChR2-EYFP. In order to restrict activity-dependent labelling to a single learning experience, mice were maintained on diet containing doxycycline (DOX).

Object Recognition

The novel object recognition task was as based on the protocol previously described by Bevins and Besheer (2006). Mice were first habituated to the testing arena (30cm L x 20cm W x 30cm H) for a 10-minute exploration period on two consecutive days. On day three, two identical objects were positioned on adjacent sides of the arena and each animal was introduced for a 10-minute exploration period. Mice were then placed directly back into their home cages. After a 24-hour or two-week inter-trial interval, one familiar object was replaced with a novel object, and each animal was introduced for a five-minute exploration period. Objects and locations were counterbalanced across groups. Object exploration was defined when the animal's nose came in contact with the object. The testing arena and objects were cleaned with a disinfectant, TriGene, between each animal and training session. Video recordings were made to allow for manual scoring of object exploration. The object discrimination index was calculated as the time spent exploring the novel object minus the time spent exploring the familiar object divided by the total time spent exploring both objects (novel – familiar / novel + familiar).

Environmental Enrichment

The enriched environment consisted of a larger home cage (50cm L x 20cm W x 40cm H). The cage was included a running wheel, tunnels, extra nesting material as well as an assortment of Lego bricks. Furthermore, the configuration of tunnels, Lego bricks and housing enrichment changed every week. Mice were placed in the enriched housing for three weeks prior to behavioral testing and remained in the housing for the duration of the experiment.

Immunohistochemistry

On completion of the behavioral testing, mice were deeply anaesthetized with sodium pentobarbital and then transcardially perfused with 4% paraformaldehyde. Brains were post-fixed for 24 hours in 4% paraformaldehyde, transferred to PBS, and stored at 4°C. Coronal sections through the DG were collected onto slides at 50 µm thickness in a 1:4 series. Coronal sections were immunostained for EYFP and c-Fos. Non-specific antibody binding was blocked using 10% normal goat serum (NGS) in a solution of PBS with 0.2% Triton–X100 and tissue sections were incubated with goat anti-EYFP (Anti-GFP chicken IgY fraction 1:1000, Invitrogen) and anti-c-Fos (Anti-c-Fos rabbit, 1:500, Synaptic System). Sections were then incubated in the appropriate AlexaFluor secondary antibody (AF488 for EYFP and AF596 for c-Fos) and then with Dapi (1:1000; Sigma) to stain nuclei. Lastly, sections were washed, mounted, and coverslipped with anti-fade mounting media (Vectashield-DAPI). Images were obtained using a Leica SP8 gated STED confocal microscope at 40x magnification.

For BrdU/NeuN immunohistochemistry, hippocampal sections were washed, denatured in HCl (2 N) for 45 min at 37 °C and renatured in 0.1 M sodium tetraborate. Sections were then washed in PBS and blocked in 10% normal goat serum (NGS; Sigma) diluted in 0.1% Triton-X 100 PBS to prevent non-specific binding. Sections were incubated with rat anti-BrdU antibody (1:100, Abcam) in 1% NGS diluted in 0.1% Triton-X 100 PBS, washed, and then incubated in AlexaFluor secondary antibody (AF598 for BrdU and AF88 for NeuN) and then with Dapi (1:1000; Sigma) to stain nuclei. Lastly, sections were washed, mounted, and coverslipped with anti-fade mounting media (Vectashield-DAPI). Images were obtained using a Leica SP8 gated STED confocal microscope at 40x magnification.

Morphological Analysis

In order to obtain engram dendritic spine density, the dentate gyrus was imaged using the Leica SP8 gated STED confocal microscope and images were collected with the Leica Application Suite X (LasX) software. Z-stacks were taken bidirectional under a 40x lens. Dendritic spine analysis was carried out using the Imaris software (Oxford Instruments, Imaris v9.5). The dendrites of EYFP-positive cells were traced using a semiautomated neurofilament tracer tool and dendritic spines were individually highlighted and manually traced with the software. The image processing feature of Imaris was used to apply the Gaussian and Median filters to Z-stack images to remove background EYFP staining and allow for better resolution and visualisation of dendritic fragments and associated spines. Following the labelling of spines on traced dendritic fragments, parameters for spine volume, spine head volume and dendritic spine density (/10µm) were collected.

Statistical analyses

All data were analysed using SPSS statistical software (SPSS, Chicago, IL). Behavioral data and the number of ChR2-EYFP-positive and c-Fos cells were graphed as means ±SEM. Data were analysed by Student's t-test or ANOVA where appropriate. An alpha level of 0.05 was used as a criterion for statistical significance, and probability levels were quoted for non-significance. Standard errors of the mean (SEM) were used with all graphical output.

Computational Modelling

We used an error-driven learning rule inspired by the Rescorla-Wagner model (51, 52). The model offers a mechanistic explanation for the key behavioral results of our experiments (Figure 7 and 8). In line with the idea of the Rescorla-Wagner model that learning is an error-driven updating process, the model computes the relevancy of an engram e_t on each day t of the experiment. The engram relevancy is updated as a function of the prediction error ($o_t - e_t$; where o_t denotes the object observation). The degree to which the prediction error changes the computed engram relevancy is determined by the learning rate α_t. To update the engram relevancy, the model relies on a two-speeded mechanism through which the relevancy quickly increases when an object is present (higher learning rate) and slowly decays when the object is absent (lower learning rate). In particular, the appearance of an object yields a positive prediction error and triggers the fast-updating process, while the absence of an object is associated with negative prediction errors and slow decay in the engram relevancy. Depending on the time between the acquisition phase and the retrieval test, the model predicts different levels of memory performance, where a longer time interval between acquisition and test is associated with less object recognition.

Task Model

To model mouse behavior in the object-based memory task, we first formulated a model of the experimental paradigm.

- $T := 21$ denotes the maximum number of days, which are indexed as $t = 0,1, \dots, T$.
- $x \in \{0,1\}$ denotes the objects of the task. $x = 0$ refers to the novel object; $x = 1$ refers to the acquisition object that is presented together with the novel object.

- $O \in \{0,1\}$ indicates whether an object is presented. When the acquisition (and therefore familiar) object $x = 1$ is presented in the acquisition phase, $o_t^{x=1} = 1$. Moreover, $o_t^{x=1} = 1$ when the retrieval test takes place, which depending on the task condition is after one day, one week, two weeks or three weeks. In all other cases, $o_t = 0$.

Learning Model

To formalize the learning and memory processes of mice in the object-based memory task, we developed a simple reinforcement learning model. Here,

- $e_t \in [0,1]$ denotes relevancy of an engram, which is updated on each trial. Higher values indicate higher object relevancy.

The key assumption of the model is that once engrams are formed, they endure but might be inaccessible when they cannot be reactivated following natural forgetting. The expressibility of the engram depends on the inferred relevancy of the respective object, where objects that are subjectively more likely to be encountered in the current environment (higher relevancy), are more easily accessible. The inferred relevancy of the (familiar) acquisition object $x = 1$ governing the accessibility of the engram was computed according to the delta rule

$$e_{t+1} = e_t + \alpha_t(o_t - e_t), \qquad \text{(eq. 1)}$$

where e_t denotes the relevancy of the engram, α_t the learning rate, and $o_t - e_t =: \delta_t$ the prediction error. For clarity, we omit the dependency on x here. Moreover, we used separate learning rates for positive and negative prediction errors

$$\alpha_t := \begin{cases} 1, \delta_t \geq 0 \\ \alpha^-, \delta_t < 0. \end{cases} \qquad \text{(eq. 2)}$$

That is, positive prediction errors indicating the presence of an object lead to a rapid increase in the relevancy of the engram. Negative prediction errors indicating the absence of an object lead to a slower decrease in the relevancy (when $\alpha^{-} < 1$).

Exploration model

To translate the modeled engram relevancy into exploration behavior, we used the softmax and beta functions. The softmax function was used to compute the average exploration probability for the familiar object

$$\mu = \frac{\exp(\beta e_t^{x=1})}{\sum_x \exp(\beta e_t^{x})} \quad \text{(eq. 3)}$$

where the inverse-temperature parameter β determines the slope of the function. $e_t^{x=1}$ denotes the engram relevancy of the familiar object that is dynamically updated according to the delta rule (eq. 1). $e_t^{x=0}$corresponds to the relevancy of the novel object, where, since the object has not been experienced before, we assume an engram relevancy of 0. Subsequently, we utilized the beta distribution to model exploration variability across individuals. The beta distribution for $\mu \in [0,1]$ is defined by

$$Beta(\mu; a, b) := \frac{1}{B(a,b)} \mu^{a-1}(1-\mu)^{b-1} \quad \text{(eq. 4)}$$

with the shape parameters a and b. In order to compute the distribution over exploration probability conditional on the mean μ, we exploited that the sum of a and b indicates the concentration κ of the distribution. That is, when $\kappa = a + b$ gets larger, the beta distribution is more concentrated, and the predicted exploration variability is lower. When κ is lower, the distribution is wider and the model predicts greater exploration variability (for more details, see (77)).

Therefore, to compute the exploration probabilities for object $x = 1$ using the beta distribution conditional on the computed average exploration probability μ and concentration κ, we computed the shape parameters a and b in the following way:

$$a = \mu\kappa \text{ and } b = (1 - \mu)\kappa. \quad \text{(eq. 5)}$$

Parameter estimation

We implemented the following free parameters. The learning rate conditional on negative prediction errors α^- (see eq. 2), the inverse-temperature parameter β of the softmax distribution (see eq. 3), and the κ parameter of the beta distribution (see eq. 4 and 5). To estimate these parameters, we computed the probability of the observed exploration probabilities $\hat{\mu}$ for the familiar object $x = 1$ conditional on a and b on the test day using the beta distribution (eq. 4). Based upon this, we computed the model fit across subjects of the experimental and control group by summing the log-likelihoods

$$\ell = \sum_s \log p\,(\hat{\mu}_s | a_s, b_s) \quad \text{(eq. 6)}$$

where s denotes the subject. The free parameters were estimated using the bound-constrained optimization algorithm L-BFGS-B of the SciPy library in Python 3.10. The parameter boundaries were [0, 0.25] for α^-, [-10, 0] for β, and [1, 100] for κ.

Model comparison

We systematically compared our model to a baseline model that explored the two objects with the same probability ($\mu = 0.5$), where we computed the log-likelihood based on $a = 1$ and $b = 1$. We compared the Bayesian information criterion (BIC; (78)) of the two models defined by

$$BIC \coloneqq \ell - \frac{k}{2} ln(S) \qquad \text{(eq. 7)}$$

where k denotes the number of free parameters and S the number of subjects of the respective group.

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Author Contributions

J.OL. and T.R. conceived the original scientific design. J.OL. and L.A. conducted the experiments and analysed the data. J.OL., R.B., L.A. and T.R. interpreted the results. R.B. conducted all modelling work. J.OL., R.B. and T.R. wrote the manuscript draft. J.OL., R.B., L.A. and T.R. reviewed and edited the manuscript.

Declaration of Interests

The authors declare no competing interests.

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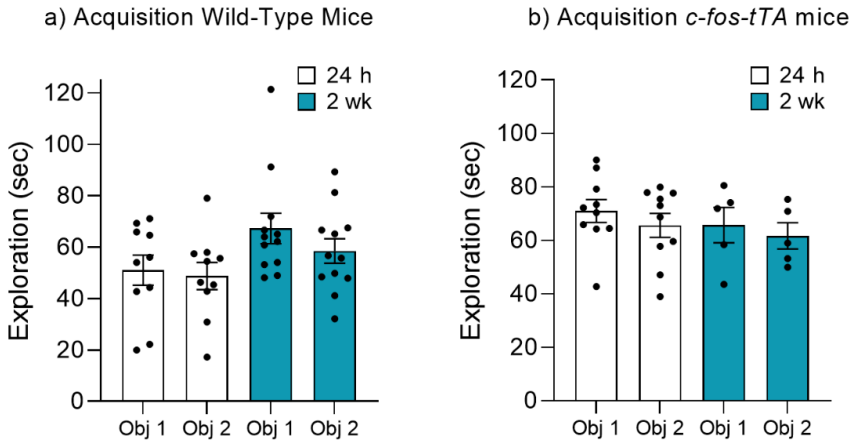
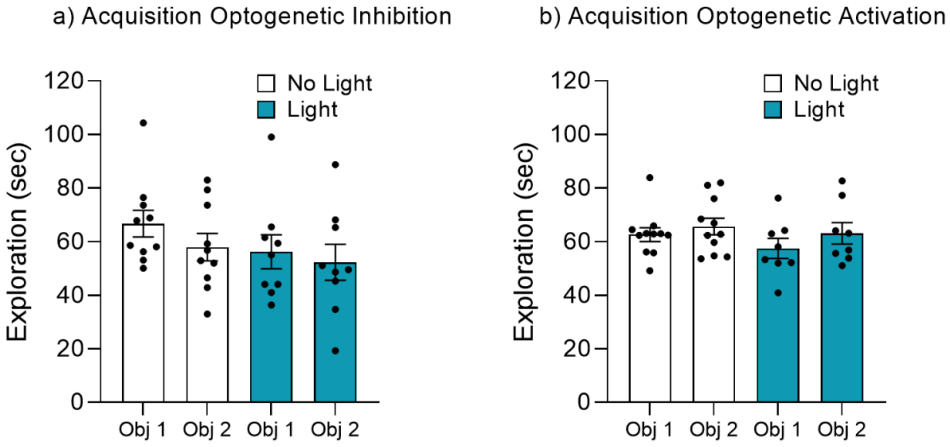
Supplementary information

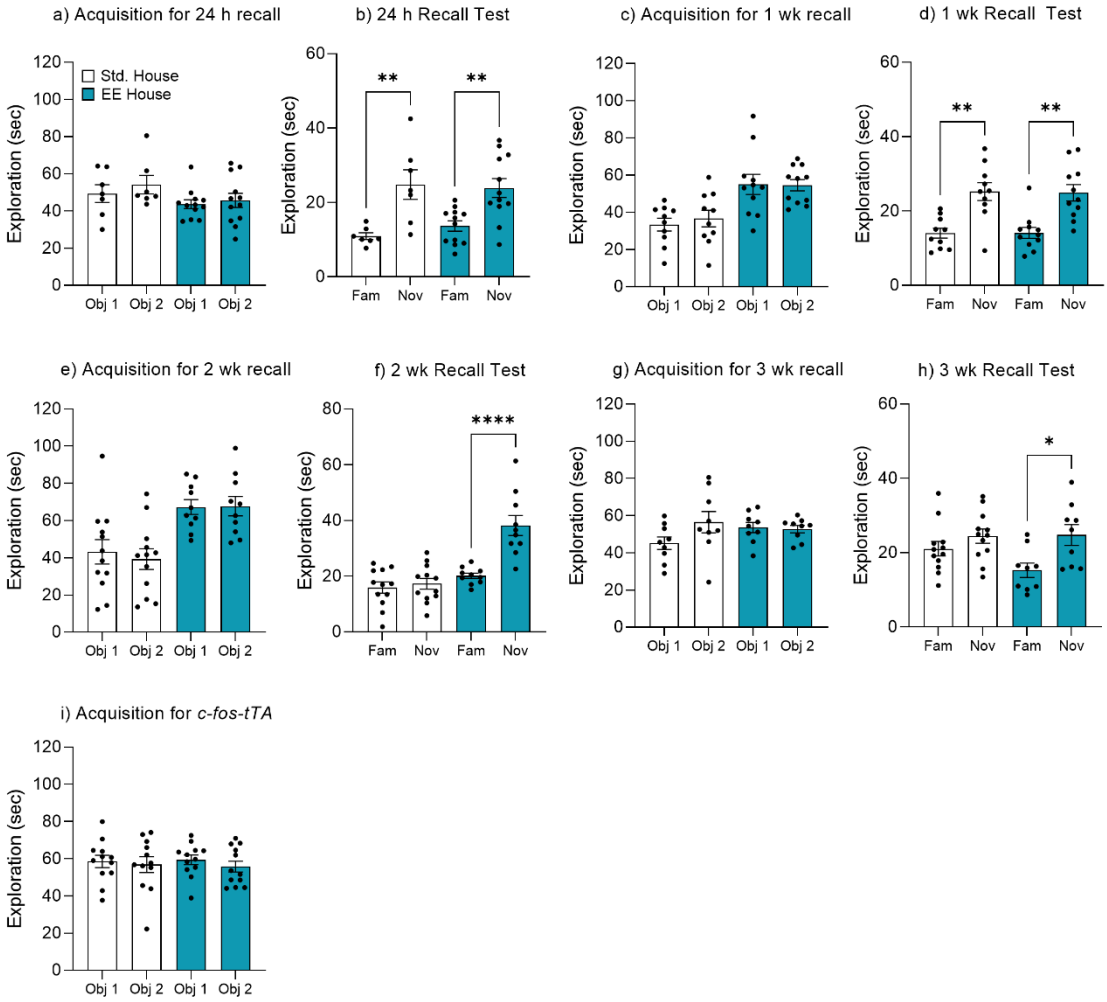
Supplementary Figures



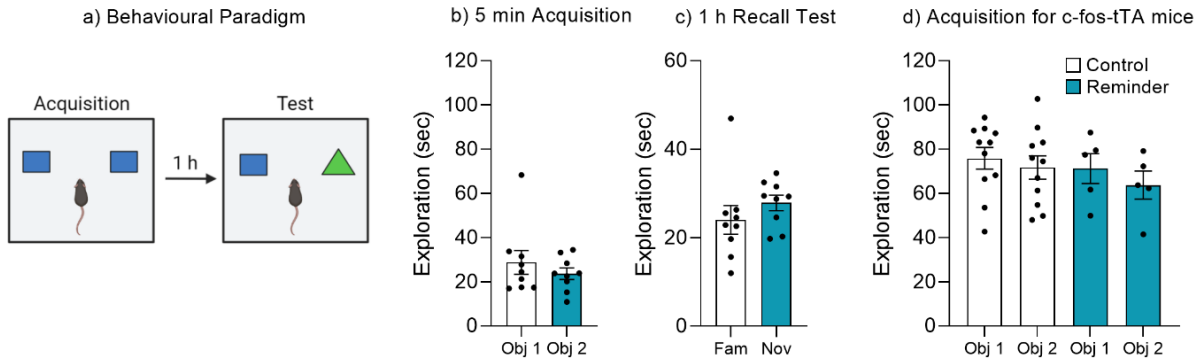
Supplementary Figure 1*: Object exploration during acquisition training for* ***(a)*** *Wild-Type mice and* ***(b)*** *c-fos-tTA mice. Bar graphs indicates average values in n = 5-12 per group. Data graphed as means ± SEM.*



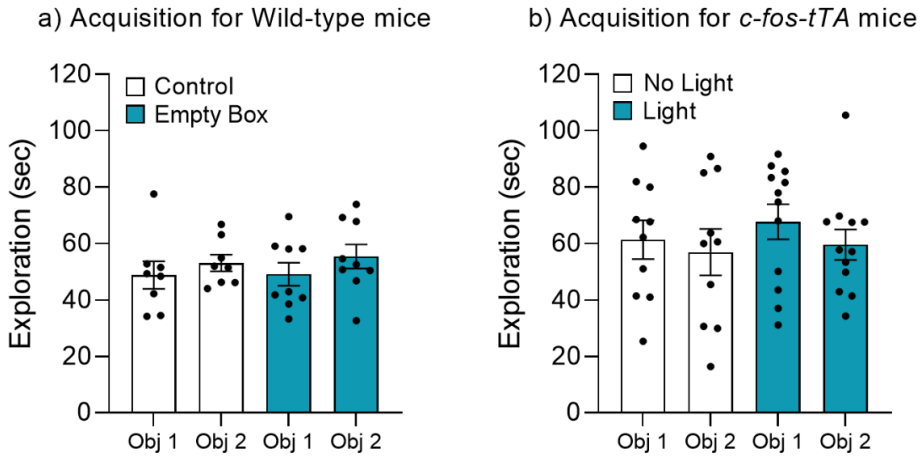
Supplementary Figure 2: *Object exploration during acquisition training for **(a)** ArchT-GFP inhibition and **(b)** ChR2-EYFP activation. Bar graphs indicates average values in n = 9-12 per group. Data graphed as means ± SEM.*



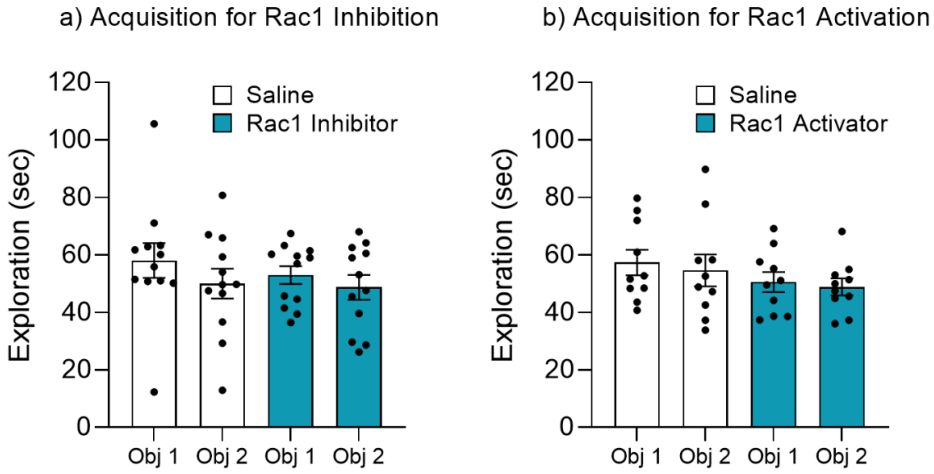
Supplementary Figure 3: (a) *Object exploration for 24h acquisition training and* ***(b)*** *24 h recall group* ***(c)*** *Object exploration for 1 wk acquisition training and* ***(d)*** *1 wk recall group* ***(e)*** *Object exploration for 2 wk acquisition training and* ***(f)*** *2 wk recall group* ***(g)*** *Object exploration for 3 wk acquisition training and* ***(h)*** *3 wk recall group* ***(i)*** *Object exploration during acquisition training for c-fos-tTA mice. Bar graphs indicates average values in n = 7-12 per group. Data graphed as means ± SEM.*



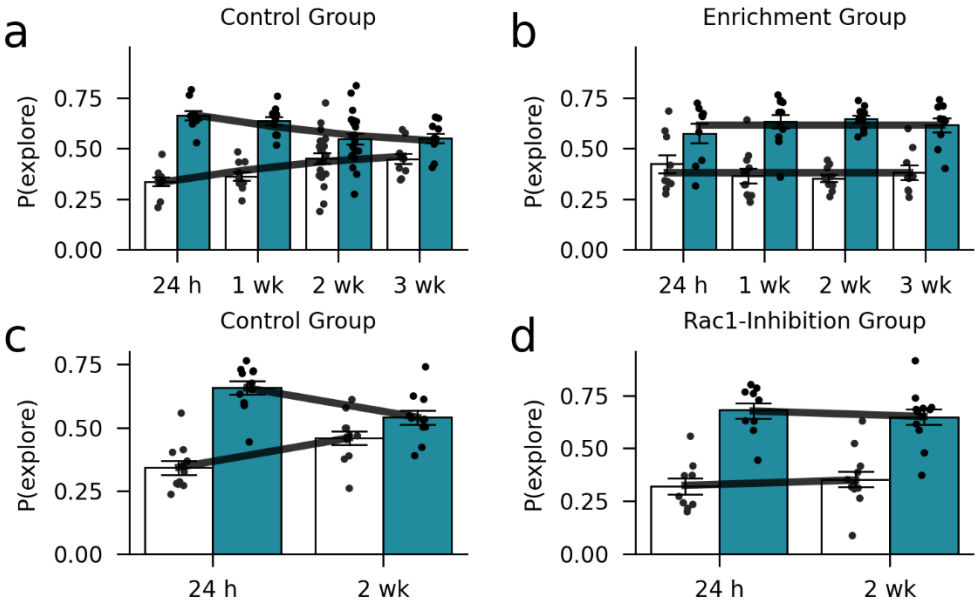
Supplementary Figure 4: ***(a)*** *Behavioral paradigm for brief reminder trial* ***(b)*** *Object exploration during 5 min acquisition training* ***(c)*** *Object exploration during 1 h recall test* ***(d)*** *Object exploration during acquisition training for c-fos-tTA mice. Bar graphs indicates average values in n = 5-10 per group. Data graphed as means ± SEM.*



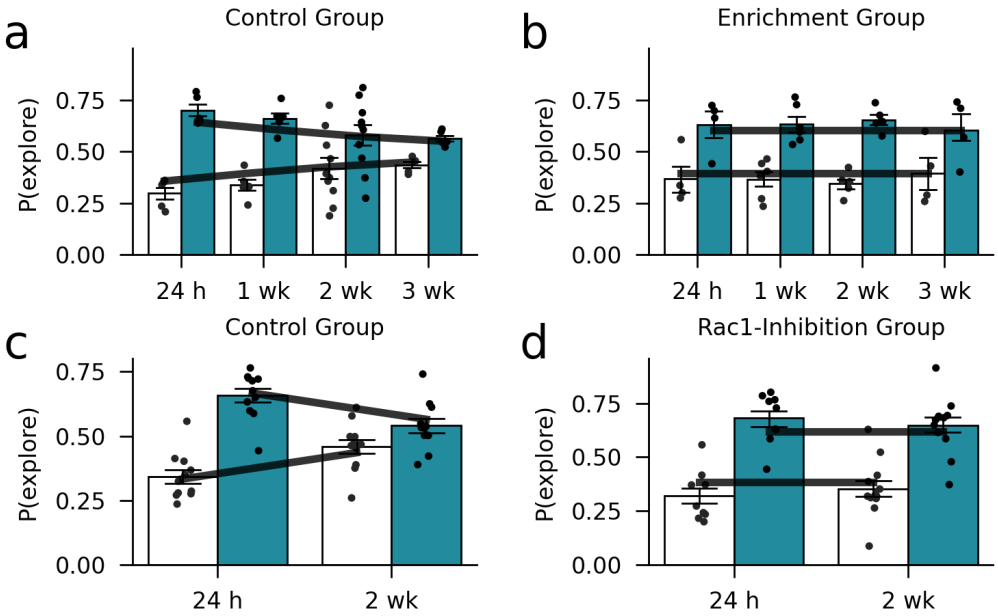
Supplementary Figure 5: ***(a)*** *Object exploration during acquisition training for Wild-Type mice and* ***(b)*** *c-fos-tTA mice. Bar graphs indicates average values in n = 8-10 per group. Data graphed as means ± SEM.*



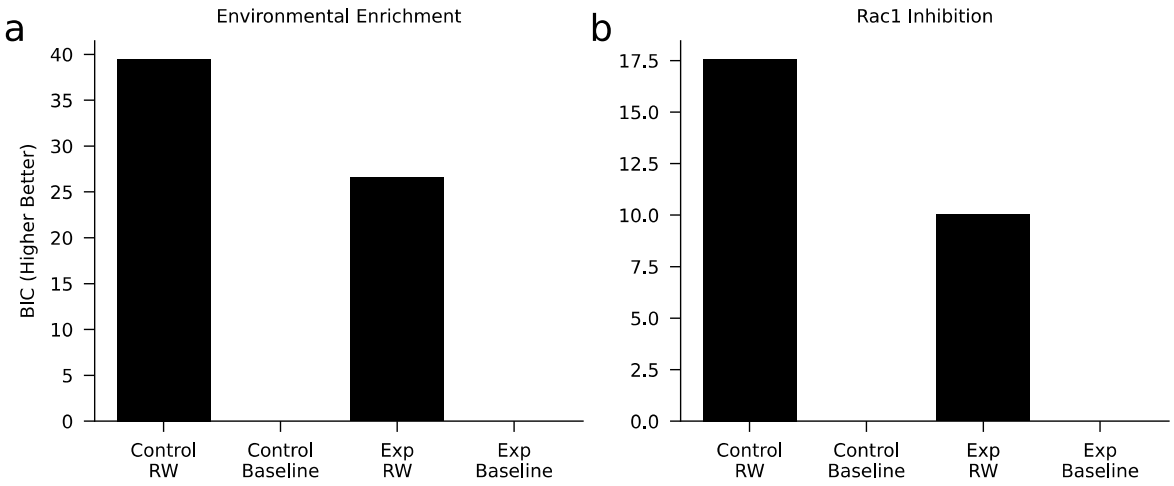
Supplementary Figure 6: *Object exploration during acquisition training for **(a)** Rac1 Inhibition **(b)** Rac1 Activation. Bar graphs indicates average values in n = 10-12 per group. Data graphed as means ± SEM.*



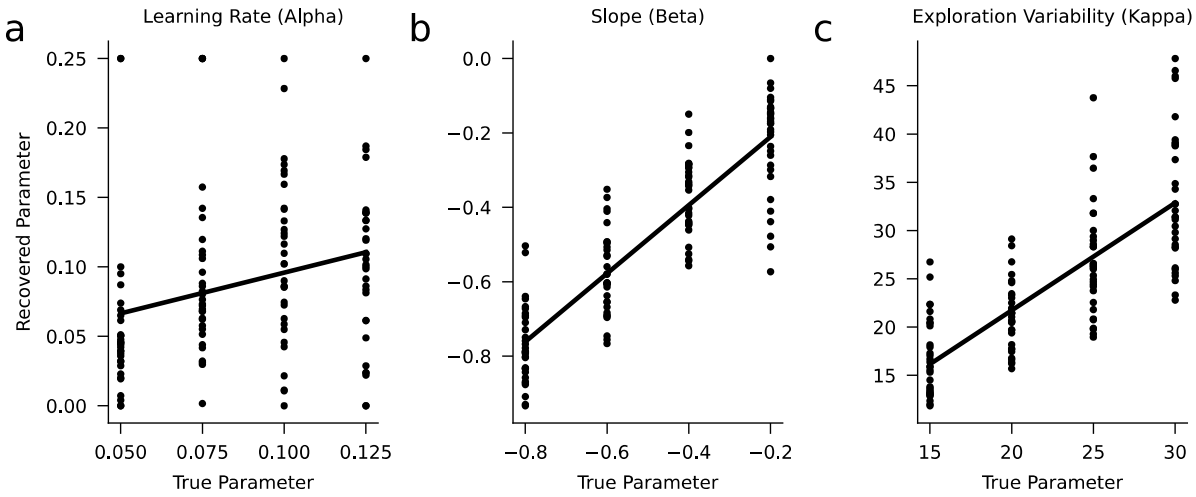
Supplementary Figure 7. In-Sample Model Validation. *A comparison between the forgetting data and the forgetting curve predicted by the model suggests that the model captures the data accurately.* ***(a)*** *Control group (standard housing condition) of the environmental enrichment experiment.* ***(b)*** *Enrichment group of the environmental-enrichment experiment.* ***(c)*** *Control group of the Rac1-inhibition experiment.* ***(d)*** *Rac1-inhibition group of the Rac1-inhibition experiment. Markers and bars show exploration probabilities of the mice. Lines show the average exploration probability for the familiar and novel object according to the model.*



Supplementary Figure 8. Out-of-Sample Model Validation. *We examined the model fit using a simple cross-validation procedure. For the environmental-enrichment data set, we estimated the free parameters based on half of the subjects. Then, we compared the predicted exploration behavior (dark lines) to the data of the other half of the subjects.* ***(a)*** *Control group.* ***(b)*** *Enrichment group (markers and bars show exploration probabilities of the mice). Furthermore, we compared the predicted exploration probabilities conditional on the parameter estimates from the environmental-enrichment experiment to the observed exploration probabilities in the Rac1-inhibition experiment.* ***(c)*** *Control group.* ***(d)*** *Rac1-inhibition group.*



Supplementary Figure 9. Model Comparison. *We tested if our computational model (Rescorla-Wagner, "RW") describes the data better than a control model ("Baseline") that explores each object with a probability of 0.5. The model comparison was based on a comparison of the cumulated Bayesian information criterion (BIC). Here, higher values indicate a better model fit. In both the environmental-enrichment experiment (**(a)**; experimental and control condition) and the Rac1-inhibition experiment (**(b)**; experimental and control condition), the model comparison favored the RW-learning model. These results show that our model explained the data above chance-level.*



Supplementary Figure 10: Parameter Recovery. *We performed a parameter-recovery study to examine whether the free parameters of our learning model could be estimated accurately. Similar to the enrichment experiment, we assumed N=12 mice per inter-test interval (24 hours, 1 week, 2 weeks, 3 weeks). The recovery study indicates that the **(a)** learning-rate, **(b)** slope, and **(c)** exploration-variability parameters can sufficiently be estimated given the limited amount of data for model fitting. We validated that the variability of the parameters, especially of the learning rate, is lower when more subjects are included (e.g., N=50; not shown).*

Supplementary Table 1. Parameter Estimates. Control group (Cont) and Experimental group (Exp).

	Alpha	**Beta**	**Kappa**
Enrichment	Cont: 0.072; Exp: 0.0	Cont: -0.695; Exp: -0.48	Cont: 23.12; Exp: 19.668
Rac1 inhibition	Cont: 0.101; Exp: 0.013	Cont: -0.651; Exp: -0.736	Cont: 29.209; Exp: 13.841