Basolateral amygdala bidirectionally modulates stress-induced hippocampal learning and memory deficits through a p25/Cdk5-dependent pathway

Damien Rei[a], Xenos Mason[a,1], Jinsoo Seo[a,1], Johannes Gräff[a,2], Andrii Rudenko[a], Jun Wang[a], Richard Rueda[a], Sandra Siegert[a], Sukhee Cho[a], Rebecca G. Canter[a], Alison E. Mungenast[a], Karl Deisseroth[b,c], and Li-Huei Tsai[a,3]

[a]Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139 and [b]Department of Bioengineering and [c]Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305

Edited* by Ann M. Graybiel, Massachusetts Institute of Technology, Cambridge, MA, and approved March 24, 2015 (received for review August 15, 2014)

Repeated stress has been suggested to underlie learning and memory deficits via the basolateral amygdala (BLA) and the hippocampus; however, the functional contribution of BLA inputs to the hippocampus and their molecular repercussions are not well understood. Here we show that repeated stress is accompanied by generation of the Cdk5 (cyclin-dependent kinase 5)-activator p25, up-regulation and phosphorylation of glucocorticoid receptors, increased HDAC2 expression, and reduced expression of memory-related genes in the hippocampus. A combination of optogenetic and pharmacosynthetic approaches shows that BLA activation is both necessary and sufficient for stress-associated molecular changes and memory impairments. Furthermore, we show that this effect relies on direct glutamatergic projections from the BLA to the dorsal hippocampus. Finally, we show that p25 generation is necessary for the stress-induced memory dysfunction. Taken together, our data provide a neural circuit model for stress-induced hippocampal memory deficits through BLA activity-dependent p25 generation.

basolateral amygdala | behavioral stress | cognitive dysfunction | p25/Cdk5 | HDAC2

Chronic stress can have devastating psychological consequences that include depression and cognitive impairment (1–3). Decades of research suggest that the hippocampus, a structure important for learning and memory and implicated in depression, is particularly sensitive to the effects of chronic stress. In animal models, for example, chronic stress impairs hippocampus-dependent forms of learning and memory (2). This sensitivity is partially conferred by a dense concentration of glucocorticoid receptor (GR) in the hippocampus (4), as well as through hippocampal connectivity to important stress response coordinators, such as the amygdala, from which the hippocampus receives abundant glutamatergic inputs (5–7). Following chronic stress, the hippocampus shows marked reductions in dendritic arborization and neurogenesis, along with impaired plasticity (2). Many of these effects have been attributed to connections between the hippocampus and a specific amygdalar subregion, the basolateral amygdala (BLA) (8–10).

Abundant evidence suggests that these BLA inputs have a major impact on hippocampus function; for example, the hippocampus and BLA synchronize their activity during fear memory retrieval and fear extinction (11, 12), whereas electrical stimulation of the BLA disrupts the induction of long-term potentiation (LTP), a measure of synaptic plasticity, in the hippocampal CA1 subregion (13). Lesions of the BLA have been shown to block the detrimental effects of repeated stress, a model of chronic stress in rodents, on LTP and spatial memory (8, 10), as well as the deleterious effect of hippocampal GR activation on hippocampus-dependent memory (9). Although the BLA sends abundant projections to the hippocampus (5–7), this region also projects diffusely throughout the brain and thereby regulates a myriad of behaviors, including valence or social interaction (14), as well as hormonal cascades (15). Because of this complexity, whether BLA activity affects hippocampus-dependent learning and memory directly or indirectly through distinct relay brain regions or other downstream mediators, such as stress hormones, remains unclear.

Cdk5 (cyclin-dependent kinase 5) plays a pleiotropic role in the nervous system (16). This enzyme is essential for proper brain development and regulates synaptic plasticity and cognitive function. Activation of Cdk5 requires association with a regulatory subunit known as p35. p35 is subjected to calpain-mediated cleavage into p25 in a process dependent on the activation of glutamate receptors, specifically NR2B-containing NMDA receptors, following neurotoxic stimulation, such as exposure to β-amyloid peptides, oxidative stress, or

Significance

Chronic stress has emerged in the epidemiologic literature as a risk factor for both psychiatric and neurodegenerative diseases. Thus, neurologic maladaptation to chronic stress is highly relevant to the pathogenesis of human diseases such as depression and Alzheimer's disease, yet it remains poorly understood. Here we report a study of the neural circuits and molecular pathways that govern the relationship between stress and cognition. We present data demonstrating that behavioral stress impairs cognitive function via activation of a specific direct neural circuit from the basolateral amygdala to the dorsal hippocampus. Moreover, we delineate a molecular mechanism by which behavioral stress is translated to hippocampal dysfunction via a p25/Cdk5 (cyclin-dependent kinase 5)-dependent pathway and epigenetic alterations of neuroplasticity-related gene expression.

Author contributions: This study was designed by D.R. and L.-H.T., and directed and coordinated by L.-H.T.; D.R. provided the samples and S.S. performed and analyzed the RT-PCR experiment; K.D. provided the virus as well as expertise for the optogenetic experiments; D.R. performed all stereotaxic injections, behavior (except when otherwise stated), and immunohistochemistry and mainly performed RFS, optogenetic stimulation, and CNO treatment before RFS with the help of X.M.; all work on Δp35 mice was done by D.R.; J.G. performed the Western blot analysis in Fig. 1 and novel object recognition quantification in Figs. 1 and 3; novel location recognition was run by D.R. and X.M. and quantified blindly by X.M.; J.S. performed hippocampal dissections for p25 Western blot and Western blots; S.C. performed the electrophysiology analysis on the Gi-DREADD BLA brain slices; immunohistochemistry analysis of HDAC2 and SYP were performed by D.R., X.M., A.R., and R.R.; fiber optic placements were determined by X.M.; revisions were performed by A.R., J.W., R.R., R.G.C., and A.E.M.; and the manuscript was written by D.R., X.M., A.E.M., and L.-H.T.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

[1]X.M. and J.S. contributed equally to this work.

[2]Present address: Ecole Polytechnique Fédérale Lausanne, School of Life Sciences, Brain Mind Institute, CH-1015 Lausanne, Switzerland.

[3]To whom correspondence should be addressed. Email: lhtsai@mit.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1415845112/-/DCSupplemental.

NEUROSCIENCE

excitotoxicity, as well as in response to physiological neuronal activity (16).

A number of studies have implicated p25 production in Alzheimer's disease (AD)-like phenotypes, including learning and memory impairments (16), and long-term overexpression of p25 in the forebrain is known to lead to cognitive deficits (16). Furthermore, stress and the heightened sensitivity to stress are known risk factors for the development of AD (1). The role of p25 production after repeated stress remains undetermined, however.

One pathway through which p25/Cdk5 might be implicated in stress-induced cognitive dysfunction is stress hormone receptor-mediated epigenetic signaling in the hippocampus. Indeed, it was previously shown that GR is activated by p25/Cdk5-dependent phosphorylation on Ser211 (17, 18), and that increased GR phosphorylation leads to increased expression of histone deacetylase 2 (HDAC2) in a mouse model of AD (18). HDAC2 in turn suppresses the expression of genes important for learning and memory (18, 19), suggesting a mechanism by which elevated p25 generation leads to cognitive impairment. Although GR activation has been shown to be required for stress-induced hippocampal dysfunction and is dependent upon its phosphorylation (20–22), and HDAC2 has been shown to be up-regulated in the ventral striatum of mice following chronic stress (23), the possible up-regulation of HDAC2 in the hippocampus after repeated stress, and the role of p25/Cdk5 signaling in this process, are unknown. We tested the hypothesis that p25 is generated in the hippocampus after repeated stress in an amygdala-dependent manner and contributes to stress-associated learning and memory deficits. Blockade of p25 generation would then protect the hippocampus from the detrimental effects of repeated stress.

Here we identify that the activity of a specific BLA to dorsal hippocampus neural circuit mediates the detrimental effects of repeated stress on hippocampal learning and memory via a molecular pathway dependent on p25 generation.

Results

Repeated Stress Leads to p25 Production, HDAC2 Elevation, and Learning and Memory Deficits in the Hippocampus. To characterize the effects of stress on hippocampus-dependent learning and memory pathways, we modified a learned-helplessness paradigm, originally developed in rats (24), termed repetitive foot shock (RFS), for use in Swiss Webster mice, a strain known for its susceptibility to stress (25). This protocol is depicted in Fig. S1*A* and described in *SI Experimental Procedures*.

Because different stress induction protocols have been shown to either facilitate or impair learning and memory (3), we first sought to establish the effects of our paradigm on hippocampus-dependent tasks. To do this, we used two relatively low-stress cognitive tests that have been validated in studies of hippocampal function (26): the novel object recognition task, which in addition to the hippocampus also relies on perirhinal and prefrontal cortices (27), and novel location recognition (28). Detailed descriptions of these tasks can be found in *SI Experimental Procedures*. During the first object training session, animals in both the control and RFS-treated groups exhibited similar locomotor features, such as total distance moved and velocity, and spent a comparable amount of time investigating the objects (Fig. S1*B*). In these paradigms, control mice showed a significant preference for the novel over the familiar object or location, whereas RFS-treated mice performed no better than chance (Fig. 1*A*). These data indicate that the RFS stress-induction paradigm led to a deficit in hippocampus-dependent learning and memory tasks, which is consistent with previous observations suggesting that inescapable, uncontrollable repeated stress leads to memory impairment (2, 8, 10).

To gain insight into the molecular mechanisms mediating the deleterious effect of repeated stress on hippocampal function, we examined the generation of p25 after RFS. We did so because p25 is known to be generated by neuronal activity (29, 30), and its sustained expression is known to be detrimental to learning and memory (16). We analyzed the hippocampi of RFS-treated mice and found increased p25 levels compared with control animals (Fig. 1*B*).

We next tested whether the increase in p25 generation following repeated stress was accompanied by an up-regulation of HDAC2 and consequent decreases in memory-related genes (18, 19). These events are associated with p25 overexpression, decreases in learning and memory genes, and memory deficits in neurotoxic conditions (18). We concentrated on the dorsal hippocampus and its CA1 subregion, because the dorsal hippocampus is involved in memory and spatial processing (31), and its CA1 subregion is the output structure of the hippocampus and is considered essential for novelty detection (32). We found that RFS-treated animals exhibited increased HDAC2 immunoreactivity in the hippocampus. Furthermore, changes in HDAC2 were accompanied by decreased expression of known HDAC2-regulated genes (19) in dorsal hippocampal CA1, including Synaptophysin (SYP, Fig. 1*C*), a presynaptic marker of functional synapses, and Synapsin II (Syn. II) and Homer (Fig. S1*C*, *Left* and *Right*, respectively), pre- and post-synaptic markers of functional synapses, respectively. This stress paradigm was also shown to decrease mRNA levels of other HDAC2-regulated genes (Fig. S1*D*). A similar decrease in Synaptophysin and an increase in HDAC2 were observed in dorsal hippocampal CA3 as well (Fig. S1 *E* and *F*). This effect was confirmed using a restraint stress paradigm (33). After 8 d of unpredictable daily restraint, generation of p25, HDAC2 up-regulation, and down-regulation of Synaptophysin were also evident in hippocampal lysates from restrained animals compared with controls (Fig. S1*G*). In contrast, RFS treatment did not alter p25 generation or HDAC2 and Synaptophysin expression in the BLA compared with controls (Fig. S1 *H* and *I*). This suggests that the observed changes are brain region-specific.

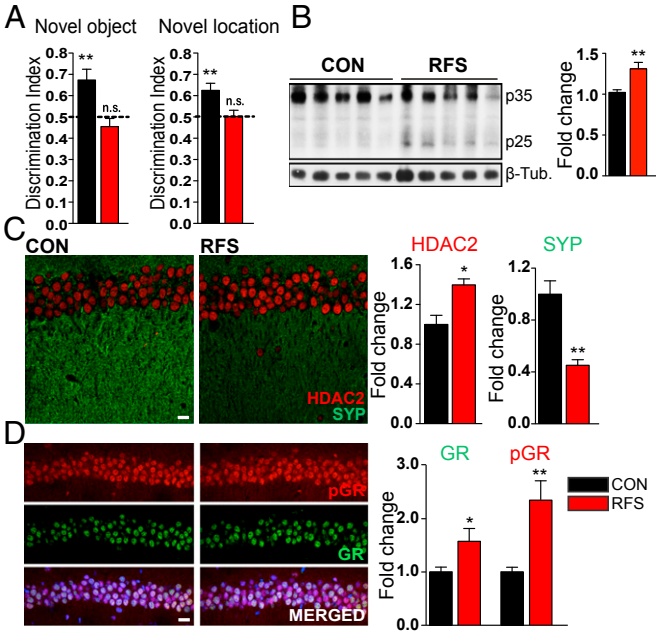

Fig. 1. Repeated stress induces hippocampal molecular changes and learning and memory deficits. (*A*) Effect of RFS treatment on learning and memory abilities in the novel object recognition (n = 10 and 16) and novel location recognition tasks (n = 10 per group; one-tailed t test). (*B*) Western blot images and quantification of the effect of repeated stress on p25 generation in the hippocampus (n = 5 per group; unpaired t test). (*C* and *D*) Representative immunohistochemical images and quantitative analysis of the effect of stress on HDAC2 and Synaptophysin (*C*) and GR and pGR (*D*) expression levels in the dorsal hippocampal CA1 subregion (n = 4 per group; unpaired t test) (Synaptophysin and GR in green; HDAC2 and pGR in red; DAPI in blue). Values are mean ± SEM. n.s., nonsignificant; $P > 0.05$; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. (Scale bars: 20 μm.)

We next examined the expression levels of GR, which has a low affinity for corticosterone and thus is typically activated only after stress (4). GR can be activated via Cdk5-dependent phosphorylation on serine 211 (17) after behavioral stress (34) or neurotoxic stress (18). Samples from the dorsal CA1 of RFS-treated animals exhibited an up-regulation in both protein expression and phosphorylation of GR on Ser211 [phospho-GR (pGR)] compared with controls (Fig. 1*D*). This indicates an increase in the total amount of activated GR in dorsal CA1 neurons. After p25 generation, the Cdk5-mediated phosphorylation of GR has been shown to lead to the up-regulation of HDAC2 and a subsequent down-regulation of genes associated with learning and memory (18).

Our findings demonstrate that RFS treatment can serve as a model of stress induction with reliable effects on hippocampal function that can be measured using behavioral tests as well as molecular indicators. Moreover, we describe the activation of an RFS molecular pathway consisting of p25 generation; increased GR, pGR, and HDAC2 expression; and decreases in HDAC2-regulated learning and memory genes in the hippocampus.

Pharmacosynthetic Inhibition of Glutamatergic Cells in the BLA Blocks the Detrimental Effect of Stress on the Hippocampus. We next sought to map the upstream regulators implicated in the stress-induced impairment of hippocampal function. We examined whether BLA activation is necessary for the effects of stress on the hippocampus. We sought to chronically decrease the activity of excitatory BLA neurons during RFS treatment using DREADD (designer receptor exclusively activated by a designer drug) technology (35). The inhibitory DREADD was transduced in glutamatergic cells of the BLA using an adeno-associated virus serotype 5 (AAV_5) expressing the mutated G_i-coupled receptor Gi-DREADD under control of the CaMKIIα promoter (AAV_5-CaMKIIa-HM4Di-IRES-mCitrine). Control animals received the same virus expressing enhanced yellow fluorescent protein (eYFP) only (AAV_5-CaMKIIa-eYFP) (Fig. 2*A*). The experimental procedure is depicted in Fig. S2*A*. The efficacy of the Gi-DREADD–mediated inhibition of BLA neurons was verified by quantifying the effect of clozapine-*N*-oxide (CNO) treatment upon c-fos expression in the transduced BLA cells, freezing levels following fear conditioning, and by ex vivo slice recording (Fig. S2 *B–F* and Table S1).

We then investigated the effect of BLA inhibition on hippocampal learning and memory following repeated stress. Importantly, we observed that BLA inactivation during RFS rescued the effects of stress on both cognitive function and molecular pathology in the hippocampus. Indeed, expression levels of p25, HDAC2, Synaptophysin, GR, and pGR were normalized in hippocampi of Gi-RFS mice, as demonstrated by Western blot and immunohistochemistry analyses (Fig. 2 *B–D*). The performance of Gi-RFS mice in novel object recognition and novel location recognition tasks was also indistinguishable from that of unstressed control mice (Fig. 2*E*). These results suggest that the activity of glutamatergic neurons in the BLA is necessary for stress-induced hippocampal dysfunction and associated cognitive deficits.

Chronic BLA Cell Body Photostimulation Reproduces the Effect of Repeated Stress on Learning and Memory. Next, to test whether BLA activation per se is sufficient to induce hippocampus-related deficits in the absence of RFS, we expressed a channel rhodopsin-2 (ChR2)-eYFP fusion protein or eYFP alone in BLA pyramidal neurons (Fig. 3*A* and Fig. S3*A*, *Left*), combined with implantation of a bilateral optical fiber over the BLA (Fig. 3*A*). eYFP expression allowed us to identify the transduced BLA efferents at their destination in the dorsal hippocampus. Most of the labeled afferents appeared to terminate in area CA3, with a smaller proportion in CA1 (Fig. S3*A*). The dentate gyrus was essentially devoid of any BLA efferents (not shown). We used an 8-d photostimulation protocol to mimic the RFS procedure (Fig. S3*B*). This protocol was adapted from a previous study showing that pairing the optogenetic activation of glutamatergic BLA cells with a tone led to an association with fear learning (36). Restricted expression in CamKII-positive cells and photostimulation was verified by examining eYFP coexpression, c-fos expression, and action potential firing in the BLA (Fig. S3 *C–F*), and the site of implantation above the BLA was verified (Fig. S3*G*).

After repeated glutamatergic BLA cell body photostimulation, we found increased p25 generation in the hippocampus (Fig. 3*B*), increased HDAC2 expression, down-regulation of Synaptophysin expression (Fig. 3*C*), and increased GR and pGR expression (Fig. 3*D*) in the dorsal hippocampal CA1 subregion of ChR2 mice compared with eYFP controls. Importantly, BLA activation also impaired hippocampus-dependent memory formation, as measured by novel object and novel location recognition tasks (Fig. 3*E*). These results show that selective chronic activation of BLA glutamatergic cell bodies is sufficient to reproduce molecular and behavioral effects previously associated with the RFS treatment.

Chronic Photostimulation of BLA Axon Terminals in the Dorsal Hippocampus Reproduces the Effect of Stress on Learning and Memory. To determine whether BLA projections to the hippocampus directly mediate the effects of BLA activation on cognitive performance, we conducted photostimulation of BLA axon terminals within the hippocampus and asked whether this could recapitulate the effects of RFS. Glutamatergic neurons of the BLA were transduced with AAV_5-CaMKIIα-ChR2-eYFP or eYFP only. Unlike before, optical fibers were implanted above either the dorsal area or ventral area CA3 (Fig. S4*A*). These regions receive an extensive network of projections from the BLA (Fig. S4*B*) and, in the case of the dorsal hippocampus, are likely to activate BLA fibers en route to the dorsal CA1 (5, 6). BLA terminals were stimulated in the hippocampus using a protocol aimed at mimicking the RFS procedure (Fig. S4*C*).

We tested the efficiency of this procedure by measuring circulating corticosterone levels after stimulation, freezing levels during light stimulation, and c-fos expression at the site of stimulation (Fig. S5 *A–C*). Only photostimulation of the dorsal hippocampus led to an increase in the number of c-fos–positive cells in the dorsal hippocampus of ChR2 mice compared with

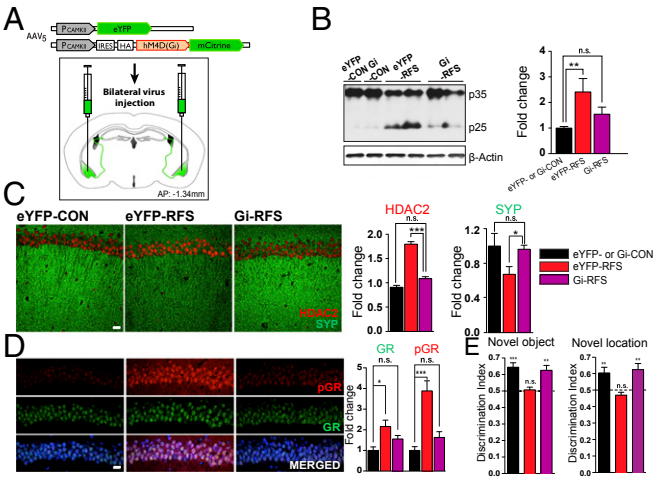

Fig. 2. BLA function is necessary for repeated stress-induced hippocampal molecular changes and learning and memory deficits. (*A*) Schematic of the AAV_5-CaMKIIa-eYFP control and AAV_5-CaMKIIa-HM4Di-IRES-mCitrine constructs and mode of virus administration. (*B–D*) Effect of DREADD-induced BLA inhibition during repeated stress on p25 generation (*B*) and of expression levels of HDAC2 and Synaptophysin (*C*) and GR and pGR (*D*) in the dorsal hippocampal CA1 subregion ($n = 4$, 5, and 6 per group, one-way ANOVA with Tukey's post hoc analysis) (pGR and HDAC2 in red; GR and Synaptophysin in green; DAPI in blue). (*E*) Effect of DREADD-induced BLA inhibition on the performance of RFS-treated mice in novel object recognition and novel location recognition tasks ($n = 10$, 10, and 7). Values are mean ± SEM. n.s., nonsignificant; $P > 0.05$; $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$, one-tailed *t* test. (Scale bars: 20 μm.)

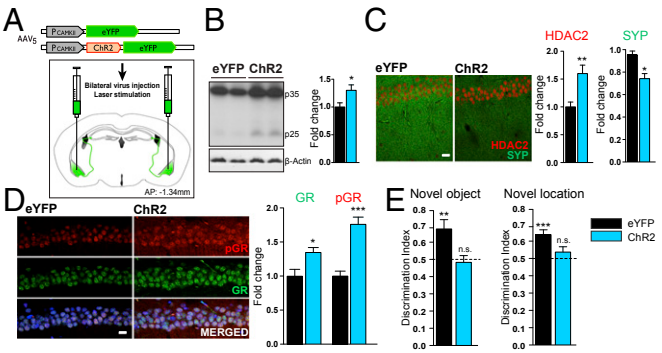

Fig. 3. BLA activation is sufficient to reproduce repeated stress-induced hippocampal molecular changes and learning and memory deficits. (*A*) Schematic of the AAV_5-CaMKIIa-eYFP and AAV_5-CaMKIIa-ChR2-eYFP constructs, mode of virus administration, and fiber optic placement. (*B*) Effect of BLA photostimulation on p25 generation in the hippocampus ($n = 5$ per group; unpaired *t* test). (*C* and *D*) Representative immunohistochemical images and quantitative analysis of HDAC2 and Synaptophysin (*C*) and GR and pGR (*D*) expression levels in the dorsal hippocampal CA1 subregion from eYFP and ChR2 mice ($n = 5$ per group; unpaired *t* test) (HDAC2 and pGR in red; Synaptophysin and GR in green; DAPI in blue). (*E*) Effect of optogenetic BLA activation on performance in novel object recognition and novel location recognition behavioral tasks ($n = 10$ CON mice and 7 ChR2 mice; one-tailed *t* test). Values are mean ± SEM. n.s., nonsignificant; $P > 0.05$; $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$. (Scale bars: 20 μm.)

control mice (Fig. S5*C*), consistent with the notion that the two hippocampal domains act independently of each other (31). Photostimulation of the BLA inputs to the dorsal hippocampus (Fig. 4*A*), but not the ventral hippocampus (Fig. S5*D*), increased p25 generation in ChR2-expressing mice compared with eYFP controls. Dorsal, but not ventral, photostimulation of BLA terminals was also associated with increased immunoreactivity for HDAC2, reduced expression of Synaptophysin, and increased GR and pGR expression in the dorsal CA1 of ChR2 mice compared to eYFP controls (Fig. 4 *B* and *C* and Fig. S5 *E* and *F*). In the case of the dorsally stimulated mice, this was accompanied by an impaired performance in both the novel object and novel location recognition tasks (Fig. 4*D*). In contrast, illumination of the ventral hippocampus in ChR2-transduced animals impaired performance in the novel object recognition task only (Fig. S5*G*). These results suggest that selective activation of glutamatergic projections from the BLA primarily to the dorsal, but not the ventral, hippocampus reproduced the effects of repeated stress on the hippocampal CA1 subregion and behavioral measures of learning and memory.

Generation of p25 Is Necessary for the Detrimental Effect of Stress on Learning and Memory. We directly examined the role of p25 generation in stress-induced learning and memory dysfunction by taking advantage of a novel mouse model, the Δp35 knock-in (KI) mouse (Δp35KI mice), in which p25 generation is abolished (30) (Fig. S6*A*). Δp35KI and WT littermate mice were subjected to RFS, as before. We found that RFS treatment did not result in p25 generation in the Δp35KI hippocampus (Fig. S6*B*). Nor did RFS affect Synaptophysin (Fig. 5*A*), GR, or pGR expression levels in these mice (Fig. 5*B*). Additionally, HDAC2 up-regulation following stress was considerably reduced in the p35KI mice compared to control littermates (Fig. 5*A*). Remarkably, these mice also appeared to be resilient to RFS-induced impairments in hippocampus-dependent memory formation, with the performance of RFS-treated Δp35KI mice indistinguishable from that of unstressed controls (Fig. 5*C*). These in vivo experiments suggest that production of p25 in the hippocampus is necessary for the behavioral and molecular phenotypes that manifest in the hippocampus following repeated stress.

Discussion

The data presented here demonstrate that repeated stress activates a molecular pathway in the hippocampus consisting of p25 generation, GR up-regulation and phosphorylation, and HDAC2 up-regulation. These phenotypes are accompanied by the down-regulation of memory-related markers in the hippocampus and impairments of learning and memory. We found that this pathway is activated by direct glutamatergic projections from the BLA to the dorsal hippocampus, and that these phenotypes are rescued in the absence of p25 generation. This work details the mechanisms of how repeated stress impacts hippocampus-associated learning and memory at the neural circuit and molecular levels (see the proposed model in Fig. S7).

Modulation of Hippocampal Function by the BLA and the Importance of the Direct BLA to Dorsal Hippocampus Connections. Previous studies have concluded that BLA stimulation leads to LTP deficits in hippocampal CA1, and that the BLA is necessary for the detrimental effects of chronic stress on spatial memory (8, 10,

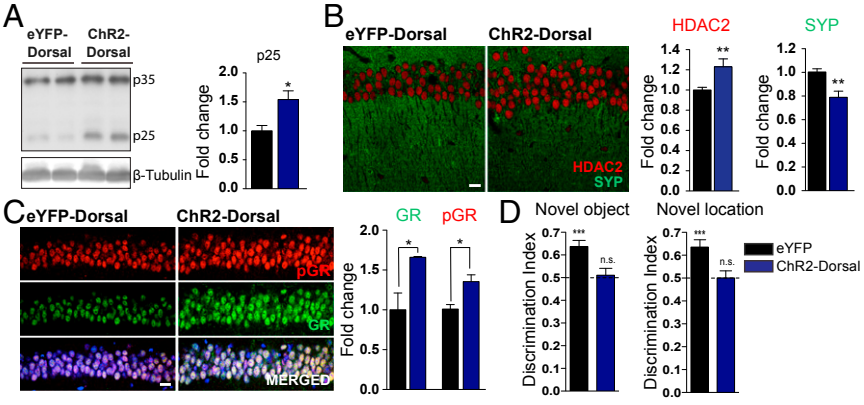

Fig. 4. Activation of direct projections from the BLA to the dorsal hippocampus is sufficient to reproduce repeated stress-induced hippocampal molecular changes and learning and memory deficits. (*A*) Effect of BLA terminal photostimulation on p25 expression level, measured by Western blot analysis from hippocampal lysates (unpaired *t* test). (*B* and *C*) Representative immunohistochemical images and quantitative analysis of HDAC2 and Synaptophysin (*B*) and GR and pGR (*C*) expression levels in dorsal CA1 after BLA terminal photostimulation in the dorsal hippocampus ($n = 5$ per group; unpaired *t* test for HDAC2 and Synaptophysin, one-way ANOVA with Tukey's post hoc analysis for GR/pGR) (pGR and HDAC2 in red; GR and Synaptophysin in green; DAPI in blue). (*D*) Effect of dorsal BLA terminal photostimulation on novel object (NO) and novel location (NL) recognition behavioral tasks (one-tailed *t* test). $n = 16$ for eYFP and 10 for ChR2. Values are mean ± SEM. n.s., nonsignificant; $P > 0.05$; $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$. (Scale bars: 20 μm.)

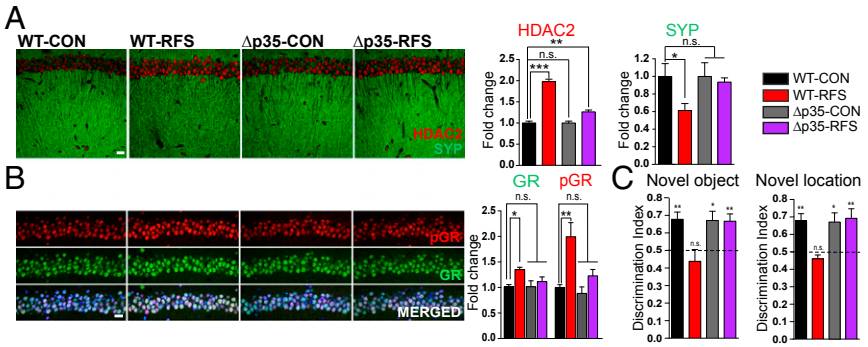

Fig. 5. p25 generation is necessary for repeated stress-induced hippocampal molecular changes and learning and memory deficits. (*A* and *B*) Absence of RFS-induced changes in HDAC2 and Synaptophysin (*A*) and GR and pGR (*B*) expression levels in the hippocampal CA1 subregion in the Δp35KI hippocampus ($n = 4$ per group; one-way ANOVA Tukey's post hoc analysis) (HDAC2 and pGR in red; Synaptophysin and GR in green; DAPI in blue). (*C*) Absence of repeated stress-induced learning and memory deficits in the Δp35KI mouse. ($n = 8$ for WT CON and RFS; $n = 10$ and 11 for Δp35 CON and RFS, respectively; one-sample one-tailed t test). Values are mean ± SEM. n.s., nonsignificant; $P > 0.05$; $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. (Scale bars: 20 μm.)

13). In the present study, we used genetic and pharmacosynthetic methods to examine the function of the BLA in a paradigm reminiscent of repeated stress and its impact on cognition, with a degree of cell- and circuit-specific modulation not attainable by previous electric stimulation and pharmacologic or physical cell inactivation paradigms (8, 13, 37). Here we describe the pivotal role of glutamatergic cell activity in the BLA to modulate the stress-related hippocampal phenotypes. We show that their activation is necessary for the detrimental effect of repeated stress on hippocampal-associated learning and memory, and that repeated optogenetic activation of those cells (in the absence of a stressor) is sufficient to reproduce the effects of repeated stress on the hippocampus. Taking advantage of the anterograde transport of ChR2 along the axons of BLA neurons to the hippocampus, we were able to show via terminal photostimulation that the effects of BLA activation on the hippocampus are mediated directly, as opposed to being reliant on an intermediate structure or circulating hormones. Consistent with this, we found that BLA terminal stimulation in the dorsal hippocampus and ventral hippocampus led to an increase in circulating levels of corticosterone, whereas only the former recapitulated the effect of stress on the hippocampus. This is consistent with the notion that changes in hippocampal function affect glucocorticoid secretion (15). Furthermore, the fact that ventral stimulation induced an increase in corticosterone without fully affecting hippocampal function confirms earlier work showing that the increase in circulating corticosterone levels alone is insufficient to induce hippocampal dysfunction in the absence of a functional amygdala (9, 22). We now show that this requirement is due to the necessary BLA input onto the dorsal hippocampus. The question of whether or not corticosterone is a necessary or permissive factor in the impact of this circuit on hippocampal function remains to be formally addressed.

Subregion-Specific Effects of BLA Inputs into the Hippocampus. We were surprised to find that the specific activation of BLA inputs into the dorsal, but not the ventral, hippocampus recapitulated the effect of repeated stress on hippocampal function. Functional differences between hippocampal subdomains have previously been suggested by the finding of a higher density of place cells in the dorsal hippocampus compared with the ventral hippocampus, which provides finer spatial tuning (31). In addition, the dorsal CA1 area is thought to be essential for novelty detection (32, 38). This could explain why the novel location recognition task was unaffected in the ventrally stimulated animals. These two hippocampal regions also exhibit a number of differences in their connectivity patterns; for example, dorsal CA1 has extensive reciprocal connections to association cortices, whereas ventral CA1 has a greater degree of connectivity to subcortical areas, such as the hypothalamus and amygdala (39). Accordingly, lesions of the dorsal hippocampus impair memory and spatial processing, whereas lesions of the ventral hippocampus impair emotional, social, and endocrine regulation (31). Patterns of corticosteroid receptor expression also differ between these regions, with a higher level of GR expression in the dorsal CA1 (4). Perhaps a higher GR tone in this region can amplify input from the BLA, resulting in greater sensitivity to glutamate and p25 generation compared with that in the ventral region.

p25 Generation Is Necessary for the Negative Effects of Repeated Stress on the Hippocampus and Is Modulated at the Neural Circuit Level. We have shown that the activity of specific BLA inputs leads to p25 generation and increased HDAC2 expression in the dorsal hippocampus and that p25 generation is necessary to induce hippocampus-dependent learning and memory deficits following repeated stress. The pathway from p25 generation to HDAC2-associated decreases in learning and memory genes, which lead to cognitive impairment, has been demonstrated previously (18, 19). The present study shows that p25 generation links the activation of a specific neural circuit following stress with epigenetic changes associated with learning and memory impairment.

Tracing studies have uncovered a highly conserved amygdalo-hippocampal circuitry in rodents and nonhuman primates that likely is similar in humans (5, 40). This conservation raises the potential that specific therapies aimed at restraining the activity of the BLA to inhibit p25 generation, or to reduce the associated Cdk5 overactivation, may effectively alleviate cognitive symptoms in the host of neurologic, psychiatric, and systemic diseases for which stress is emerging as both a causative and exacerbating factor.

Experimental Procedures

All mouse work was approved by the Committee for Animal Care of the Division of Comparative Medicine at Massachusetts Institute of Technology. RFS procedure consisted of submitting the mice to the delivery of 10 foot shocks at random intervals during an hour, daily for 8 d. DREADD-BLA inhibition was induced during the RFS paradigm. Photostimulation of the BLA or its fibers in the hippocampus was made at 20 Hz for 2 or 20 s, respectively, and repeated 10 times daily for 8 d. Detailed information on materials and methods, including information on animals and the RFS paradigm, behavioral assays, Western blot analysis, immunohistochemistry, qRT-PCR, Gi-DREADD, stereotaxic ChR2 injection and optical fiber placement, optogenetic stimulation, corticosterone assays, generation of the Δp35KI mice, and statistical analysis, is provided in *SI Experimental Procedures*.

ACKNOWLEDGMENTS. We thank Alexander Nott, Adam Bero, Susan Su, and Kay Tye for their careful reading of the manuscript. This study was supported by National Institutes of Health Grants AG047661 and NS051874, and funding from the JPB Foundation (to L.-H.T.). X.M. was a 2012–2013 Howard Hughes Medical Institute Medical Research Fellow. J.G. was supported by a Bard Richmond Fellowship and a Swiss National Science Foundation grant for prospective

researchers. S.S. was supported by a Human Frontier Science Program long-term postdoctoral fellowship. K.D. is an investigator at the Howard Hughes Medical Institute and the National Institutes of Health. L.-H.T. is an investigator at the Picower Institute for Learning and Memory.

1. Aznar S, Knudsen GM (2011) Depression and Alzheimer's disease: Is stress the initiating factor in a common neuropathological cascade? *J Alzheimers Dis* 23(2): 177–193.
2. Sandi C, Pinelo-Nava MT (2007) Stress and memory: Behavioral effects and neurobiological mechanisms. *Neural plasticity* 2007:78970.
3. Lupien SJ, McEwen BS, Gunnar MR, Heim C (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci* 10(6):434–445.
4. Reul JM, de Kloet ER (1985) Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. *Endocrinology* 117(6):2505–2511.
5. Pikkarainen M, Ronkko S, Savander V, Insausti R, Pitkanen A (1999) Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. *J Comp Neurol* 403(2):229–260.
6. Pitkanen A, Pikkarainen M, Nurminen N, Ylinen A (2000) Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Ann N Y Acad Sci* 911:369–391.
7. Felix-Ortiz AC, et al. (2013) BLA to vHPC inputs modulate anxiety-related behaviors. *Neuron* 79(4):658–664.
8. Kim JJ, Koo JW, Lee HJ, Han JS (2005) Amygdalar inactivation blocks stress-induced impairments in hippocampal long-term potentiation and spatial memory. *J Neurosci* 25(6):1532–1539.
9. Roozendaal B, McEwen BS, Chattarji S (2009) Stress, memory and the amygdala. *Nat Rev Neurosci* 10(6):423–433.
10. Kim JJ, Lee HJ, Han JS, Packard MG (2001) Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. *J Neurosci* 21(14):5222–5228.
11. Seidenbecher T, Laxmi TR, Stork O, Pape HC (2003) Amygdalar and hippocampal theta rhythm synchronization during fear memory retrieval. *Science* 301(5634):846–850.
12. Lesting J, et al. (2011) Patterns of coupled theta activity in amygdala-hippocampal-prefrontal cortical circuits during fear extinction. *PLoS One* 6(6):e21714.
13. Vouimba RM, Richter-Levin G (2005) Physiological dissociation in hippocampal subregions in response to amygdala stimulation. *Cereb Cortex* 15(11):1815–1821.
14. Fernando AB, Murray JE, Milton AL (2013) The amygdala: Securing pleasure and avoiding pain. *Front Behav Neurosci* 7:190.
15. Herman JP, Ostrander MM, Mueller NK, Figueiredo H (2005) Limbic system mechanisms of stress regulation: Hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry* 29(8):1201–1213.
16. Su SC, Tsai LH (2011) Cyclin-dependent kinases in brain development and disease. *Annu Rev Cell Dev Biol* 27:465–491.
17. Kino T, et al. (2007) Cyclin-dependent kinase 5 differentially regulates the transcriptional activity of the glucocorticoid receptor through phosphorylation: Clinical implications for the nervous system response to glucocorticoids and stress. *Mol Endocrinol* 21(7):1552–1568.
18. Gräff J, et al. (2012) An epigenetic blockade of cognitive functions in the neurodegenerating brain. *Nature* 483(7388):222–226.
19. Guan JS, et al. (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature* 459(7243):55–60.
20. Kamal A, Ramakers GM, Altinbilek B, Kas MJ (2014) Social isolation stress reduces hippocampal long-term potentiation: Effect of animal strain and involvement of glucocorticoid receptors. *Neuroscience* 256:262–270.
21. Kim JJ, Diamond DM (2002) The stressed hippocampus, synaptic plasticity and lost memories. *Nat Rev Neurosci* 3(6):453–462.
22. Krugers HJ, Goltstein PM, van der Linden S, Joels M (2006) Blockade of glucocorticoid receptors rapidly restores hippocampal CA1 synaptic plasticity after exposure to chronic stress. *Eur J Neurosci* 23(11):3051–3055.
23. Uchida S, et al. (2011) Epigenetic status of Gdnf in the ventral striatum determines susceptibility and adaptation to daily stressful events. *Neuron* 69(2):359–372.
24. Grahn RE, Watkins LR, Maier SF (2000) Impaired escape performance and enhanced conditioned fear in rats following exposure to an uncontrollable stressor are mediated by glutamate and nitric oxide in the dorsal raphe nucleus. *Behav Brain Res* 112(1-2):33–41.
25. Adamec R, Walling S, Burton P (2004) Long-lasting, selective, anxiogenic effects of feline predator stress in mice. *Physiol Behav* 83(3):401–410.
26. Manns JR, Eichenbaum H (2009) A cognitive map for object memory in the hippocampus. *Learn Mem* 16(10):616–624.
27. Broadbent NJ, Gaskin S, Squire LR, Clark RE (2010) Object recognition memory and the rodent hippocampus. *Learn Mem* 17(1):5–11.
28. Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H (2002) Hippocampal damage and exploratory preferences in rats: Memory for objects, places, and contexts. *Learn Mem* 9(2):49–57.
29. Engmann O, et al. (2011) Cyclin-dependent kinase 5 activator p25 is generated during memory formation and is reduced at an early stage in Alzheimer's disease. *Biol Psychiatry* 70(2):159–168.
30. Seo J, et al. (2014) Activity-dependent p25 generation regulates synaptic plasticity and Aβ-induced cognitive impairment. *Cell* 157(2):486–498.
31. Fanselow MS, Dong HW (2010) Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* 65(1):7–19.
32. Lisman JE, Otmakhova NA (2001) Storage, recall, and novelty detection of sequences by the hippocampus: Elaborating on the SOCRATIC model to account for normal and aberrant effects of dopamine. *Hippocampus* 11(5):551–568.
33. Buynitsky T, Mostofsky DI (2009) Restraint stress in biobehavioral research: Recent developments. *Neurosci Biobehav Rev* 33(7):1089–1098.
34. Adzic M, et al. (2009) Acute or chronic stress induce cell compartment-specific phosphorylation of glucocorticoid receptor and alter its transcriptional activity in Wistar rat brain. *J Endocrinol* 202(1):87–97.
35. Sternson SM, Roth BL (2014) Chemogenetic tools to interrogate brain functions. *Annu Rev Neurosci* 37:387–407.
36. Johansen JP, et al. (2010) Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. *Proc Natl Acad Sci USA* 107(28):12692–12697.
37. Kim EJ, Kim ES, Park M, Cho J, Kim JJ (2012) Amygdalar stimulation produces alterations on firing properties of hippocampal place cells. *J Neurosci* 32(33):11424–11434.
38. Blum KI, Abbott LF (1996) A model of spatial map formation in the hippocampus of the rat. *Neural Comput* 8(1):85–93.
39. Naber PA, Witter MP (1998) Subicular efferents are organized mostly as parallel projections: a double-labeling, retrograde-tracing study in the rat. *J Comp Neurol* 393(3):284–297.
40. Aggleton JP (1986) A description of the amygdalo-hippocampal interconnections in the macaque monkey. *Exp Brain Res* 64(3):515–526.