Brief Communications

Genetic Deletion of *gadd45b*, a Regulator of Active DNA Demethylation, Enhances Long-Term Memory and Synaptic Plasticity

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Dynamic epigenetic mechanisms including histone and DNA modifications regulate animal behavior and memory. While numerous enzymes regulating these mechanisms have been linked to memory formation, the regulation of active DNA demethylation (i.e., cytosine-5 demethylation) has only recently been investigated. New discoveries aim toward the Growth arrest and DNA damage-inducible 45 (Gadd45) family, particularly Gadd45b, in activity-dependent demethylation in the adult CNS. This study found memory-associated expression of *gadd45b* in the hippocampus and characterized the behavioral phenotype of *gadd45b*^{−/−} mice. Results indicate normal baseline behaviors and initial learning but enhanced persisting memory in mutants in tasks of motor performance, aversive conditioning and spatial navigation. Furthermore, we showed facilitation of hippocampal long-term potentiation in mutants. These results implicate Gadd45b as a learning-induced gene and a regulator of memory formation and are consistent with its potential role in active DNA demethylation in memory.

Introduction

Alterations in neuronal gene expression play a necessary role in memory consolidation (Miyashita et al., 2008). Although studies have identified a host of memory-related genes, our understanding of upstream pretranscriptional regulatory processes is incomplete. A growing body of evidence implicates molecular epigenetic mechanisms, which regulate transcription without modifying gene sequences, in memory formation and associated gene transcription (Sultan and Day, 2011). While initial studies focused on histone covalent modifications, a smaller but burgeoning literature similarly uncovered the role of active DNA methylation dynamics in adult memory and synaptic plasticity (Feng et al., 2010). Although *de novo* hypermethylation has been well characterized, the mechanism of active DNA demethylation, that occurring in the absence of cell division, remains elusive (Wu and Zhang, 2010).

Studies implicate the Growth arrest and DNA damage-inducible 45 (Gadd45) family in active demethylation (Barreto et al., 2007; Niehrs and Schäfer, 2012), and a landmark study showed Gadd45b mediates gene-specific demethylation in the dentate gyrus following seizure (Ma et al., 2009). These results

along with the finding of activity-induced *gadd45b* in the hippocampus led us to hypothesize that Gadd45b modulates memory and synaptic plasticity. We tested our hypothesis through molecular studies of experience-dependent *gadd45b* regulation and behavioral and physiological assessment of *gadd45b*^{−/−} mutants. We found selective enhancements in long-term memory and synaptic plasticity in mutants.

Materials and Methods

Animals and genotyping. Mice for behavioral studies were bred at the University of Alabama at Birmingham. Gadd45b knock-outs on a B6:129VJ background were generated as described previously (Gupta et al., 2005). Wild-type and mutant male offspring of heterozygotes between two and 6 months of age were used for behavior and physiology. Animals were singly housed at least 3 d before the start of experiments. Back-crossed mice were generated by breeding heterozygotes with C57BL/6 wild-types for at least six generations. For expression studies (Fig. 1A–D), adult male C57BL/6 mice (Harlan) 2–3 months of age were used. Animals were housed under a 12 h light/dark schedule and provided access to food and water *ad libitum*. All studies were performed in compliance with the University of Alabama Institutional Animal Care and Use Committee.

Behavioral tasks. Baseline tasks were modified from the work of Chwang et al. (2007). Morris water maze was done as described except that animals first underwent cued training, in which the platform was marked with a visible flag, with four trials per day for 2 d. For hidden training, animals were given four trials per day with a 30 min intertrial interval. Fear conditioning was adapted from Vecsey et al. (2007). For training, after 2 min of habituation, a tone (2.8 kHz, 80 dB) was played for 30 s and coterminated with a footshock (0.4 mA, 1 s for mild and 2 s for moderate training). The animal was removed after an additional 30 s. For robust training, the animal received three such tone-shock pairings (0.75 mA, 2 s) every 2 min and was removed after a total of 7 min. For

contextual memory testing, the animal was reintroduced to the chamber for 10 min for extinction and 3 min for all other tests at the indicated time points after training. For cued testing, the animal was placed in a modified chamber for 3 min; the tone was played during the last 90 s. Percentage freezing was scored manually at 3 or 5 s intervals. For training in gene expression experiments (Fig. 1 *B–D*), mice received 3 footshocks (1 s, 0.5 mA) every 2 min and were removed from the chamber after 7 min. Statistics for all behavioral studies were performed with two-way ANOVA and unpaired Student's *t* test.

Fluorescence in situ *hybridization.* Male C57BL/6 mice were perfused and debrained 1 h after fear conditioning. Brains were fixed in Bouin's solution, washed with 70% ethanol, processed and embedded in paraffin. Slices (8 μm) were cut and deparaffinized. The fluorescence in situ hybridization (FISH) protocol was adapted from Ma et al. (2009). Hybridization was performed with digoxigenin (DIG)-labeled antisense Gadd45b probe (Exiqon), and anti-DIG Ab (Millipore Bioscience Research Reagents) was used for amplification. Fluorescent signal was obtained with Cy3 Plus (PerkinElmer).

Primary neuron culture. Hippocampi from hybrid-background P0-P2 brains were dissected, digested in papain, dissociated, and plated in Neurobasal media as described previously (Invitrogen) with minor modifications. Experiments were performed on 3 days *in vitro* cultures.

Tissue collection and gene expression. Area CA1 was collected as described previously (Miller and Sweatt, 2007). Other brain regions were dissected from frozen 1 mm brain slices cut on a brain matrix. RNA was extracted with AllPrep DNA/RNA Mini kit (Qiagen) and was used to synthesize cDNA with oligo-$(dT)_{18}$ primers; RevertAid First Strand cDNA Synthesis Kit (Fermentas). Quantitative reverse transcriptase PCR was performed with SYBR Green (Bio-Rad) and 300 nM primer. Primers were designed to span exon-exon junctions using PrimerQuest (Integrated DNA Technologies), and hypoxanthine-guanine phosphoribosyltransferase was used as an internal control. Data were analyzed by one-way ANOVA with Fischer's *post hoc.*

Electrophysiology. Electrophysiology on hybrid-background mice was done as described previously (Feng et al., 2010). Field EPSP slopes over various

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left in home cages (Naive). Expression analysis in CA1 was performed at 30 min, 1 h, or 24 h. *Gadd45b, gadd45g* and *Arc* expression was significantly regulated by experience. *Gadd45b* expression was significantly suppressed compared with the context group at 30 min only ($p < 0.05$). *D*, Expression of *gadd45b* and *gadd45g* but not *gadd45a* was enhanced in the amygdala 1 h after training ($p < 0.05$). *E*, WT and KO primary hippocampal neuron cultures depolarized with 50 mM KCl for 2 h showed enhanced *gadd45g* in both genotypes. *Gadd45b* was upregulated in WTs ($p < 0.01$) ($n = 4$–15/group).
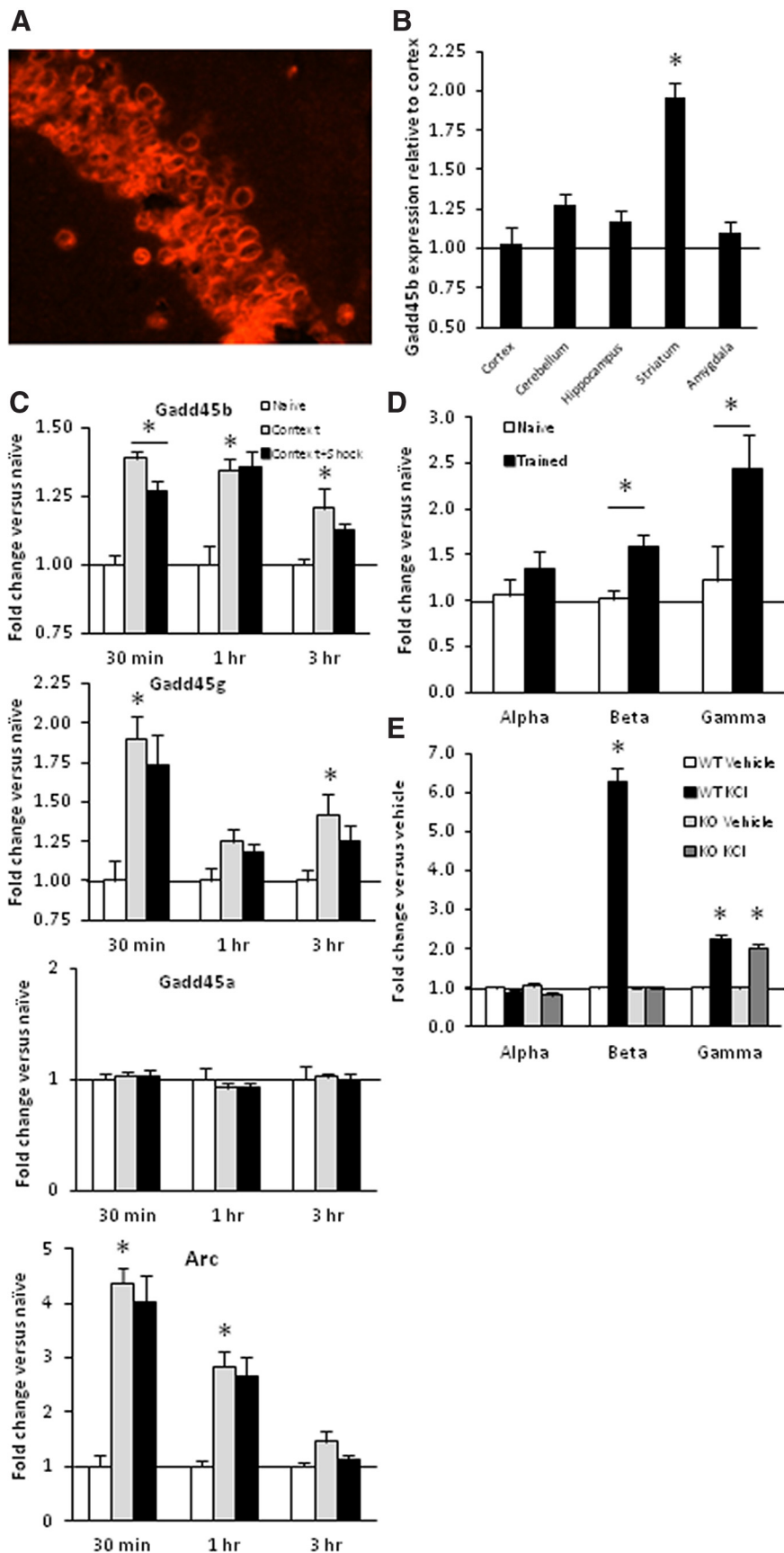
Figure 1. Memory-associated *gadd45b* transcription. *A*, FISH analysis of *gadd45b* expression in area CA1 in naive mouse brain. *B*, Quantitative PCR analysis of *gadd45b* reveals enrichment in striatum ($p < 0.01$). *C*, Mice were trained (3 footshock protocol described in Materials and Methods) in contextual fear conditioning (Context + Shock), exposed to the chamber only (Context) or
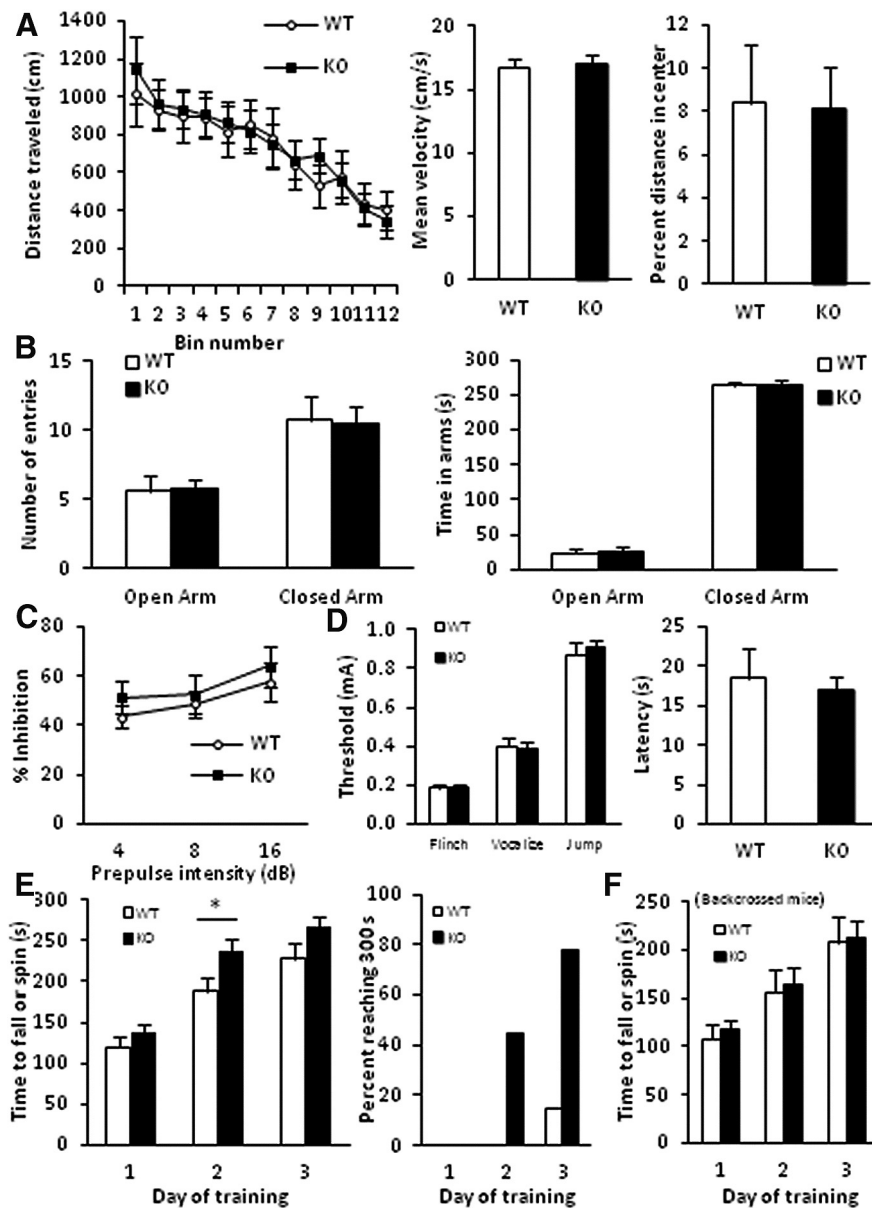
Figure 2. *Gadd45b* $^{-/-}$ mutants exhibit normal baseline behavior and elevated motor learning. **A**, Open field. No significant differences were found in horizontal activity (left), velocity (middle) or thigmotaxis (right). **B**, Elevated plus maze. No significant effect of genotype was found on open or closed arm entries (left) or total time spent in open and closed arms (right). **C**, Prepulse inhibition. No effect of genotype was found at 4, 8 or 16 dB prepulse intensities. **D** (Left) Shock threshold test. Similar thresholds to flinch, vocalize and jump were found between genotypes. (Right) Hot plate test. No significant difference in latency to lift or lick the animal's hindpaw was found. **E**, Accelerating rotarod. Mean latency of four trails per day over 3 d (left) and percentage of animals per genotype reaching 300 s maximum (right) are reported. Knock-outs showed significantly elevated performance on day 2 ($p < 0.05$). **F**, Accelerating rotarod on backcrossed mice. No significant ($p > 0.05$) effect of genotype was found ($n = 7$–15/group).

stimulus intensities (1–30 mV) were used to assess baseline synaptic transmission. Subsequent stimuli were set to an intensity that evoked a field EPSP (fEPSP) that had a slope of 50% or 25% of the maximum fEPSP slope. Paired-pulse facilitation (PPF) was measured at various interstimulus intervals (10–300 ms). Long-term potentiation (LTP) was induced by administering one 100 Hz tetanus (0.1–1 s). Synaptic efficacy was assessed for 3 h following stimulation by recording fEP-SPs every 20 s (traces were averaged for every 2 min interval). Analysis was performed with two-way ANOVA and unpaired Student's *t* test.

Results

Regulation of gadd45b during memory consolidation
To assess *gadd45b* transcription in the brain, we first found broad expression of *gadd45b* in area CA1 (Fig. 1*A*) and enrichment in the

striatum (Fig. 1*B*). Seizure activity upregulates expression in the pyramidal and granule cell layers of the hippocampus (Ma et al., 2009), and DNA methyltransferase (DNMT) transcripts are regulated in the hippocampus during memory consolidation (Levenson et al., 2006; Miller and Sweatt, 2007). To determine whether transcription of the *gadd45* genes is similarly modulated after fear conditioning, we performed contextual fear conditioning and measured transcripts in area CA1 30 min, 1 and 3 h after training. Transcription of *gadd45b* was significantly upregulated by both context exposure learning and associative fear training (Fig. 1*C*). A small but significant reduction in expression was found in the trained group compared with the context group at 30 min. This effect had attenuated by 1 h. Similarly, *gadd45g* (Gadd45γ) expression was augmented in the context learning group, but its expression was not further affected by association training. No experience-associated regulation of *gadd45a* (Gadd45α) was found. Consistent with previous results, *arc* expression was regulated by activity and not selectively regulated by context-shock association (Huff et al., 2006). In addition, broadly enhanced *gadd45b* expression in the pyramidal cell layer of CA1 1 h after training was confirmed by FISH (data not shown).

Because the amygdala is necessary for fear memory, we assessed *gadd45* transcription in the amygdala 1 h after training and found selective enhancements in *gadd45b* and *gadd45g* (Fig. 1*D*). To assess whether *gadd45b* ablation affects *gadd45a* and *gadd45g*, we cultured hippocampal neurons from wild-type (WT) and knock-out (KO) mice. After depolarization, *gadd45b* was upregulated in WT neurons, and *gadd45g* was similarly enhanced in both genotypes (Fig. 1*E*). Similar baseline expression of *gadd45a* and *gadd45g* ($p > 0.05$) was also found in *gadd45b* KO and WT neurons (data not shown).

Normal baseline behaviors in *gadd45b* $^{-/-}$ mice
We performed tests to determine whether *gadd45b* ablation affects baseline behavior. In the open field task, hybrid-background animals were placed in a novel chamber for 1 h. We found no effect on total distance traveled, habituation or mean velocity (Fig. 2*A*). Additionally, a similar degree of thigmotaxis was found between genotypes. Mutants also exhibited a similar fraction of time in open and closed arms of the elevated plus maze and a similar number of crossings into open arms over a 5 min period (Fig. 2*B*). We next performed prepulse inhibition, a test of sensorimotor gating. WT and KO mice showed robust inhibition upon exposure to prepulses of 4, 8 and 16 dB, and no significant effect of genotype were found (Fig. 2*C*). Since fear conditioning,

an important memory task, depends on normal perception and response to a mild footshock, two tests of nociception were performed. In the shock threshold task, we found no differences between genotypes in thresholds to flinch, vocalize or jump (Fig. 2 *D*). Likewise, normal responses to a 55°C surface were found in the hotplate test (Fig. 2 *D*). Backcrossed animals also exhibited no effect of genotype on these tasks (data not shown). These results suggest normal nociception in *gadd45b*$^{-/-}$ mice.

We next assessed performance in the accelerating rotarod. Mice were trained over 3 d with four consecutive trials per day. Day 1 performance is indicative of a rapid phase of motor learning (Bureau et al., 2010). We found no significant differences in mean performance during this phase of learning (Fig. 2 *E,F*). This implies *gadd45b* ablation fails to affect short-term motor memory or baseline motor function and coordination. However, 24 h after training, *gadd45b*$^{-/-}$ mice on the hybrid but not C57BL/6 background demonstrated significantly enhanced performance versus wild-types (Fig. 2 *E,F*). Mutants on both backgrounds showed similar performance to wild-types on day 3. However, a ceiling effect may have masked augmented performance by hybrid-background knock-outs, as a larger fraction of these mice reached the 300 s (40 rpm) maximum on days 2 and 3 (Fig. 2 *E*). These results implicate *gadd45b* in motor memory consolidation but not initial acquisition.

Enhanced hippocampus-dependent memory in gadd45b$^{-/-}$ mice

Active DNA demethylation has been documented in the consolidation of hippocampus-dependent memory (Day and Sweatt, 2010). In light of the function of Gadd45b in this molecular process, we sought to determine the cognitive effects of its deletion. In cue-plus-context fear conditioning, the rodent learns in a single-trial training session to associate a novel context and auditory cue with a mild footshock. We trained WT and KO hybrid-background mice with mild, moderate and robust paradigms and reexposed them to the chamber 1 or 24 h later to assess short-term and long-term contextual fear memory. Significantly enhanced performance of KO compared with WT mice was found at 24 h with mild and moderate but not robust training (Fig. 3*A*). Normal acquisition and short-term memory were found with moderate training (Fig. 3*B*). Both genotypes also exhibited normal and similar extinction learning (Fig. 3*C*). Since dynamic modulation of DNA methylation has also been associated with remote memory (Miller et al., 2010), we assessed contextual fear memory 28 d after modified mild training and found significantly enhanced freezing in knock-outs (Fig. 3*D*). We next confirmed the enhanced memory consolidation phenotype in backcrossed knock-out mice (Fig. 3*E*). Similar performance between genotypes was found in short-term memory assessment.

Since contextual fear memory relies on intact functioning of the hippocampus and amygdala, we next tested memory for the cue-shock association, a hippocampus-independent process. We found no significant effect of genotype during cued testing in hybrid-background animals (Fig. 3*F*).

We next trained mice in the Morris water maze, a measure of spatial learning and memory. Animals were first trained in the cued version of the task, in which the submerged platform was demarcated by a visible flag. Although wild-type and mutant mice successfully learned the task within 1 d and showed robust performance on the second day, *gadd45b*$^{-/-}$ mice showed significantly enhanced latencies on the first 3 trials (Fig. 3*G*). During the hidden platform task, no significant differences were found in latencies to locate the escape, sug-

gesting normal acquisition of spatial memory. Performance on the first probe trial indicated that *gadd45b*$^{-/-}$ rodents crossed the platform location significantly more times than *gadd45b*$^{+/+}$ mice. After the last training session, a second probe trial was administered; no differences were found in platform crossings. Additionally, animals of each genotype showed similar ($p > 0.05$) swim speeds during training and probe trials (data not shown). Backcrossed mice demonstrated no effect of genotype on cued or hidden platform training (data not shown) but significantly enhanced platform crossings on the first but not second probe trial (Fig. 3*H*).

Enhanced hippocampal synaptic plasticity in gadd45b$^{-/-}$ mice

Lasting synaptic plasticity supports memory consolidation (Sweatt, 2010). To determine whether the memory-augmenting effect of *gadd45b* deletion correlated with enhanced synaptic plasticity, we measured activity-induced LTP *ex vivo* in area CA1 induced by stimuli of varying strength. A significant enhancement in late-phase LTP was found after stimulation with a near-threshold stimulus (Fig. 4*A*). No differences were found in LTP magnitude when slices were stimulated by 100 Hz pulses with normal intensities (Fig. 4*B–D*). Normal baseline synaptic transmission was found in input-output studies (Fig. 4*E*). Surprisingly, a significant effect of genotype was found in paired-pulse facilitation induced by low- but not normal-intensity stimulation (Fig. 4*F,G*). Knock-out slices exhibited small but significant deficits in PPF at interstimulus intervals of 250 and 300 ms.

Discussion

We verified that *gadd45b* is dynamically tuned during memory formation and that *gadd45b* deletion facilitates memory and synaptic plasticity. Expression data show *gadd45b* is widely expressed in the adult brain and undergoes rapid, activity-driven transcription in the hippocampus and amygdala (Fig. 1). *Gadd45b*$^{-/-}$ neurons do not show compensatory regulation of other *gadd45* isoforms and show normal induction of *gadd45g* by activity (Fig. 1 *E*). Additionally, *gadd45b* exhibits selective, memory-associated suppression during fear memory formation, suggesting it may act as a negative memory regulator (Fig. 1*C*). In contrast, *dnmt3a* and *dnmt3b* are enhanced by context-shock association in the same training paradigm, and inhibition of DNMT function impairs memory (Miller and Sweatt, 2007; Feng et al., 2010). These results are not surprising in light of the opposing function of these epigenetic regulators (although DNMTs have paradoxically also been implicated in DNA demethylation) (Métivier et al., 2008). Consistent with this interpretation, behavioral assessment of *gadd45b* mutants showed no effects on baseline function and enhanced consolidation of motor memory and hippocampus-dependent contextual memory (Figs. 2, 3). This memory augmentation was first observed 24 h after training and persisted for 28 d. Normal performance of mutants in a hippocampus-independent cued fear memory task suggests Gadd45b does not contribute to amygdala-dependent learning (Fig. 3*F*).

Our data demonstrate selective enhancement of late-phase LTP magnitude in knock-out hippocampi upon induction by near-threshold stimulus (Fig. 4*A–D*). These data are highly consistent with fear conditioning data which show the most robust phenotypes following modest fear conditioning training (Fig. 3*A–E*). Additionally, mutants performed better on the first Mor-
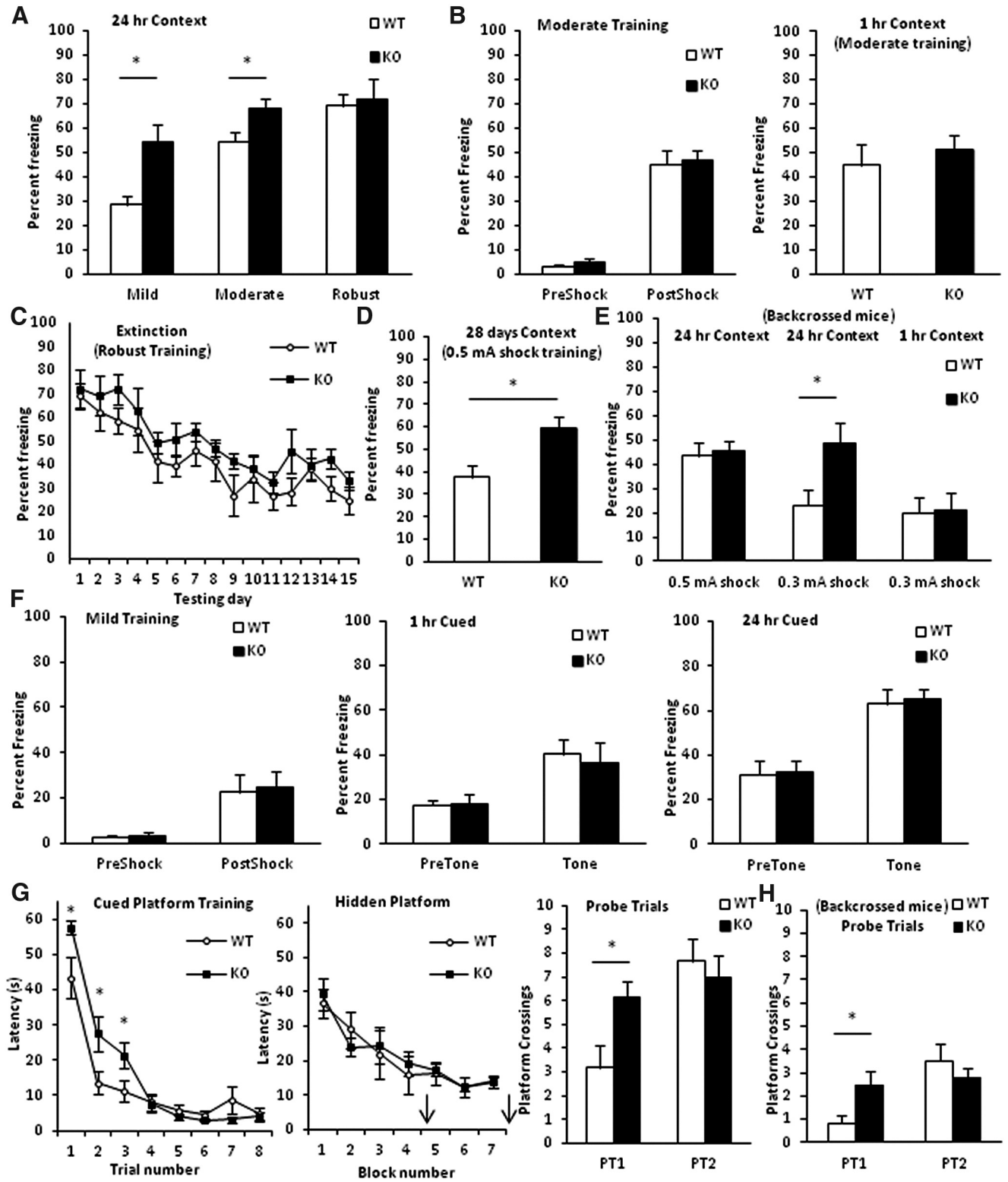
Figure 3. Enhanced long-term memory in *gadd45b* $^{-/-}$ mice. **A**, Mice were trained with mild, moderate and robust cue-plus-context fear conditioning paradigms and returned to the training chamber in the absence of a footshock after 24 h. Knock-outs exhibited significantly higher percentage freezing ($p < 0.05$) after mild and moderate but not robust training. **B**, Mice trained with the moderate paradigm were tested for contextual memory at 1 h. No significant effects of genotype were found during training (left) or testing (right). **C**, Mice trained with the robust paradigm underwent extinction training for 15 d. Both genotypes exhibited significant fear extinction ($p < 0.05$). No main effect of genotype was found ($p > 0.05$). **D**, Mice trained with a modified mild paradigm with 0.5 mA shock were tested for contextual memory at 28 d. Mutants showed significantly ($p < 0.05$) higher freezing. **E**, Backcrossed mice were trained with modified mild paradigm (0.5 or 0.3 mA shock) and tested after 1 or 24 h. Mutants exhibited normal short-term memory and significant ($p < 0.05$) enhancement in long-term memory only after 0.3 mA shock training. **F**, Hybrid-background mice were trained with the mild paradigm and tested 1 or 24 h later in a novel context for tone-shock association. No effect of genotype ($p > 0.05$) was found. **G**, Morris water maze. Hybrid-background knock-outs exhibited significant ($p < 0.05$) deficits in the cued visible platform task and similar ($p > 0.05$) performance to wild-types during hidden platform training. Probe trials, denoted by arrows, were given at the beginning of days 5 and 8. Mutants also demonstrated augmented performance in the first but not second probe trial (right, $p < 0.05$). **H**, Backcrossed mutants trained similarly showed enhanced performance to wild-types on the first but not second probe trial ($p < 0.05$) ($n = 6$–15/group).

ris water maze probe trial, but both genotypes showed robust performance on the second trial (Fig. 3G,H).

Gadd45b$^{-/-}$ hippocampal slices surprisingly show PPF deficits at long interstimulus intervals (Fig. 4G), suggesting presynaptic mechanisms may be altered. Mutants may have reduced residual calcium following low-intensity activation. Alternatively, mutants may experience reduced function of proteins mediating short-term plasticity such as NCS-1 (Jeromin et al., 2006). Transcription of genes supporting each of these mechanisms may be subject to epigenetic regulation by Gadd45b whose net activity may predominate at lower activity. Although mutant slices exhibit this difference in short-term plasticity, this finding is still consistent with the lack of observed phenotypes in tasks of short-term memory, as current models do not posit a role for short-term plasticity on the millisecond time scale as underlying short-term memory in the 1–2 h time frame (Sweatt, 2010).

The finding that mutants exhibit selective long-term memory phenotypes but normal learning is consistent with an epigenetic hypothesis, as *de novo* transcription modulates the former but not latter phase. Gadd45b has been shown to modulate activity-associated DNA demethylation (Ma et al., 2009) and, during memory formation, may similarly target loci such as *reelin* and *bdnf* (Day and Sweatt, 2010). Of note, Gadd45b binds these loci upon mGlu2/3 receptor activation, an antipsychotic pharmacological paradigm associated with demethylation (Matrisciano et al., 2011). Still, impairments in demethylation and transcriptional upregulation of these memory-enhancing transcripts would suggest cognitive impairments in mutants. However, the breadth of demethylation target loci is unknown. In particular, mutants may also experience heightened hypermethylation and downregulation of memory-suppressor genes such as *PP1β* and *calcineurin* (Miller and Sweatt, 2007; Miller et al., 2010). Similarly, mGlu2/3 activation enhanced Gadd45b binding to and demethylation of *GAD67*, a regulator of inhibitory tone (Matrisciano et al., 2011). Alternatively, Gadd45b may modulate expression of other epigenetic regulators including DNMTs and numerous histone-modifying enzymes. It is also possible that active neurogenesis may regulate the memory phenotype, but this appears unlikely because of the long time scale needed for cell maturation and integration into the hippocampal circuit and because neurogenesis has been positively, not negatively, linked to cognition (Ma et al., 2009; Koehl and Abrous, 2011). Additionally, we can-
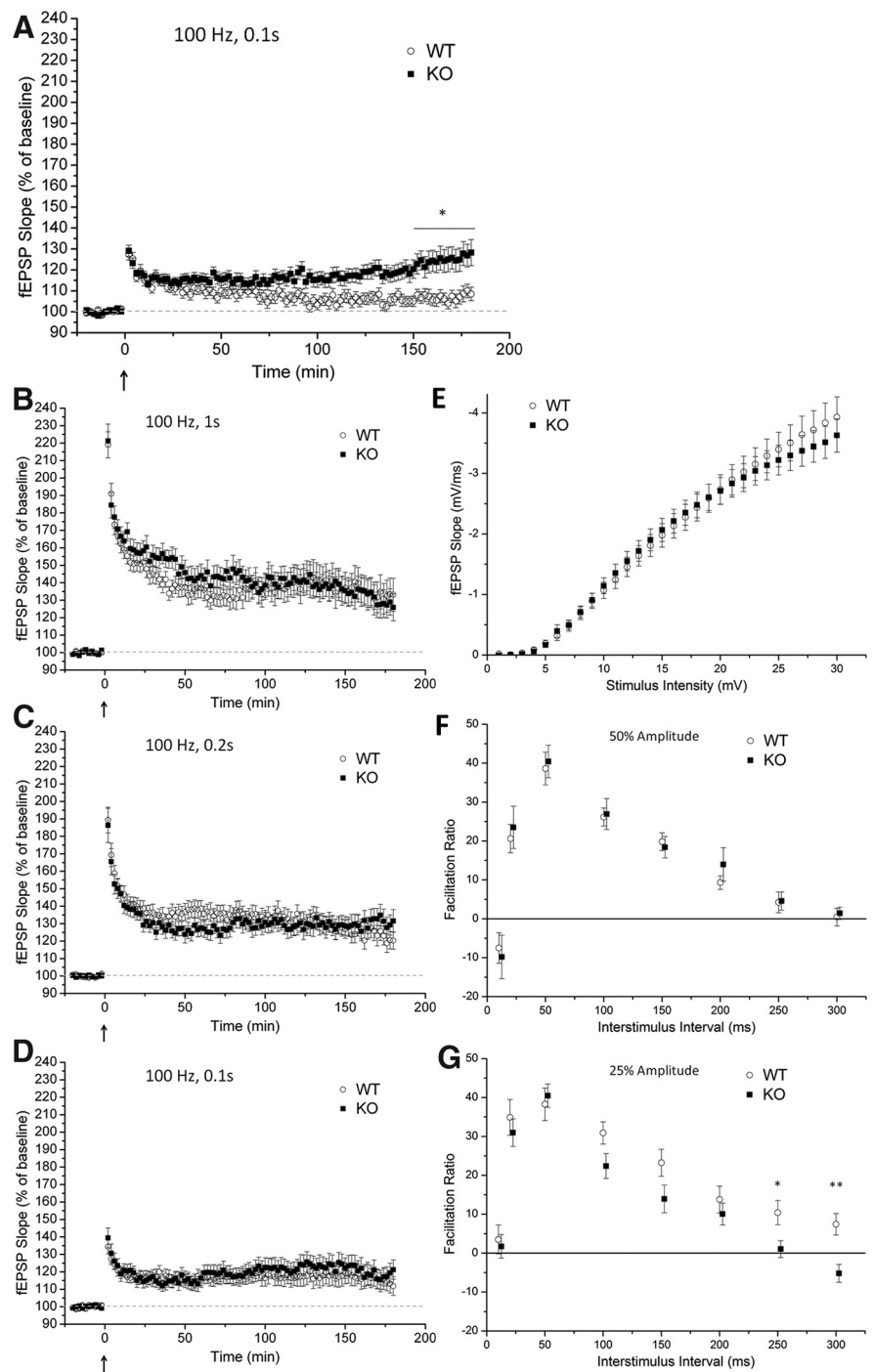

Figure 4. Facilitation of long-term potentiation in *gadd45b*$^{-/-}$ mutants. ***A–D***, Field EPSP recordings from area CA1 before and after stimulation of Schaffer collaterals with listed paradigms. Stimuli were set to an intensity eliciting a fEPSP with 25% of the maximum slope in ***A*** and 50% in ***B–D***. Mutant slices demonstrated enhanced LTP ($p < 0.05$) during the last 30 min of recording only after low-intensity induction. ***E***, Input-output relationship of evoked fEPSP slope versus stimulus intensity in WT and KO slices revealed no effect of the mutation on baseline synaptic transmission. ***F, G***, Paired-pulse facilitation studies across different interstimulus intervals revealed no effect of genotype when using normal-intensity stimuli (***F***) but a significant ($p < 0.01$) deficit in KOs under low-intensity stimuli (***G***) ($n = 6$–15 mice/group, 4–10 slices/mouse).

not discount the possible role of numerous non-epigenetic signaling factors mediated by Gadd45b (Papa et al., 2004).

In addition, we note that near-threshold slice stimulation produced relatively stable LTP for ∼2 h following the tetanus and that field slopes appeared to ramp up slightly for the next 1 h (Fig.

4*A*). This surprising result suggests Gadd45b may play a role not only in the stabilization of potentiated synaptic weights but also in the long-term stability of these weights. Potentially, stimulation may initiate a cascade of epigenetic reprogramming events in the neuron which influence distinct rounds of target gene expression, and novel protein products may affect different components of LTP maintenance. Identification of these epigenetically modulated gene products will be the subject of future investigations into the breadth of the complex, temporally and regionally defined epigenomic interactome within the memory-subserving neuron.

We note that enhanced remote memory in mutant mice (Fig. 3*D*) indicates the persisting effect of *gadd45b* deletion on memory retention. This finding implies cortical epigenetic mechanisms, perhaps distinct from those in the hippocampus, may be influenced by Gadd45b in the storage of lasting fear memory. Indeed, DNA methylation has been shown to subserve memory storage in the anterior cingulate cortex (Miller et al., 2010). However, this finding may alternatively result from "spillover" from enhanced consolidation of fear memory in the hippocampus.

While Gadd45b is necessary for seizure-induced demethylation of *bdnf* (Ma et al., 2009), this does not rule out the possibility that Gadd45g or Gadd45a play overlapping roles with Gadd45b in regulating other loci. This possibility may explain why mutants respond similarly to wild-types upon robust behavioral and synaptic experience. Gadd45b-mediated mechanisms may predominate at lower activity, leading to the near-threshold phenotypes observed. This hypothesis is consistent with the finding that context-shock association appears to suppress *gadd45b* induction in CA1 shortly after training (Fig. 1*C*). It is possible that, before onset of the fear memory consolidation window, the neuron modulates its epigenetic tone to promote methylating and reduce demethylating activity and that it achieves this in part by attenuating Gadd45b function. Although mRNA induction does not appear to vary between trained and context-only control mice 1 h after foreground conditioning, overall demethylation function could still be modulated by other means such as translational regulation of Gadd45b. While the mechanism of demethylation is not known, it is unlikely that Gadd45b acts alone in this process; rather, the orchestrated activity of a number of demethylation regulators is probably necessary, and the interaction of Gadd45b with these proteins may be differentially regulated during memory consolidation (Rai et al., 2008; Wu and Zhang, 2010; Guo et al., 2011).

We finally note that one of our results conflicts with those of the recently published Leach et al. (2012) manuscript. Our findings are consistent with those of this study regarding experience-dependent regulation of *gadd45b* transcription and a lack of phenotype in cued fear conditioning. However, Leach et al. (2012) observed a deficit in contextual fear conditioning whereas we observed an enhancement using near-threshold training stimulation. The reasons for these differing observations are not clear; one possibility is subtle background strain differences that may have arisen during backcrossing. Indeed, we feel that a parsimonious explanation is that effects of the loss of a potent regulator of the epigenome such as *gadd45b* might be exquisitely sensitive to both the background genome in which the gene product resides and the prior experiential history of the animals under study. In light of the prolific role of context-dependent epigenetic tuning of animal behavior by experience, it is possible that differences in the housing or training facility environments may have amplified subtle differences in background genome or epigenome in

mutant mice (Crews, 2011). Additionally, Leach et al. (2012) used a foreground training paradigm for contextual memory assessment whereas our study predominantly used background training. Regardless, our studies and those of Leach et al. (2012) are consistent with the hypothesis that *gadd45b* is transcriptionally regulated by experience and also regulates memory capacity.

In conclusion, these results suggest Gadd45b and potentially other modulators of DNA demethylation regulate memory function and may be viable therapeutic targets in cognitive disease.

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