UC Irvine

UC Irvine Previously Published Works

Title

Noradrenergic activation of the basolateral amygdala maintains hippocampus-dependent accuracy of remote memory

Permalink
https://escholarship.org/uc/item/1157q53j

Journal
Proceedings of the National Academy of Sciences of the United States of America, 114(34)

ISSN
0027-8424

Authors
Atucha, Erika
Vukojevic, Vanja
Fornari, Raquel V
et al.

Publication Date
2017-08-22

DOI
10.1073/pnas.1710819114

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Noradrenergic activation of the basolateral amygdala maintains hippocampus-dependent accuracy of remote memory

Erika Atucha[a,b,c,1], Vanja Vukojevic[d,e,f,1], Raquel V. Fornari[g], Giacomo Ronzoni[a,b], Philippe Demougin[d,e], Fabian Peter[d,e], Piray Atsak[a,b], Marcel W. Coolen[h], Andreas Papassotiropoulos[d,e,f,i], James L. McGaugh[j,2], Dominique J.-F. de Quervain[f,i,k], and Benno Roozendaal[a,b,2]

[a]Department of Cognitive Neuroscience, Radboud University Medical Center, 6500 HB Nijmegen, The Netherlands; [b]Donders Institute for Brain, Cognition and Behaviour, Radboud University, 6525 EN Nijmegen, The Netherlands; [c]Functional Architecture of Memory Department, Leibniz Institute for Neurobiology, 39118 Magdeburg, Germany; [d]Department of Psychology, Division of Molecular Neuroscience, University of Basel, CH-4056 Basel, Switzerland; [e]Biozentrum, University of Basel, CH-4056 Basel, Switzerland; [f]Transfacultary Research Platform, University of Basel, CH-4056 Basel, Switzerland; [g]Centro de Matemática, Computação e Cognição, Universidade Federal do ABC, São Bernardo do Campo, 09606-070, Brazil; [h]Department of Human Genetics, Radboud University Medical Center, 6500 HB Nijmegen, The Netherlands; [i]Psychiatric University Clinics, University of Basel, CH-4056 Basel, Switzerland; [j]Center for the Neurobiology of Learning and Memory, Department of Neurobiology and Behavior, University of California, Irvine, CA 92697-3800; and [k]Department of Psychology, Division of Cognitive Neuroscience, University of Basel, CH-4056 Basel, Switzerland

Contributed by James L. McGaugh, July 18, 2017 (sent for review June 15, 2017; reviewed by Ivan Izquierdo and Carmen Sandi)

Emotional enhancement of memory by noradrenergic mechanisms is well-described, but the long-term consequences of such enhancement are poorly understood. Over time, memory traces are thought to undergo a neural reorganization, that is, a systems consolidation, during which they are, at least partly, transferred from the hippocampus to neocortical networks. This transfer is accompanied by a decrease in episodic detailedness. Here we investigated whether norepinephrine (NE) administration into the basolateral amygdala after training on an inhibitory avoidance discrimination task, comprising two distinct training contexts, alters systems consolidation dynamics to maintain episodic-like accuracy and hippocampus dependency of remote memory. At a 2-d retention test, both saline- and NE-treated rats accurately discriminated the training context in which they had received footshock. Hippocampal inactivation with muscimol before retention testing disrupted discrimination of the shock context in both treatment groups. At 28 d, saline-treated rats showed hippocampus-independent retrieval and lack of discrimination. In contrast, NE-treated rats continued to display accurate memory of the shock–context association. Hippocampal inactivation at this remote retention test blocked episodic-like accuracy and induced a general memory impairment. These findings suggest that the NE treatment altered systems consolidation dynamics by maintaining hippocampal involvement in the memory. This shift in systems consolidation was paralleled by time-regulated DNA methylation and transcriptional changes of memory-related genes, namely *Reln* and *Pkmζ*, in the hippocampus and neocortex. The findings provide evidence suggesting that consolidation of emotional memories by noradrenergic mechanisms alters systems consolidation dynamics and, as a consequence, influences the maintenance of long-term episodic-like accuracy of memory.

basolateral amygdala | norepinephrine | memory accuracy | hippocampus | systems consolidation

Emotionally arousing experiences are well-retained in memory (1, 2). Beyond their increased strength, emotionally enhanced memories are also often characterized by increased vividness and the subjective feeling of remembering (3, 4). Both animal and human research indicate that noradrenergic activation of the basolateral amygdala (BLA) enhances memory of emotionally arousing experiences by regulating neural plasticity and information storage processes in other brain regions (5–12). A majority of studies investigating the effects of noradrenergic activation of the BLA in memory have focused on episodic (declarative) or contextual tasks that involve functioning of the hippocampus (13–17). The BLA sends extensive projections to the hippocampus (18) and has a major impact on hippocampal functioning (19–21). There is extensive evidence that the memory enhancement induced by BLA activation by norepinephrine (NE) administration or emotional arousal during or shortly after learning involves hippocampal activation (20, 22–24). This activation may increase the strength of episodic-like or context-specific memories (22, 25), enabling a more distinct separation of memory traces for individual items or similar datasets (26, 27).

Thus, it is by now well-established that noradrenergic activation of the BLA enhances the initial formation of hippocampus-dependent memories. However, an understanding of how noradrenergic activation after encoding affects the later specificity or accuracy of the enhanced episodic-like memory remains elusive. Over time, memory traces are thought to undergo a neural reorganization, referred to as systems consolidation, during which they are, at least partly, transferred from the hippocampus to neocortical networks (28–32). This temporal shift in memory representation is associated with a transformation in the quality of memory (30, 33). Retrieval of remote memories mainly relies on semantic or gist-like representations in extrahippocampal

Significance

Emotional arousal creates lasting and vivid memories. According to the systems consolidation theory, the hippocampus has a time-limited role in memory, and retrieval of remote memories mainly relies on neocortical networks. Here we show that this systems consolidation and associated change in memory specificity constitute a dynamically regulated process that can be modified by emotional arousal status. Norepinephrine administration into the basolateral amygdala after an episodic-like training experience maintained accuracy and hippocampus dependency of remote memory. This altered systems consolidation was paralleled by time-regulated epigenetically driven transcriptional changes of memory-related genes in the hippocampus and neocortex.

Author contributions: E.A., V.V., R.V.F., M.W.C., A.P., J.L.M., D.J.-F.d.Q., and B.R. designed research; E.A., V.V., R.V.F., G.R., P.D., F.P., and P.A. performed research; E.A., V.V., M.W.C., A.P., D.J.-F.d.Q., and B.R. analyzed data; and E.A., V.V., J.L.M., D.J.-F.d.Q., and B.R. wrote the paper.

Reviewers: I.I., Pontifical Catholic University of Rio Grande do Sul; and C.S., Swiss Federal Institute of Technology (EPFL).

The authors declare no conflict of interest.

[1]E.A. and V.V. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: james.mcgaugh@uci.edu or benno.roozendaal@radboudumc.nl.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1710819114/-/DCSupplemental.

structures, for example the anterior cingulate cortex (ACC), that are likely to maintain core information but few contextual details (29, 30, 33). Such dynamic shift of neural representation in systems consolidation and accompanied change in the nature of the memory are supported by time- and brain region-specific epigenetic modifications (34, 35). The underlying molecular mechanisms such as DNA methylation, for example, expression silencing by covalent modifications of DNA, provide pathways for self-perpetuating transcriptional alternations driven by environmental signals. Previous findings have indicated that such epigenetic changes of memory-related genes play a crucial role in both the formation and maintenance of memory (35–37). For example, lower methylation levels of reelin (*Reln*) and increased methylation levels of protein phosphatase 1 (*Pp1*) in the hippocampus led to successful memory retention 1 d later (36), whereas cortical expression of protein kinase M zeta (PKMζ), the N-truncated form of protein kinase C zeta, is required for maintenance of long-term memory (37). However, whether the dynamics of systems consolidation and time-dependent change in the specificity of memory are actively regulated by environmental conditions, such as emotional arousal status, has not, as yet, been investigated.

The present experiments investigated, in rats, whether noradrenergic activation of the BLA after training on an episodic-like discrimination task affects systems consolidation dynamics and associated epigenetic and transcriptional changes to maintain episodic-like accuracy and hippocampus dependency of remote memory. Such NE-induced changes in systems consolidation may represent a critical mechanism underlying the long-term vividness of emotional memories (3, 4).

Results

Noradrenergic Activation of the BLA Posttraining Maintains Accuracy of Remote Memory. We first investigated the effect of posttraining NE infusions into the BLA on episodic-like accuracy of recent and remote memory. Male Sprague–Dawley rats were trained on a modified version of the inhibitory avoidance task, termed the inhibitory avoidance discrimination task (38), in which they explored two contextually distinct inhibitory avoidance apparatuses within a 2-min interval but footshock was delivered only in the latter context (Fig. 1*A*). NE (1.0 μg in 0.2 μL) or saline was microinfused bilaterally into the BLA immediately after the training session. Either 2 d (recent) or 28 d (remote) later, retention latencies were tested in the two training contexts (i.e., "shock box" and "nonshock" box) as well as in a "novel" box to assess whether rats accurately discriminated the training context in which they had received footshock.

We first determined the effect of posttraining NE infusions on memory accuracy at a 2-d retention test. Two-way ANOVA for retention latencies in the three test environments indicated significant NE ($F_{1,38}$ = 5.25; P = 0.03), context ($F_{2,38}$ = 45.02; P < 0.0001), and interaction effects ($F_{2,38}$ = 6.66; P = 0.003). As shown in Fig. 1*B*, rats treated with either saline or NE in the BLA after the training session displayed significantly longer retention latencies in the shock box than in the nonshock box (*P*s < 0.01) or novel box (*P*s < 0.001), indicating accurate discrimination of the shock context. Moreover, NE-treated rats, relative to saline controls, had longer retention latencies in the shock box (P < 0.05), indicating increased memory strength. Delayed infusions of NE given 3 h posttraining were ineffective (Fig. 1*C*), supporting the view that the NE effect is dependent on a consolidation process (8).

Next, we examined, in separate groups of rats, the effect of posttraining NE infusions on memory accuracy at a 28-d retention test. Two-way ANOVA for retention latencies revealed no NE effect ($F_{1,46}$ = 0.16; P = 0.69) but a significant context effect ($F_{2,46}$ = 18.27; P < 0.0001) as well as a significant interaction between NE and context ($F_{2,46}$ = 8.09; P = 0.001). As shown in Fig. 1*D*, saline-treated rats no longer accurately discriminated the shock context, as indicated by similar retention latencies in the shock box and nonshock box (P = 0.89). Unlike saline controls, NE-treated rats continued to display accurate memory of the shock–context association, as shown by significantly longer retention latencies in the shock box than in the nonshock box (P < 0.001) or novel box (P < 0.001). Moreover, NE-treated rats, relative to saline-treated controls, had longer retention latencies in the shock box (P < 0.01), indicating that the NE-induced memory enhancement was also still present 4 wk later.

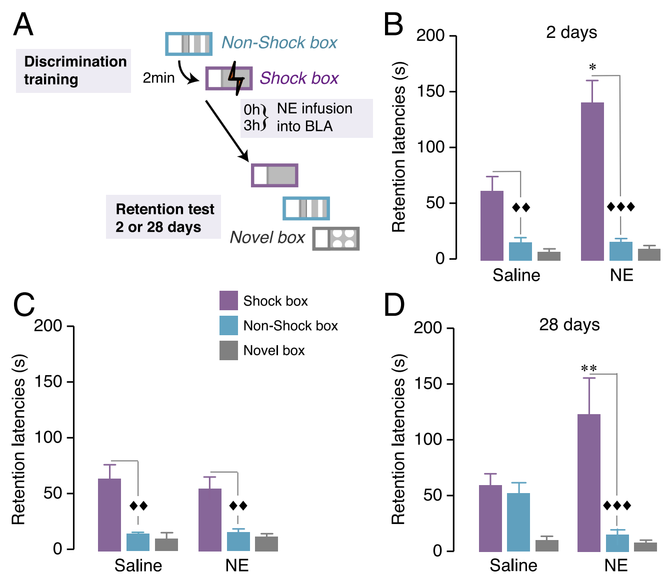

Fig. 1. Noradrenergic activation of the BLA maintains accurate remote memory. (*A*) Experimental design of the inhibitory avoidance discrimination task (*SI Methods* and Fig. S1). (*B*) At 2 d, rats treated with either saline or NE (1.0 μg in 0.2 μL) showed significantly longer retention latencies (mean ± SEM in s) in the shock box than in the nonshock box (◆◆P < 0.01, ◆◆◆P < 0.001), indicating discrimination of the shock context. Moreover, NE-treated rats had enhanced memory, as indicated by longer retention latencies in the shock box (*P < 0.05 vs. saline). n = 10 to 12 rats per group. (*C*) Delayed infusions of NE into the BLA given 3 h after training were ineffective. ◆◆P < 0.01. n = 9 rats per group. (*D*) At 28 d, saline-treated rats no longer had accurate memory of the shock–context association. By contrast, NE-treated rats continued to display accurate memory of the shock–context association (◆◆◆P < 0.001). The NE-induced memory enhancement was also still present at this remote retention test (**P < 0.01 vs. saline). n = 12 to 13 rats per group.

Noradrenergic Activation of the BLA Posttraining Maintains Hippocampus Dependency of Remote Memory. We next investigated whether the accurate episodic-like remote memory displayed by the NE-treated rats continues to rely on the hippocampus. For this, NE or saline was administered into the BLA immediately after training, as above, and hippocampal involvement in the expression of the memory was determined by inactivation of the dorsal hippocampus with the GABAergic receptor agonist muscimol (0.5 μg in 0.5 μL) 20 min before retention testing (39).

At 2 d, three-way ANOVA for retention latencies revealed significant NE ($F_{1,96}$ = 6.37; P = 0.002) and muscimol effects ($F_{1,96}$ = 12.33; P < 0.0001) but no significant interaction between these factors ($F_{1,96}$ = 2.37; P = 0.19). As shown in Fig. 2*A*, hippocampal inactivation of both saline- and NE-treated rats disrupted the expression of accurate memory of the shock–context association, illustrated by similar retention latencies in the shock box and nonshock box (*P*s ≥ 0.94). The muscimol infusion neither impaired retention latencies of saline controls (P = 0.81) nor blocked the NE-induced memory enhancement (P = 0.96), indicating that the hippocampal inactivation did not disrupt the general expression of memory strength. Moreover, the muscimol infusion did not affect discrimination of the two training contexts from the novel box (P < 0.001). When these rats were tested again 24 h after the muscimol infusion (Fig. S2*A*), the expression of accurate memory of the shock–context association had returned, confirming that the muscimol-induced inactivation of the hippocampus was transient and had worn off 24 h later.

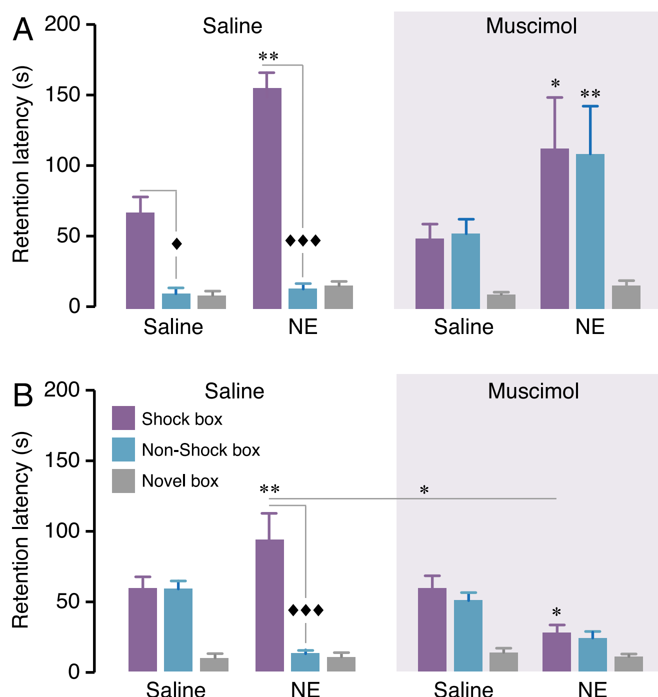

Fig. 2. Noradrenergic activation of the BLA maintains hippocampus dependency of remote memory. (*A*) The GABAergic receptor agonist muscimol (0.5 μg in 0.5 μL) administered bilaterally into the hippocampus 20 min before the 2-d retention test blocked the expression of accurate memory of the shock–context association in both saline- and NE-treated rats but did not block NE-induced memory enhancement. Retention latencies are shown as mean ± SEM in s. $*P < 0.05$, $**P < 0.01$ vs. BLA-saline group, ◆$P < 0.05$, ◆◆◆$P < 0.001$. n = 11 to 14 rats per group. (*B*) At 28 d, muscimol inactivation did not alter retention latencies of saline-treated rats but significantly impaired the accuracy and strength of memory of NE-treated rats. $*P < 0.05$, $**P < 0.01$ vs. BLA-saline group, ◆◆◆$P < 0.001$. n = 11 or 12 rats per group.

At 28 d, three-way ANOVA for retention latencies showed significant NE ($F_{1,84} = 14.07$; $P < 0.0001$), muscimol ($F_{1,84} = 11.87$; $P < 0.0001$), and interaction effects ($F_{1,84} = 14.29$, $P < 0.0001$). As in the first experiment, rats administered saline into the BLA after training no longer accurately discriminated the shock context at this remote retention test ($P = 0.83$; shock vs. nonshock box) (Fig. 2*B*). Hippocampal inactivation of saline-treated rats did not significantly alter retention performance, supporting the systems consolidation hypothesis that the expression of remote generalized memory is associated with a loss of hippocampal engagement. By contrast, hippocampal inactivation of NE-treated rats blocked the expression of accurate memory of the shock–context association ($P = 0.63$; shock vs. nonshock box). Moreover, and unlike at the 2-d retention test, muscimol inactivation of NE-treated rats also impaired general expression of memory. As shown in Fig. 2*B*, retention latencies in the shock box of NE-treated rats administered muscimol before retention testing were significantly shorter than those of NE-treated rats administered saline into the hippocampus ($P < 0.05$), and also than those of saline-treated rats administered muscimol before the retention test ($P < 0.05$). Further, retention latencies in the shock box did not differ significantly from those in the novel box ($P = 0.13$), indicating that these rats, without an intact hippocampus, did not show any evidence of retention. When these NE-treated rats were tested again 24 h after the muscimol inactivation (Fig. S2*B*), the original accurate discrimination of the shock context as well as memory strength had returned.

Noradrenergic Activation of the BLA Posttraining Induces Time- and Tissue-Specific Transcriptomic Changes. Our findings indicate that posttraining BLA activation promotes prolonged accuracy of episodic-like memory through a hippocampus-dependent mechanism. This change in consolidation mechanism could be supported by sustained changes at the molecular level, that is, modified gene expression. Therefore, we measured transcriptional changes in the hippocampus (dentate gyrus area) and ACC, brain structures previously associated with early and late phases of memory consolidation, respectively (29, 30). For this, we trained and tested another cohort of rats on the same task (Fig. 3*E*). To get a broad picture of time- and tissue-specific changes, we first used gene set enrichment analysis (GSEA) and focused on a memory-associated gene set (MGS; 122 genes; see *SI Methods*). These genes were previously reported as being differentially expressed in the rodent brain after training on an episodic-like memory task (adapted from ref. 40; Table S1). Posttraining NE administration into the BLA was associated with a time-dependent differential expression of the MGS in the hippocampus ($P_{\text{nominal}} = 0.007$; $P_{\text{adjusted}} < 0.02$, global test; Table S2). Next, we analyzed the MGS genes individually and examined NE-dependent changes in gene expression at the remote time point in the two brain regions. Upon applying a Bonferroni correction, *Reln* was significantly up-regulated in the hippocampus and *Pkmζ* was down-regulated in the ACC ($P_{\text{Bonferroni}} < 0.05$).

Previous studies related *Reln* to early stages of plasticity in the hippocampus (36) and *Pkmζ* to long-term memory maintenance in cortex (37). Therefore, our second-level analysis focused on the expression of these two genes in a pharmacological and time- and tissue-specific context. We first examined *Reln* and *Pkmζ* expression patterns separately for NE- and saline-treated animals (Fig. S3), followed by examining the specific NE-induced changes in the expression dynamics of these genes, after accounting for saline effects (Fig. 3). In the hippocampus of animals that received NE, *Reln* expression was stably up-regulated at both 2 and 28 d (Fig. S3*A*), as opposed to an only initial up-regulation at the 2-d time point in saline-treated animals (Fig. S3*C*). After subtracting saline effects, NE treatment was associated with a down-regulation in *Reln* expression in the hippocampus at 2 d ($P_{\text{Bonferroni}} < 0.05$) and, interestingly, an up-regulation at 28 d ($P_{\text{Bonferroni}} < 0.05$), thus indicating a time-regulated expression shift ($P = 0.0004$; Fig. 3*A* and Fig. S4*A*). In contrast, NE treatment-associated *Pkmζ* expression in the hippocampus was not significantly changed over time ($P > 0.05$; Fig. 3*A* and Fig. S4*A*; after subtracting saline effects), mostly due to similar expression dynamics in both the NE and saline groups (Fig. S3 *B* and *D*). In the ACC, *Reln* expression in both NE- and saline-treated animals was down-regulated at 28 d compared with 2 d (Fig. S3 *A* and *C*), while *Pkmζ* was time-dependently up-regulated in animals that received saline only (Fig. S3 *B* and *D*). After subtracting saline effects, NE treatment was associated with an up-regulation of both *Pkmζ* and *Reln* expression in the ACC at 2 d ($P_{\text{Bonferroni}} < 0.05$) but only transitionally for *Pkmζ* (28 vs. 2 d, $P = 0.006$), while *Reln* expression remained up-regulated (28 vs. 2 d, $P > 0.05$; Fig. 3*B* and Fig. S4*B*). These time- and tissue-specific expression dynamics are in line with our behavioral observations of an altered systems consolidation in NE-treated rats, marked by a sustained hippocampus dependency.

Noradrenergic Activation of the BLA Posttraining Induces Time- and Tissue-Specific DNA Methylation Changes in *Reln* and *Pkmζ* Gene Promoters. DNA methylation is critical for memory formation (36) as well as for memory maintenance (34, 35). Therefore, we next examined whether the changes in gene expression induced by the NE activation could be driven by epigenetic mechanisms, namely DNA methylation. Indeed, mRNA measurements of DNA methyltransferases (Dnmts), a family of enzymes that catalyzes DNA methylation (36), in the hippocampus 30 min posttraining revealed up-regulated expression of de novo *Dnmt3a* in NE-treated rats ($P < 0.01$, after subtracting saline effects; $P < 0.05$ for *Creb* transcriptional factor as a control; Fig. 3*F*). Thus, posttraining NE activation of the BLA induced changes in de novo *Dnmt3a* expression that were obvious shortly after the training session, during the initial consolidation phase.

Additionally, to examine if DNA methylation could be associated with the time- and tissue-specific expression changes of

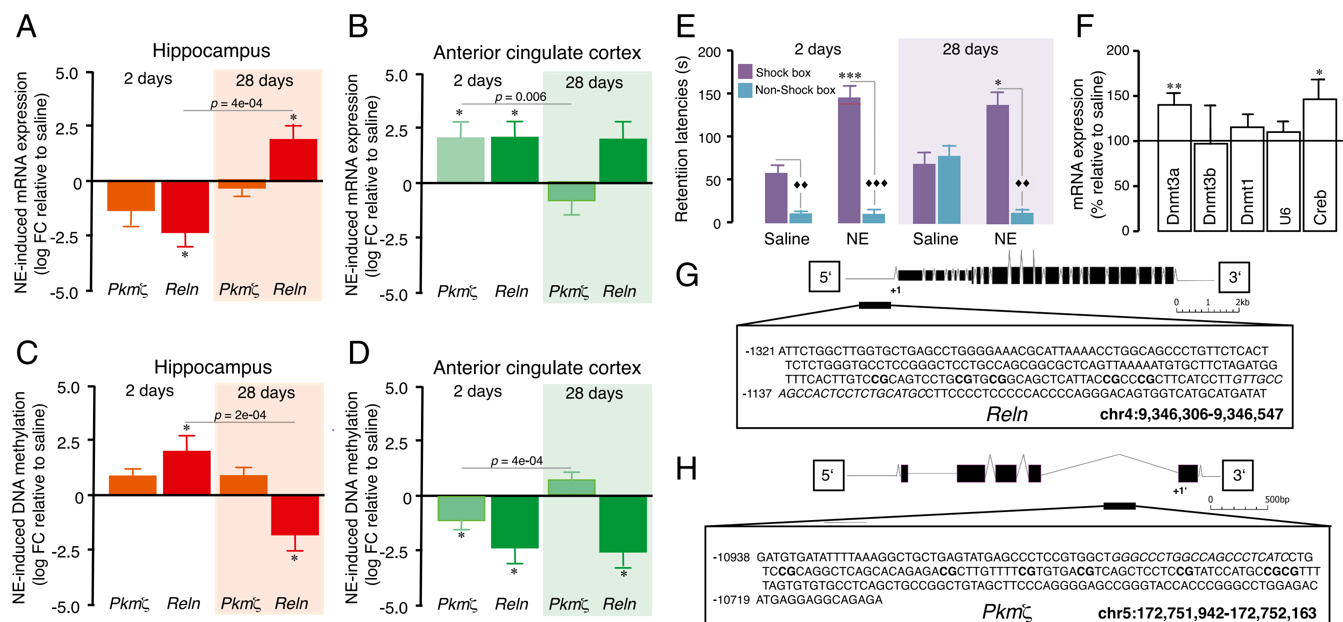

Fig. 3. Noradrenergic activation of the BLA induces time- and tissue-specific transcriptional and epigenetic changes. (*A* and *B*) Temporal specificity of NE-induced *Reln* and *Pkmζ* expression changes in the hippocampus (*A*) and ACC (*B*), based on Affymetrix Rat Gene ST 2.0 data (mean ± SEM). (*C* and *D*) Temporal specificity of NE-induced *Reln* and *Pkmζ* promoter DNA methylation in the hippocampus (*C*) and ACC (*D*) (mean ± SEM). Bars represent fold change relative expression or promoter DNA methylation of NE-treated rats compared with saline-treated rats (log FC, *t* test, $^*P_{\text{Bonferroni}} < 0.05$; $n = 3$ pooled samples per group). (*E*) Retention latencies (mean ± SEM in s) on the inhibitory avoidance discrimination task of rats used for the molecular studies shown in *A*–*D*. $^*P < 0.05$, $^{***}P < 0.001$ vs. saline; ♦♦$P < 0.01$, ♦♦♦$P < 0.001$. $n = 11$ rats per group. (*F*) Expression levels of *Dnmt3a*, *Dnmt3b*, *Dnmt1*, *U6*, and *Creb* (control) in the hippocampus of NE-treated vs. saline-treated rats 30 min posttraining ($^*P < 0.05$, $^{**}P < 0.01$; $n = 5$ to 7 rats per group). (*G* and *H*) *Reln* and *Pkmζ* gene promoter regions analyzed by bisulfite pyrosequencing (for details, see *SI Methods*).

memory-associated genes, we measured DNA methylation of the *Reln* and *Pkmζ* promoters at the recent and remote time points [averaged across CpGs in relevant promoter regions: chr4:9,346,306 to 9,346,547 for *Reln* (35) and chr5:172,751,942 to 172,752,163 for *Pkmζ* (41); Fig. 3 *G* and *H*]. Further, we examined temporal changes in *Dnmt3a* expression. In the hippocampus, *Reln* promoter DNA methylation showed opposite time-dependent dynamics in NE- and saline-treated rats (Fig. S5 *A* and *C*). After subtracting saline effects, NE treatment was associated with a hypermethylation of the *Reln* promoter in the hippocampus at 2 d but a hypomethylation at 28 d ($P_{\text{Bonferroni}} < 0.05$; Fig. 3*C*). These findings indicate that noradrenergic activation of the BLA induced a temporal shift in *Reln* promoter DNA methylation in the hippocampus ($P = 0.0002$). Furthermore, *Dnmt3a* expression in the hippocampus was accordingly down-regulated at 28 d ($P_{\text{Bonferroni}} < 0.05$, after subtracting saline effects; Fig. S6*A*), suggesting that the sustained changes in DNA methylation and gene expression could be actively maintained. In the ACC, DNA methylation at the *Reln* promoter increased over time both in NE- and saline-treated rats (Fig. S5 *A* and *C*), while it decreased at the *Pkmζ* promoter in animals that received saline only (Fig. S5*D*). DNA methylation of the *Pkmζ* promoter in the ACC of NE-treated rats did not show a change across time (Fig. S5*B*). After accounting for saline effects, NE treatment was associated with a hypomethylation of both the *Reln* and *Pkmζ* promoters in the ACC at 2 d ($P_{\text{Bonferroni}} < 0.05$; Fig. 3*D*); this effect was only transitional for *Pkmζ* (28 vs. 2 d, $P = 0.0004$), while *Reln* remained hypomethylated at 28 d ($P_{\text{Bonferroni}} < 0.05$). NE treatment did not significantly affect *Dnmt3a* expression levels in the ACC at either the recent or remote time points (Fig. S6*B*). These observed DNA methylation changes were concordant and negatively correlated with the NE treatment-associated temporal expression dynamics of *Reln* and *Pkmζ*, both in the hippocampus and ACC.

Discussion

Our findings indicate that NE administration into the BLA after inhibitory avoidance discrimination training maintained long-term hippocampus-dependent accuracy of memory. These effects were associated with time-regulated DNA methylation and transcriptional changes of memory-related genes, namely *Reln* and *Pkmζ*, in the hippocampus and neocortex. Such NE-induced changes in systems consolidation dynamics may represent a critical mechanism underlying the long-term vividness of emotional memories (3, 4).

Decades of research have provided compelling evidence that emotional arousal or brief periods of stress after encoding facilitate subsequent memory performance (2, 12). Evidence indicates that the BLA upon emotional arousal or noradrenergic activation modulates information transfer and neural plasticity mechanisms in different memory circuits to enhance the consolidation of different types of training experiences (10, 12, 42, 43). In line with the animal data, findings of human studies confirmed the key role of noradrenergic arousal in emotional memory enhancement (13, 44). Findings of human neuroimaging studies further indicated that arousal-induced activation of the amygdala enhances memory-related hippocampal activity (16, 17, 20, 22, 45). The present findings provide evidence that such posttraining noradrenergic activation also affects the later strength and accuracy of memory: NE administration into the BLA immediately after inhibitory avoidance discrimination learning maintained the strength as well as the long-term episodic-like specificity of memory. Moreover, the findings with rats given posttraining NE infusions into the BLA as well as hippocampal inactivation with muscimol during retention testing revealed this episodic-like specificity to be hippocampus-dependent at both recent and remote time points. These findings strongly suggest that noradrenergic activation of the BLA may alter the time-dependent transfer of the memory trace from the hippocampus to neocortical areas, thereby also preventing the transformation of the detailed, very specific memories into more semantic, gist-like memories.

According to the standard model of systems consolidation, memories are initially dependent on the hippocampus and are subsequently stored, in their original form, in other brain areas (28). Findings of several studies suggest that systems consolidation

NEUROSCIENCE

may be a more dynamic process that involves a transformation of the nature of the memory, including a decrease in memory specificity (30). To the extent that episodic or context-specific memories are retained, they might continue to require the hippocampus. Our findings indicate that saline control rats initially showed accurate episodic-like memory of the association of footshock with the training context, but at the 28-d retention test were unable to discriminate the shock context. Consistent with the systems consolidation theory, the results of hippocampal inactivation during retention testing indicated that discrimination at the recent time point depends on the hippocampus but that the expression of generalized memory at the remote time point no longer requires the hippocampus. Our findings further suggest that the hippocampus does not appear to play any crucial role in remembering the aversive experience or training contexts per se. Hippocampal inactivation also preserved the initial NE-induced memory enhancement, despite a lack of episodic-like accuracy. As noradrenergic activation is known to activate large-scale neural networks (46), it is likely that the NE administration into the BLA might have enhanced the initial consolidation of different aspects of the acquired information simultaneously in multiple brain regions (8). We were particularly interested in determining whether the accurate long-term memory of NE-treated rats continues to depend on the hippocampus. The evidence indicates that it does; hippocampal inactivation of these rats at the 28-d retention test blocked the expression of episodic-like accuracy of memory. Rather unexpectedly, however, hippocampal inactivation of NE-treated rats at this remote retention test, different from at the recent retention test, also resulted in a general impairment in memory. These findings thus indicate that BLA activation by posttraining NE infusions induces long-lasting accurate and strong memories by engaging the hippocampus for longer periods of time. Noradrenergic stimulation shortly after learning may not only decelerate but even reverse the transformation of memory and maintain hippocampus dependency, enabling long-term accurate memory.

This altered systems consolidation was paralleled by dynamic changes in DNA methylation and expression of memory-associated genes. It is very likely that the complex changes in systems consolidation depend on several molecular mechanisms. For instance, genes such as *Map2* and *Grin1* were also differentially regulated by the NE treatment over time in a tissue-specific manner (Table S1), supporting a role for the BLA in affecting hippocampal neurogenesis in emotional enhancement of episodic memory (47). Nevertheless, we identified here *Reln* and *Pkmζ* as the main markers. The temporal expression of these genes in the two anatomical regions, previously reported as regions important for specific and time-dependent support of memory (29, 35), was also altered, mirroring the shift in memory consolidation. The first marker, *Reln*, is involved in processes of neuronal migration and positioning in the developing brain (48), enhances synaptic plasticity by regulating long-term potentiation (49), and supports the formation of new synapses (50). Moreover, it has been reported that *Reln* expression in the hippocampus is actively, but transiently, up-regulated to facilitate the storage of new memories but returns to basal levels 24 h after fear conditioning (36). In the current study, saline-treated control rats exhibited an initial up-regulation of *Reln* expression in the hippocampus at 2 d, followed by a down-regulation to basal levels at 28 d, in line with the systems consolidation theory. In contrast, NE treatment of the BLA after training actively reversed this down-regulation of *Reln* expression in the hippocampus at the 28-d time point, thus mirroring the observed behavioral changes and supporting an enhanced involvement of the hippocampus in long-term memory maintenance. Furthermore, the noradrenergic activation also abolished the time-dependent expression changes of *Reln* in the ACC found in control animals, possibly supporting a diminished role of the cortex in long-term memory maintenance after NE treatment.

The second main marker, *Pkmζ*, has been linked with the maintenance of long-term memory in the cortex (37, 51). PKMζ is an independent catalytic domain of the full-length protein kinase C ζ and is constitutively and persistently active, without the need of a second messenger (41). Activation of this kinase enhances trafficking of AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors) in the postsynaptic membrane and affects plasticity through modulation of late-phase long-term potentiation (52, 53). Shema et al. (54) reported that the inhibition of PKMζ in the cerebral cortex abolishes long-term memory. This is in agreement with the systems consolidation theory, and points to a cortex-associated mechanism of remote memory maintenance. However, other findings suggest that PKMζ is rather dispensable and that other kinases may play a role as well (55, 56). In the current study, saline-treated control rats exhibited a time-dependent up-regulation of *Pkmζ* in the ACC, supporting an increasing role of cortical PKMζ in long-term memory maintenance. In contrast, NE treatment abolished this time-dependent cortical up-regulation of *Pkmζ* triggered by the learning experience, further supporting an altered systems consolidation and diminished role of the cortex in the long-term maintenance of the memory trace.

Thus, these findings provide evidence that posttraining noradrenergic activation of the BLA enhances strength and long-term episodic-like specificity of memory induced by sustained hippocampus dependency. Strikingly, the remote memory remained hippocampus-dependent even 4 wk after training, suggesting a shift in systems consolidation dynamics. This shift was likely supported by epigenetically driven transcriptional changes of memory-related genes in both the hippocampus and neocortex.

Methods

Subjects. Male Sprague–Dawley rats (Charles River) were kept individually in a temperature-controlled (22 °C) vivarium room (0700 to 1900 hours lights on). Training and testing were performed during the light cycle between 1000 and 1500 hours. All experimental procedures were in compliance with European Union Directive 2010/63/EU and approved by the Institutional Animal Care and Use Committee of Radboud University, Nijmegen, The Netherlands.

Inhibitory Avoidance Discrimination Task. Rats were initially placed in the starting compartment of the nonshock box and could explore the apparatus for 20 s without footshock being delivered (Fig. 1*A*). After a delay of 2 min, they were placed in the starting compartment of a contextually different inhibitory avoidance apparatus (shock box). When the rat had stepped into the dark compartment, the sliding door was closed and a single inescapable footshock (0.60 mA; 1 s) was delivered. On the retention test, either 2 or 28 d after training, they were tested, in a randomized order, in both training contexts as well as in a novel context (38) (*SI Methods*).

Tissue Collection, Homogenization, and Nucleic Acid Extraction. Brain tissue containing the hippocampus or ACC was cut into 350-μm-thick coronal slices. Bilateral punches from the dentate gyrus area of the hippocampus [anteroposterior (AP): −2.64 to −3.86 mm] and ACC (AP: +3.00 to +1.92 mm) were collected from three consecutives slices to a total of six punches. For transcriptomic and methylomic analysis, we pooled individual animals' tissue punches. Parallel DNA and RNA isolation was performed using a chaotropic lysis protocol (*SI Methods*).

Transcriptomic Analysis. Genome-wide expression changes were measured with the Rat Gene 2.0 ST Array (Affymetrix; 902124) by using standard protocols (57) (*SI Methods*).

Gene Set Enrichment Analysis. First, we created a memory-associated gene set composed of 122 genes previously implicated in memory (*SI Methods*). GSEA was performed by using a standard weighted Kolmogorov–Smirnov statistic. Finally, we tested individual genes from the MGS across significant comparisons and applied Bonferroni correction to account for multiple testing (*SI Methods*).

Quantitative PCR. Posttraining DNA methyltransferase expression measurements as well as validation of expression changes from transcriptomic analysis were performed by quantitative PCR. All reactions were performed in triplicate (*SI Methods*).

Pyrosequencing Analysis. DNA methylation of the previously reported relevant promoter regions of rat *Reln* [chr4:9,346,306 to 9,346,547; RGSC 6.0/rn6 (35); Fig. 3*G*] and *Pkmζ* [chr5:172,751,942 to 172,752,163; RGSC (Rat Genome Sequencing Consortium/Rattus norvegicus) 6.0/rn6 (41); Fig. 3*H*] genes was

quantified by direct bisulfite pyrosequencing (58). All reactions were performed in quadruplicate (*SI Methods*).

Statistics. Inhibitory avoidance retention latencies were analyzed with two- or three-way ANOVAs with retention latencies in the different test contexts as repeated measures. Post hoc comparisons used unpaired and paired t tests. For transcriptome and DNA methylation analysis, we analyzed three biological replicates formed by pooling tissue punches from 6 to 10 independent animals for each tissue/treatment combination. Bar plots show log fold change (FC) differences in expression and methylation between compared groups. *Dnmt* mRNA levels were analyzed with unpaired t tests. For all comparisons, $P < 0.05$ was accepted as statistically significant. If not noted otherwise, Bonferroni correction was applied to account for multiple testing.

ACKNOWLEDGMENTS. This research was supported by a Radboud University Topfund (to B.R.) and funds of the University of Basel (to A.P. and D.J.-F.d.Q.).

1. McGaugh JL (2003) *Memory and Emotion: The Making of Lasting Memories* (Columbia Univ Press, New York).
2. McGaugh JL (2013) Making lasting memories: Remembering the significant. *Proc Natl Acad Sci USA* 110:10402–10407.
3. Talarico JM, Rubin DC (2003) Confidence, not consistency, characterizes flashbulb memories. *Psychol Sci* 14:455–461.
4. Sharot T, Delgado MR, Phelps EA (2004) How emotion enhances the feeling of remembering. *Nat Neurosci* 7:1376–1380.
5. Izquierdo I, et al. (1992) Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat. *Behav Neural Biol* 58:16–26.
6. McGaugh JL (2006) Make mild moments memorable: Add a little arousal. *Trends Cogn Sci* 10:345–347.
7. Hurlemann R, et al. (2010) Human amygdala reactivity is diminished by the β-noradrenergic antagonist propranolol. *Psychol Med* 40:1839–1848.
8. Roozendaal B, McGaugh JL (2011) Memory modulation. *Behav Neurosci* 125:797–824.
9. Barsegyan A, McGaugh JL, Roozendaal B (2014) Noradrenergic activation of the basolateral amygdala modulates the consolidation of object-in-context recognition memory. *Front Behav Neurosci* 8:160.
10. Izquierdo I, Furini CRG, Myskiw JC (2016) Fear memory. *Physiol Rev* 96:695–750.
11. LaLumiere RT, McGaugh JL, McIntyre CK (2017) Emotional modulation of learning and memory: Pharmacological implications. *Pharmacol Rev* 69:236–255.
12. Roozendaal B, Hermans EJ (2017) Norepinephrine effects on the encoding and consolidation of emotional memory: Improving synergy between animal and human studies. *Curr Opin Behav Sci* 14:115–122.
13. Cahill L, Prins B, Weber M, McGaugh JL (1994) Beta-adrenergic activation and memory for emotional events. *Nature* 371:702–704.
14. Roozendaal B, Nguyen BT, Power AE, McGaugh JL (1999) Basolateral amygdala noradrenergic influence enables enhancement of memory consolidation induced by hippocampal glucocorticoid receptor activation. *Proc Natl Acad Sci USA* 96: 11642–11647.
15. Southwick SM, et al. (2002) Relationship of enhanced norepinephrine activity during memory consolidation to enhanced long-term memory in humans. *Am J Psychiatry* 159:1420–1422.
16. Kensinger EA, Corkin S (2003) Memory enhancement for emotional words: Are emotional words more vividly remembered than neutral words? *Mem Cognit* 31: 1169–1180.
17. Strange BA, Dolan RJ (2004) Beta-adrenergic modulation of emotional memory-evoked human amygdala and hippocampal responses. *Proc Natl Acad Sci USA* 101: 11454–11458.
18. Pikkarainen M, Rönkkö S, Savander V, Insausti R, Pitkänen A (1999) Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. *J Comp Neurol* 403:229–260.
19. Phelps EA (2004) Human emotion and memory: Interactions of the amygdala and hippocampal complex. *Curr Opin Neurobiol* 14:198–202.
20. Fastenrath M, et al. (2014) Dynamic modulation of amygdala-hippocampal connectivity by emotional arousal. *J Neurosci* 34:13935–13947.
21. Rei D, et al. (2015) Basolateral amygdala bidirectionally modulates stress-induced hippocampal learning and memory deficits through a p25/Cdk5-dependent pathway. *Proc Natl Acad Sci USA* 112:7291–7296.
22. Richardson MP, Strange BA, Dolan RJ (2004) Encoding of emotional memories depends on amygdala and hippocampus and their interactions. *Nat Neurosci* 7:278–285.
23. McIntyre CK, et al. (2005) Memory-influencing intra-basolateral amygdala drug infusions modulate expression of Arc protein in the hippocampus. *Proc Natl Acad Sci USA* 102:10718–10723.
24. Lovitz ES, Thompson LT (2015) Memory-enhancing intra-basolateral amygdala clenbuterol infusion reduces post-burst afterhyperpolarizations in hippocampal CA1 pyramidal neurons following inhibitory avoidance learning. *Neurobiol Learn Mem* 119:34–41.
25. Segal SK, Stark SM, Kattan D, Stark CE, Yassa MA (2012) Norepinephrine-mediated emotional arousal facilitates subsequent pattern separation. *Neurobiol Learn Mem* 97:465–469.
26. Smeets T, et al. (2009) Stress selectively and lastingly promotes learning of context-related high arousing information. *Psychoneuroendocrinology* 34:1152–1161.
27. Wiemers US, Sauvage MM, Schoofs D, Hamacher-Dang TC, Wolf OT (2013) What we remember from a stressful episode. *Psychoneuroendocrinology* 38:2268–2277.
28. Squire LR, Alvarez P (1995) Retrograde amnesia and memory consolidation: A neurobiological perspective. *Curr Opin Neurobiol* 5:169–177.
29. Frankland PW, Bontempi B, Talton LE, Kaczmarek L, Silva AJ (2004) The involvement of the anterior cingulate cortex in remote contextual fear memory. *Science* 304: 881–883.
30. Winocur G, Moscovitch M (2011) Memory transformation and systems consolidation. *J Int Neuropsychol Soc* 17:766–780.
31. Moscovitch M, Cabeza R, Winocur G, Nadel L (2016) Episodic memory and beyond: The hippocampus and neocortex in transformation. *Annu Rev Psychol* 67:105–134.
32. Kitamura T, et al. (2017) Engrams and circuits crucial for systems consolidation of a memory. *Science* 356:73–78.
33. Wiltgen BJ, Silva AJ (2007) Memory for context becomes less specific with time. *Learn Mem* 14:313–317.
34. Day JJ, Sweatt JD (2010) DNA methylation and memory formation. *Nat Neurosci* 13: 1319–1323.
35. Miller CA, et al. (2010) Cortical DNA methylation maintains remote memory. *Nat Neurosci* 13:664–666.
36. Miller CA, Sweatt JD (2007) Covalent modification of DNA regulates memory formation. *Neuron* 53:857–869.
37. Shema R, et al. (2011) Enhancement of consolidated long-term memory by overexpression of protein kinase Mzeta in the neocortex. *Science* 331:1207–1210.
38. Atucha E, Roozendaal B (2015) The inhibitory avoidance discrimination task to investigate accuracy of memory. *Front Behav Neurosci* 9:60.
39. Holt W, Maren S (1999) Muscimol inactivation of the dorsal hippocampus impairs contextual retrieval of fear memory. *J Neurosci* 19:9054–9062.
40. Cavallaro S, D'Agata V, Manickam P, Dufour F, Alkon DL (2002) Memory-specific temporal profiles of gene expression in the hippocampus. *Proc Natl Acad Sci USA* 99:16279–16284.
41. Hernández AI, et al. (2003) Protein kinase M zeta synthesis from a brain mRNA encoding an independent protein kinase C zeta catalytic domain. Implications for the molecular mechanism of memory. *J Biol Chem* 278:40305–40316.
42. Roozendaal B, Castello NA, Vedana G, Barsegyan A, McGaugh JL (2008) Noradrenergic activation of the basolateral amygdala modulates consolidation of object recognition memory. *Neurobiol Learn Mem* 90:576–579.
43. Paz R, Paré D (2013) Physiological basis for emotional modulation of memory circuits by the amygdala. *Curr Opin Neurobiol* 23:381–386.
44. van Stegeren AH, et al. (2005) Noradrenaline mediates amygdala activation in men and women during encoding of emotional material. *Neuroimage* 24:898–909.
45. Cahill L, et al. (1996) Amygdala activity at encoding correlated with long-term, free recall of emotional information. *Proc Natl Acad Sci USA* 93:8016–8021.
46. Hermans EJ, et al. (2011) Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. *Science* 334:1151–1153.
47. Castro JE, et al. (2010) Role of the amygdala in antidepressant effects on hippocampal cell proliferation and survival and on depression-like behavior in the rat. *PLoS One* 5: e8618.
48. Tissir F, Goffinet AM (2003) Reelin and brain development. *Nat Rev Neurosci* 4: 496–505.
49. Beffert U, et al. (2005) Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. *Neuron* 47:567–579.
50. Niu S, Yabut O, D'Arcangelo G (2008) The Reelin signaling pathway promotes dendritic spine development in hippocampal neurons. *J Neurosci* 28:10339–10348.
51. Sacktor TC (2011) How does PKMζ maintain long-term memory? *Nat Rev Neurosci* 12:9–15.
52. Pastalkova E, et al. (2006) Storage of spatial information by the maintenance mechanism of LTP. *Science* 313:1141–1144.
53. Yao Y, et al. (2008) PKM zeta maintains late long-term potentiation by *N*-ethylmaleimide-sensitive factor/GluR2-dependent trafficking of postsynaptic AMPA receptors. *J Neurosci* 28:7820–7827.
54. Shema R, Sacktor TC, Dudai Y (2007) Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKM zeta. *Science* 317:951–953.
55. Lee AM, et al. (2013) Prkcz null mice show normal learning and memory. *Nature* 493: 416–419.
56. Volk LJ, Bachman JL, Johnson R, Yu Y, Huganir RL (2013) PKM-ζ is not required for hippocampal synaptic plasticity, learning and memory. *Nature* 493:420–423.
57. Carvalho BS, Irizarry RA (2010) A framework for oligonucleotide microarray preprocessing. *Bioinformatics* 26:2363–2367.
58. Tost J, Gut IG (2007) DNA methylation analysis by pyrosequencing. *Nat Protoc* 2: 2265–2275.
59. Paxinos G, Watson C (2007) *The Rat Brain in Stereotaxic Coordinates* (Academic, San Diego), 6th Ed.
60. Ritchie ME, et al. (2015) limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 43:e47.
61. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc Series B* 57:289–300.
62. Weaver IC, et al. (2004) Epigenetic programming by maternal behavior. *Nat Neurosci* 7:847–854.
63. Subramanian A, et al. (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102: 15545–15550.
64. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45.