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A Distinct Role for Norepinephrine in Memory Retrieval

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Summary

A role for norepinephrine in learning and memory has been elusive and controversial. A longstanding hypothesis states that the adrenergic nervous system mediates enhanced memory consolidation of emotional events. We tested this hypothesis in several learning tasks using mutant mice conditionally lacking norepinephrine and epinephrine, as well as control mice and rats treated with adrenergic receptor agonists and antagonists. We find that adrenergic signaling is critical for the retrieval of intermediate-term contextual and spatial memories, but is not necessary for the retrieval or consolidation of emotional memories in general. The role of norepinephrine in retrieval requires signaling through the β_1-adrenergic receptor in the hippocampus. The results demonstrate that mechanisms of memory retrieval can vary over time and can be different from those required for acquisition or consolidation. These findings may be relevant to symptoms in several neuropsychiatric disorders as well as the treatment of cardiac failure with β blockers.

Introduction

Learning and utilizing new information is a complicated affair: one has to acquire, consolidate, retrieve, and reconsolidate memories. Studies have begun to identify the molecular and cellular events that underlie these processes (Abel and Lattal, 2001; Kandel, 2001; Morris et al., 2003; Tonegawa et al., 2003). Most studies have focused on acquisition and long-term consolidation of new information, and recently there has been renewed interest in reconsolidation of retrieved memories (Nader et al., 2000). Much less is known about the molecular mechanisms involved in memory retrieval (Abel and Lattal, 2001). The hippocampus is an important brain region involved in the acquisition and consolidation of explicit memories (e.g., knowing what, where, and when), which can be readily examined in animals using paradigms that depend on contextual (Anagnostaras et al., 2001) or spatial learning (Morris et al., 2003). The hippocampus is also involved in retrieval of such memories, as conceptualized by Hirsh (1974), although evidence indicates that retrieval becomes independent of the hippocampus over time (McClelland et al., 1995; Squire et al., 2001).

A variety of neurotransmitter systems are known to influence hippocampal function, including the adrenergic system (Bergles et al., 1996; Munro et al., 2001; Segal et al., 1991). In the CNS, this system is comprised of several brainstem nuclei that, when activated, release norepinephrine and epinephrine (NE/E) in many regions of the brain. The hippocampus has one of the denser inputs of adrenergic terminals (containing NE) in the CNS (Schroeter et al., 2000), supporting hypotheses suggesting that the adrenergic system plays a role in learning and memory (Crow, 1968; Kety, 1970). Current hypotheses have focused on memory consolidation. These hypotheses state that adrenergic signaling is important for the enhanced consolidation of memories associated with emotionally laden events (Izquierdo and Medina, 1997; McGaugh, 2000). This idea is appealing because the adrenergic system is activated during arousing, emotional experiences. In many of the studies supporting this concept, activation of the adrenergic system has been reported to enhance memory of aversively trained animals. In contrast, few studies have indicated that the adrenergic system is necessary for emotional memory consolidation (Bevilaqua et al., 1997; Gallagher et al., 1977; Lee et al., 1993). In fact, some studies suggest that the adrenergic system may not play a general role in emotional memory consolidation (Lee et al., 2001; Miserendino et al., 1990; Thomas and Palmiter, 1997a).

Numerous approaches for targeting the adrenergic system have yielded conflicting results on the role of NE in mnemonic processes, in part because of the difficulty in selectively interfering with the adrenergic system. To examine potential roles for NE in learning and memory more specifically, we created mice ($Dbh^{-/-}$) that lack NE/E by disrupting the dopamine β-hydroxylase gene (Thomas et al., 1995). A prior study using these mice in an inhibitory avoidance paradigm suggested that NE/E may not be necessary for emotional memory consolidation (Thomas and Palmiter, 1997a). In that study, mice were also tested for hippocampus-dependent spatial navigation in a water maze (Morris et al., 1982). The $Dbh^{-/-}$ mice exhibited a deficit in the probe trial at two days but not two hr after the last training trial, suggesting that consolidation but not acquisition depends on NE/E. Complicating interpretation is the extended training over days that is necessary for mice to acquire the task. Acquisition, consolidation, retrieval, and possibly reconsolidation occur repeatedly during such training.

To further test the possibility that NE is important for hippocampus-dependent memory, we subject $Dbh^{-/-}$ mice to Pavlovian fear conditioning in this study. Contextual but not cued fear memory is dependent on the hippocampus (Kim and Fanselow, 1992; Phillips and LeDoux, 1992). The single-trial paradigm permits the study of acquisition, consolidation, and retrieval by examining the behavior of animals over time. A critical feature of the $Dbh^{-/-}$ model is the ability to rapidly re-

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store NE in the mutant mice before or at various times after training by administering a synthetic amino acid precursor of NE, L-threo-3,4-dihydroxyphenylserine (L-DOPS) (Thomas et al., 1998). The present study examines the effects of the mutation, L-DOPS, and adrenergic receptor drugs on learning and memory in mice and rats.

Results

NE and Fear Conditioning in Mice

To examine adrenergic influences on learning, memory consolidation, and retrieval, *Dbh*$^{-/-}$ and control mice were trained using Pavlovian fear conditioning. In this paradigm, mice were given the opportunity to associate both a tone (cue) and the apparatus (context) with footshock in a single training trial. Memory was assessed by scoring the percent time mice spent immobile (a fear reaction) upon reexposure either to the context or the cue in a distinct context. *Dbh*$^{+/-}$ mice were used as controls because they have normal levels of NE/E and are phenotypically indistinguishable from *Dbh*$^{+/+}$ mice (Thomas et al., 1998).

Mice lacking NE/E exhibited impaired contextual but not cued fear memory one day after training (Figure 1A). There were no differences in freezing between genotypes in the context prior to shock or in a distinct context after training. Normal cued fear did not depend on whether the mice were tested for contextual fear first. The presence of normal cued fear (and inhibitory avoidance) in the *Dbh*$^{-/-}$ mice indicates that the deficit in contextual fear is not due to alterations in pain sensitivity, motivation, or performance. Because context is considered to be in the "background" relative to the cue (Phillips and LeDoux, 1994) and because of the postulated role for NE in attention (Aston-Jones et al., 2000; Robbins, 1997), fear conditioning was also performed in the absence of the cue. A similar deficit in contextual fear was apparent in the *Dbh*$^{-/-}$ mice, indicating that the deficit was not due to overshadowing. Further, the deficit was not due to rapid extinction because *Dbh*$^{-/-}$ mice froze significantly less throughout the five minute test (Figure 1B).

Importantly, the *Dbh*$^{-/-}$ mice acquired contextual fear as readily as controls, as demonstrated by normal freezing the first hour after training (Figure 1C). In contrast, contextual fear was significantly reduced two and six hr after training. While this time course is indicative of a deficit in memory consolidation beginning 1–2 hr after training, later time points suggested otherwise. For example, *Dbh*$^{-/-}$ mice exhibited normal contextual fear one week after training (Figure 1D). The reappearance of normal behavior implies that the memory was not lost due to impaired consolidation. Instead, it suggests that retrieval of the memory may have been temporarily compromised in the absence of NE. An alternate interpretation is that there exists both NE-dependent and NE-independent phases of contextual memory consolidation that operate independently of each other, with the latter having a much slower time course to develop.

To investigate the basis of the memory impairment, NE was restored in adult *Dbh*$^{-/-}$ mice using L-DOPS. NE levels peak ~5 hr after injection of L-DOPS (Thomas et al., 1998), so L-DOPS was administered 4–6 hr prior

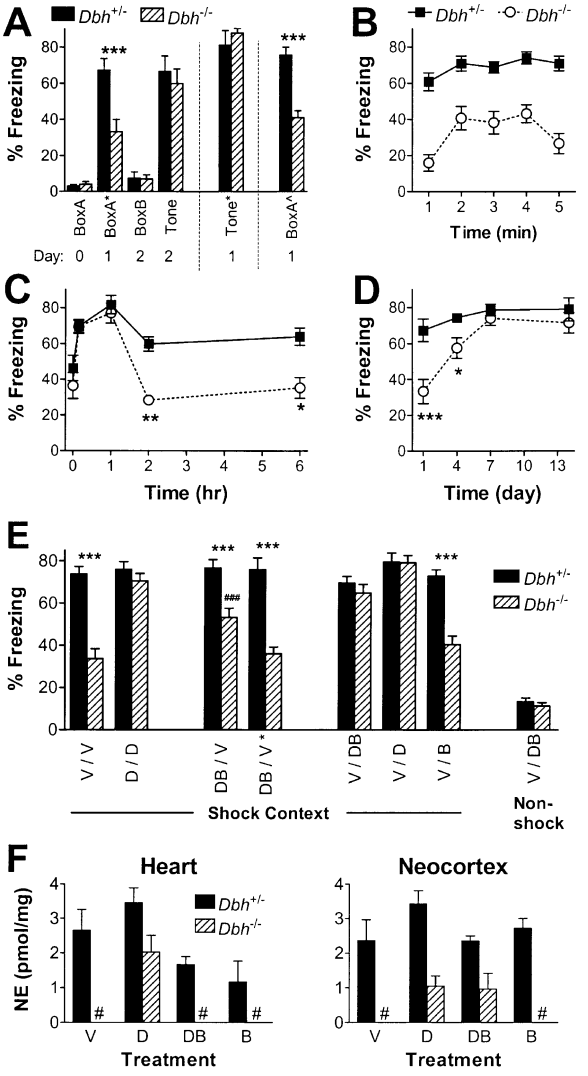

Figure 1. Fear Conditioning in Mice With (*Dbh*$^{+/-}$) and Without (*Dbh*$^{-/-}$) NE

(A) BoxA: during training before the tone; BoxA*: context one day after training. BoxB and Tone: distinct context before and during the cue (all same set of mice). Tone*: during the cue in a 2nd set of mice; BoxA^: context after training without cue, 3rd set of mice. Days after training are listed at bottom (n = 8–12 per group). For all figures, * is $p < 0.05$, ** is $p < 0.01$, and *** is $p < 0.001$.

(B) Contextual fear one day after training (n = 27 per genotype; $p <$ 0.01 at all time points).

(C and D) Contextual fear. Each time point is a separate set of mice tested only once. The first time point in (C) is freezing during the 30 s immediately after training (n = 27 per genotype). For other times, mice were returned to their home cage before testing (n = 9–20 per group).

(E) Contextual fear. Format of the labels is "1st/2nd," where 1st is injection before training and 2nd is injection before testing. V is vehicle, D is L-DOPS, B is benserazide, and DB is L-DOPS plus benserazide. Testing was one day after training except DB/V*, which was two days after. Testing context is indicated below (n = 9–12 per group; ### indicates significantly different from V/V-treated *Dbh*$^{-/-}$ mice, $p < 0.001$).

(F) Tissue levels of NE five hr after injection (n = 4 per group, # indicates undetected).

to training and again prior to testing one day later. No deficit in contextual fear was observed in the *Dbh*$^{-/-}$ mice injected with L-DOPS (Figure 1E). These results indicate that the memory deficit is not due to a developmental abnormality but rather to the loss of a physiologic role for NE.

To test whether consolidation or retrieval is selectively affected, and to test whether the action of NE is peripheral or central, mice were injected before training or before testing with L-DOPS alone or a mixture of L-DOPS plus benserazide, a peripheral aromatic L-amino acid decarboxylase inhibitor that prevents conversion of L-DOPS to NE in the periphery (Figure 1F). Importantly, *Dbh*$^{-/-}$ mice injected with L-DOPS/benserazide before testing but not before training exhibited normal levels of contextual fear (Figure 1E). Rescue was also observed with L-DOPS alone but not with benserazide alone before testing. Rescue was not due to enhancement of nonspecific freezing in the *Dbh*$^{-/-}$ mice because there was no difference between the genotypes when rescued mice were placed in a distinct context. Interestingly, there was a partial but significant enhancement of freezing in the *Dbh*$^{-/-}$ mice injected with L-DOPS/benserazide before training, even though freezing was significantly below that for controls (Figure 1E). Low levels of NE are present one but not two days after injection of L-DOPS, and there is partial rescue of other phenotypes at one but not two days (Thomas et al., 1998). Therefore, we injected mice with L-DOPS/benserazide before training and then tested two days later. In this case, freezing was equivalent to vehicle-injected *Dbh*$^{-/-}$ mice. These results suggest that NE is critical for retrieval but not for formation or consolidation of contextual fear memories, and that NE exerts this effect within the CNS.

Adrenergic Receptors and Fear Conditioning in Mice

L-DOPS did not entirely rule out the possibility that adrenergic signaling could mediate a delayed consolidation during the time between injection and testing. To address this, we examined whether adrenergic receptor antagonists alter the behavior of control mice in contextual fear conditioning. We found that 1 mg/kg of the β-adrenergic receptor antagonist (−)-propranolol (but not 3 mg/kg of less active (+)-propranolol) reduced contextual fear one day after training when given to *Dbh*$^{+/-}$ mice 30 min before testing (Figure 2A). Freezing with (−)-propranolol was comparable to that seen in untreated *Dbh*$^{-/-}$ mice, and (−)-propranolol given before testing to *Dbh*$^{-/-}$ mice did not affect freezing (Figure 2D). When given before training or before testing one week after training, (−)-propranolol did not alter contextual freezing in the *Dbh*$^{+/-}$ mice (Figure 2A). The effect of (−)-propranolol given before testing one day after training was not state-dependent because mice treated with (−)-propranolol before training and testing had a comparable deficit. Further, the effects of β blockade were CNS-dependent because nadolol and sotalol, β antagonists that do not readily cross the blood-brain barrier, had no effect on freezing when given before testing. Because control mice have the opportunity to consolidate normally during the 24 hr between training and the administration of antagonist shortly before testing, these results strongly implicate NE and β-adrenergic receptors in memory retrieval.

To test whether α-adrenergic receptors participate in contextual memory retrieval and to determine the identity of the β receptor(s) involved, additional pharmacologic and genetic experiments were performed. The α_1 antagonist prazosin had no effect on contextual freezing when given before testing despite causing ptosis as expected (Figure 2A). Interestingly, the α_2 antagonist atipamezole caused a significant increase in freezing. These results indicate that α receptors are not necessary for retrieval. The increase in freezing seen with atipamezole could be due either to blockade of postsynaptic α_2 receptors that oppose actions mediated by β receptors or to blockade of presynaptic α_2 receptors that regulate NE release. The latter effect would potentiate signaling at postsynaptic β receptors.

Because (−)-propranolol is considerably less potent at β_3 receptors (Blin et al., 1994), the results suggest that stimulation of β_1 and/or β_2 receptors mediates the effect of NE on retrieval. To assess their contributions, we treated *Dbh*$^{+/-}$ mice before testing with β subtype-selective antagonists (CGP 20712A, betaxolol, and atenolol for β_1; ICI 118,551for β_2). Both CGP 20712A and betaxolol caused maximal reductions in contextual fear at 1 mg/kg, while there was no effect of atenolol or ICI 118,551 (Figure 2B). Atenolol does not readily cross the blood-brain barrier, so it provides further support for a CNS site of action. Disruption of retrieval by betaxolol indicates that the behavioral effects of (−)-propranolol are mediated by antagonism of β-adrenergic rather than serotonergic receptors because betaxolol is devoid of serotonin receptor effects (Middlemiss, 1984; Tricklebank et al., 1987). Finally, we examined mice with a targeted disruption of the β_1 receptor and found that they also exhibited reduced contextual fear at one day but not one week after training, while cued fear was normal (Figure 2C). Taken together, these results indicate that CNS β_1-adrenergic receptors are necessary for contextual memory retrieval.

To determine whether β_1 receptor stimulation is sufficient to mediate the effect of adrenergic signaling on retrieval, we administered β subtype-selective agonists to *Dbh*$^{-/-}$ mice before testing. Xamoterol and procaterol were chosen because they have been shown in other systems to have selectivity for β_1 and β_2 receptors, respectively (Hicks et al., 1987; Waelbroeck et al., 1983). We found that xamoterol but not procaterol caused a dose-dependent, context-specific enhancement of freezing one day after training (Figure 2D). Their effectiveness correlated with their ability to stimulate adenylyl cyclase via murine β_1 but not β_2 receptors (Figure 2F). To further test the idea that adrenergic signaling is required for retrieval but not consolidation, xamoterol was administered to *Dbh*$^{-/-}$ mice one hr before testing three days after training. Xamoterol restored normal contextual freezing in the mutant mice despite the absence of NE for three days after training (Figure 2E). In total, these results demonstrate that activation of β_1 receptors is sufficient to restore contextual memory retrieval in the *Dbh*$^{-/-}$ mice but do not provide evidence suggesting a role for NE in consolidation.

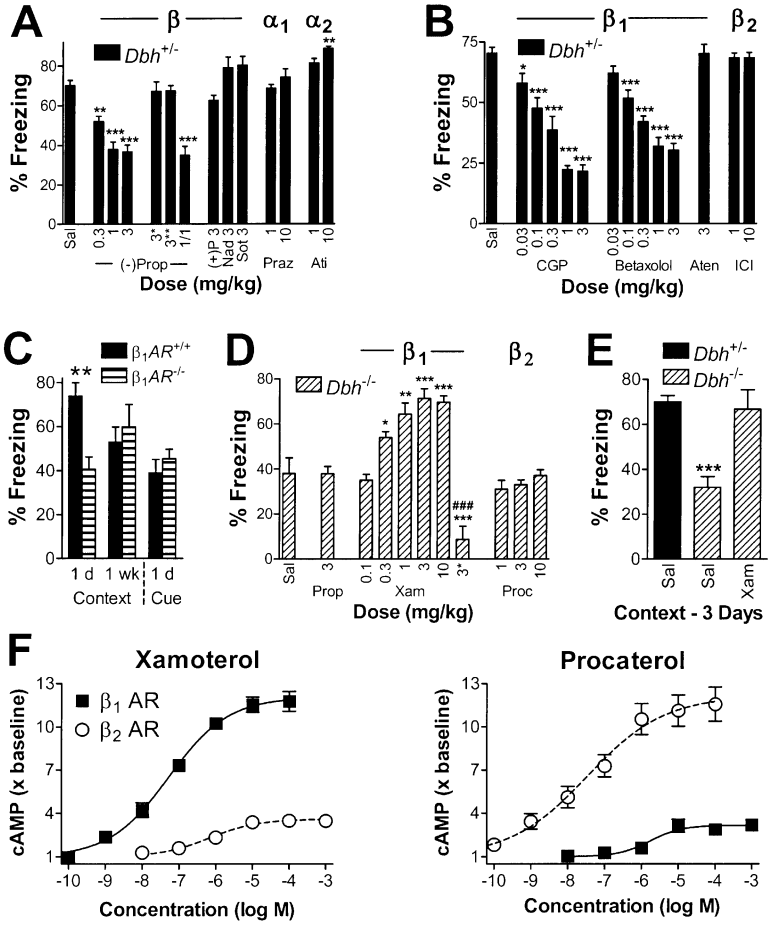

Figure 2. Adrenergic Drugs and Contextual Memory in Mice

(A) Adrenergic antagonists. Sal: saline; (−)Prop: (−)-propranolol; (+)-P: (+)-propranolol; Nad: nadolol; Sot: sotalol; Praz: prazosin; Ati: atipamezole. Mice received saline prior to training except for one group (1/1) that received (−)-Prop before training and testing. Drugs were given 30 min before testing except 3*, which was 30 min before training. Testing was one day after training except for 3**, which was one week after (n = 4–12 per group).

(B) Selective β receptor antagonists. CGP: CGP 20712A, Aten: (−)-atenolol, ICI: ICI 118,551. Testing was one day after training, 30 min after drug (n = 5–10 per group).

(C) β_1-adrenergic receptor-deficient mice ($\beta_1AR^{-/-}$). Testing was one day or one week after training (n = 5–6 per group, which were separate).

(D) $Dbh^{-/-}$ mice and contextual testing. Xam: xamoterol; Proc: procaterol. Testing was one day after training and 30 min after Prop or one hr after Xam or Proc. Group 3* was tested in a distinct context (### is $p < 0.001$ compared to 3 mg/kg Xam-treated mice in the shock context; n = 5–10 per group).

(E) Contextual fear three days after training. Mice were given Sal or 3 mg/kg Xam one hr before testing (n = 5 per group).

(F) Dose-response for β agonists at the cloned mouse β_1 and β_2 receptors, which stimulate adenylyl cyclase. Levels are relative to baseline. Each point is 4–6 separate groups of cells. For Xam at β_1: $EC_{50} = -7.3$ ($\log_{10}$M), $E_{max} = 12.1$. For Xam at β_2: $EC_{50} = -6.2$, $E_{max} = 3.6$. For Proc at β_1: $EC_{50} = -5.8$, $E_{max} = 3.2$. For Proc at β_2: $EC_{50} = -7.5$, $E_{max} = 12.2$.

β-Adrenergic Receptors and Fear Conditioning in Rats

Many of the studies examining adrenergic modulation of memory consolidation have been performed using rats. Therefore, we examined whether our results using mice would generalize to rats. We observed a dose-dependent decrease in contextual fear with (−)-propranolol that was equivalent to that for mice (Figure 3A). Cued fear was unaffected (saline: 56.2 ± 9%; 1 mg/kg (−)-propranolol: 54.6 ± 8.2%; n = 5 each, p = 0.9), consistent with a previous study (Davis et al., 1979). The effect of (−)-propranolol was mimicked by betaxolol (β_1), but not ICI 118,551 (β_2) or the peripheral β antagonist sotalol. Even more striking, the time course of the dependence of contextual memory retrieval on β-adrenergic signaling was nearly identical to that observed in mice (Figures 3B and 3C).

β-Adrenergic Receptors and Spatial Navigation in Rats

The role of NE in contextual but not cued fear suggests a role for adrenergic signaling in hippocampus-dependent memory retrieval. To test this possibility further, we used the Morris water maze, in which spatial reference memory depends on the hippocampus (Morris et al., 1982; Moser and Moser, 1998; Riedel et al., 1999). Acquisition of and short-term memory for this task do not depend on NE (Hagan et al., 1983; Thomas and Palmiter, 1997a). Because of the interest in testing retrieval, a rapid (90 min) training protocol was employed (Frick et al., 2000). As anticipated, acquisition did not depend on β-adrenergic signaling (Figures 4A and 4B). Contextual fear testing in rats indicated that CNS β blockade lasted for at least two hr (data not shown).

We next examined whether β signaling is necessary for spatial memory retrieval by injecting rats with (−)-propranolol prior to a probe trial one day after training. There was a dose-dependent reduction in quadrant preference that was significant for both 1 mg/kg (data not shown) and 3 mg/kg of (−)-propranolol (Figure 4D). Swim speed and time spent at the perimeter of the pool were not affected. (−)-Propranolol had no effect on the cued version of the water maze one day after training (Figure 4F). Finally, the time dependence of spatial memory retrieval on β signaling was examined by testing other groups of rats with a probe trial one hr or one week after training. There was no significant effect of (−)-propranolol on quadrant preference at these times (Figures 4C and 4E), demonstrating a lack of effect on motivation or performance. These results indicate that β-adrenergic signaling has a time-limited role in spatial memory retrieval.

β-Adrenergic Signaling in the Hippocampus and Memory Retrieval

Given the deficits in contextual and spatial reference memory with systemic manipulations, it was of interest

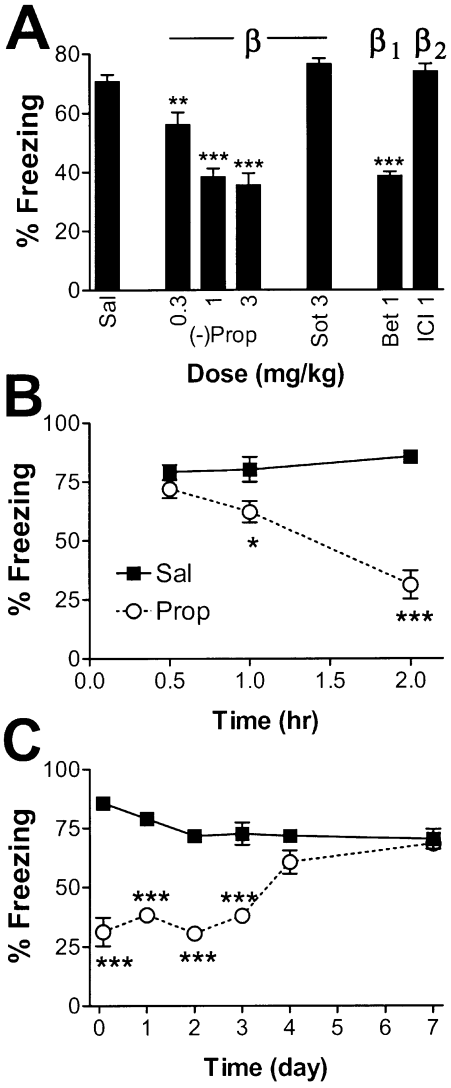

Figure 3. Adrenergic Drugs and Contextual Memory in Rats

(A) Rats were injected one day after training, 30 min before testing ($n = 5$ per group).

(B and C) Prop: 1 mg/kg (−)-propranolol. Each time point represents a separate set of rats ($n = 5$ per group).

to test whether β-adrenergic signaling in the hippocampus is necessary for retrieval. Toward this goal, cannulas were chronically implanted in control mice so that bilateral dorsal hippocampal infusions could be performed (Figure 5A). One week after surgery, cannulated mice were fear conditioned. One day later either artificial cerebrospinal fluid (aCSF) or (−)-propranolol was infused 15 min before testing. There was a dose-dependent decrease in contextual freezing that, at 10 μg, was similar in magnitude to the reduction in freezing observed after systemic treatment (Figure 5B). This effect was stereo- and site-specific: 10 μg of (+)-propranolol in the hippocampus and 10 μg of (−)-propranolol in the overlying cortex or lateral ventricles were without effect. In addition, 10 μg of (−)-propranolol had no effect on retrieval of either cued fear one day after training or contextual fear one week after training (Figure 5D).

Systemic propranolol administered immediately after contextual fear conditioning has no effect on memory consolidation (Lee et al., 2001). However, we sought to rule out possible differential effects of local verses systemic delivery on memory consolidation. To do this, 10 μg of (−)-propranolol was infused bilaterally into the hippocampus five min after training and the mice were tested one day later. No effect on freezing was observed (Figure 5D). We also tested the prediction that antagonists that do not readily cross the blood-brain barrier and have no effect when given systemically (Figures 2A and 2B) would be more potent when infused into the hippocampus than antagonists that do cross the blood-brain barrier. For mixed β blockers, nadolol was ~5 times more potent than (−)-propranolol; for β_1-selective blockers, atenolol was ~100 times more potent than betaxolol, confirming the prediction (Figure 5C). Finally, we sought to determine whether stimulation of hippocampal β receptors would be sufficient to restore retrieval in *Dbh*$^{-/-}$ mice. Infusion of the β agonist (±)-isoproterenol resulted in a dose-dependent enhancement of freezing to normal levels. Taken together, these findings indicate that β_1-adrenergic signaling within the hippocampus is necessary and sufficient for contextual memory retrieval but is not required for consolidation.

NE and Hippocampal Synaptic Plasticity

Synaptic plasticity at CA1 pyramidal neurons of the hippocampus appears to be crucial for hippocampus-dependent memory consolidation. In mice with a knockout of the NMDA receptor subunit 1 that is specific for CA1 pyramidal neurons, long-term memory for contextual fear conditioning and the Morris water maze is impaired (Shimizu et al., 2000). Reductions in hippocampal synaptic plasticity at the perforant path synapses of the dentate gyrus, the mossy fiber synapses of CA3, or the commissural/associational synapses of CA3 do not affect performance in fully cued versions of these tasks (Hensbroek et al., 2003; Nakazawa et al., 2002; Nosten-Bertrand et al., 1996). Compared to consolidation, retrieval is very rapid and may not depend on concomitant modification of synaptic strength. For example, NMDA receptor antagonists do not affect performance of hippocampus-dependent tasks (Day et al., 2003; Morris, 1989). Based on our results implicating NE in retrieval but not consolidation, we predicted that long-term synaptic plasticity in CA1 would be intact. Basal synaptic physiology in CA1 was nearly equivalent between genotypes (Figures 6A and 6B). Further, there was no significant difference in the early or late phases of long-term potentiation (LTP) (Figures 6C and 6D). These data are consistent with the idea that the memory impairment that develops 1–2 hr after training in the *Dbh*$^{-/-}$ mice is based on a deficit in retrieval rather than consolidation.

Discussion

Adrenergic Signaling, Attention, and Emotional Memory Consolidation

Despite hypothesized roles for NE in arousal and attention (Aston-Jones et al., 2000; Robbins, 1997), we did not find evidence for alterations in attention as assessed by acquisition of fear conditioning. The tone signaling shock had no impact on the contextual fear deficit exhibited by the *Dbh*$^{-/-}$ mice. Further, normal contextual fear

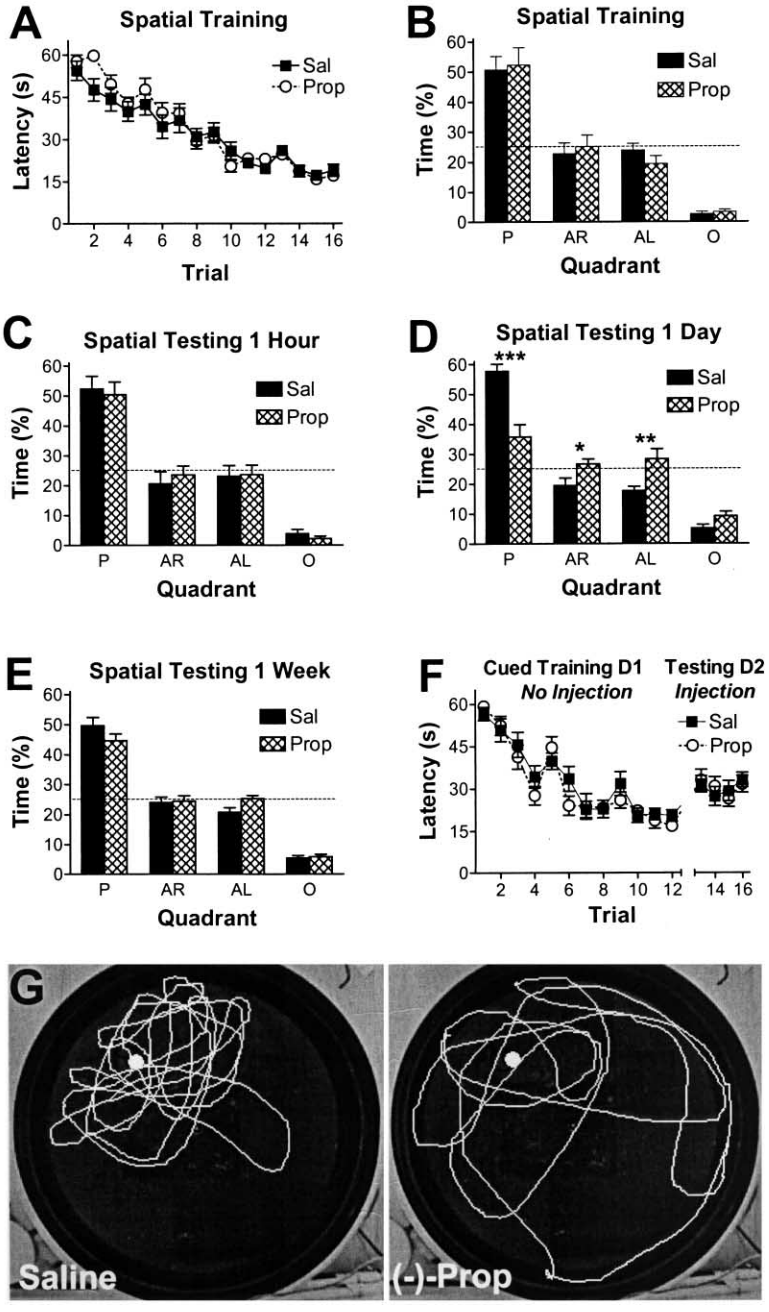

Figure 4. Spatial Memory in Rats

(A) Spatial acquisition in the Morris water maze. Rats were injected with saline (Sal) or 3 mg/kg (−)-propranolol (Prop) 30 min before training. The latency to climb onto the hidden platform (fixed location) is given (n = 10 per group).

(B–E) Probe trials.

(B) Rats trained with Sal or Prop (A) were tested one day later, 30 min after Sal injection.

(C–E) Rats were tested at the times indicated after training, 30 min after Sal or Prop (n = 10 per group). Quadrants: P, platform during training, AR, adjacent right; AL, adjacent left; O, opposite.

(F) Cued version of the water maze. No injections were given before training. One group received Sal and the other Prop 30 min before testing (n = 10 per group).

(G) Representative probe-trial swim paths for rats treated one day after training (D).

one hr and one week after training argues against alterations in attention as being responsible for the phenotype. Our results do not support a broadening of attention in the absence of adrenergic signaling, as others have proposed based on fear conditioning (Selden et al., 1990).

Given that the *Dbh*$^{-/-}$ mice exhibit normal cued fear conditioning and inhibitory avoidance (Thomas and Palmiter, 1997a), evidence for a critical role of adrenergic signaling in emotional memory consolidation (Izquierdo and Medina, 1997; McGaugh, 2000) is lacking in our studies. One could hypothesize that compensation for the loss of adrenergic signaling masks a role in consolidation. However, neither systemic nor intracerebroventricular injection of adrenergic receptor antagonists shortly before or after fear conditioning alters subsequent cued or contextual fear in retention tests (Lee et al., 2001; O. Stiedl, personal communication). These results argue strongly against a general role for adrenergic signaling in emotional memory.

Adrenergic Signaling and Explicit Memory Retrieval

The most likely explanation for our results is that NE promotes retrieval of contextual and spatial memories during a specific stage of consolidation. Once these memories have consolidated for ~4 days, retrieval becomes independent of NE. A role for NE in memory

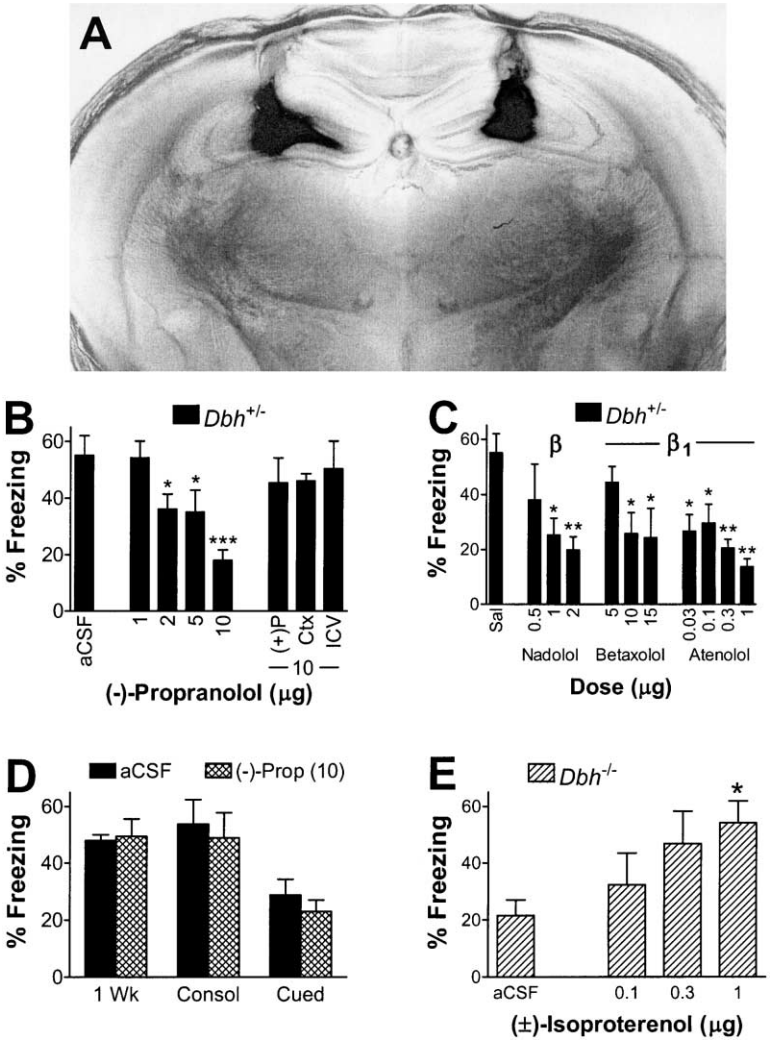

Figure 5. Fear Memory and Hippocampal Infusion of Adrenergic Drugs in Mice

(A) Representative bilateral infusion into the dorsal hippocampus (1 μl methylene blue).

(B and C) Contextual fear one day after training, 15 min after infusion.

(B) (−)-Propranolol was used for all infusions except aCSF and 10(+) (10 μg of (+)-propranolol). Infusions were into the dorsal hippocampus except "Ctx" and "ICV", where 10 μg of (−)-propranolol was infused into the neocortex above the dorsal hippocampus or into the lateral ventricles (n = 6–11 per group).

(C) Nadolol and atenolol were dissolved in 0.9% saline (Sal) and infused into the dorsal hippocampus (n = 4–11 per group).

(D) Infusion of 10 μg of (−)-propranolol into the dorsal hippocampus either 15 min prior to testing contextual fear one week after training (1 Wk), five min after fear training (tested one day later; Consol) or 15 min prior to cued fear testing (Cue) one day after training (n = 6–11 per group).

(E) Infusion of (±)-isoproterenol into the dorsal hippocampus of *Dbh*$^{-/-}$ mice 30 min before contextual testing one day after training (n = 5–8 per group).

consolidation (in addition to retrieval) cannot be completely ruled out. One could postulate that the β_1 agonist xamoterol mediates a delayed consolidation during the hour between injection and retention testing. If this were true, it would mean that the absence of NE-dependent consolidation for three days after training could be reversed within one hr of restoring β_1 signaling. A readily reversible role for NE in consolidation would not be consistent with any previous study suggesting a requirement for NE in memory consolidation (Bevilaqua et al., 1997; Gallagher et al., 1977; Lee et al., 1993). Those studies used antagonists to interfere with adrenergic signaling for much shorter time periods within minutes to hours after training, and they reported long-lasting effects under conditions of normal adrenergic signaling during testing.

Systemic or intrahippocampal glucocorticoid receptor stimulation 30–60 min before a probe trial impairs spatial navigation in the Morris water maze (de Quervain et al., 1998), and basal levels of corticosterone are moderately elevated in the *Dbh*$^{-/-}$ mice (Alaniz et al., 1999). However, acute administration of propranolol, which mimics the retrieval phenotype of the *Dbh*$^{-/-}$ mice, does not alter plasma corticosterone levels in mice or rats (Gala and Haisenleder, 1986; Gorman and Dunn, 1993). These findings argue against an elevation in corticosterone as causing impaired memory retrieval in the *Dbh*$^{-/-}$ mice.

Our results indicate that β_1-adrenergic signaling in the hippocampus is necessary and sufficient for context retrieval, although an adjacent region cannot be completely ruled out. This is consistent with a number of lesion studies showing that the hippocampus is required for consolidation and retrieval of contextual fear memories (Anagnostaras et al., 2001; Kim and Fanselow, 1992; Phillips and LeDoux, 1992). Of note, temporary inactivation of the dorsal hippocampus using the $GABA_A$ agonist muscimol shortly before testing blocks context-specific latent inhibition of contextual fear (Holt and Maren, 1999) and context-specific extinction of cued fear (Corcoran and Maren, 2001). Interestingly, at the dose used in those experiments, no effect was observed on retrieval of contextual fear itself. However, if context retrieval requires less hippocampus than context discrimination to drive behavior, then a larger volume of the hippocampus would need to be inactivated to block context retrieval. In our studies, 1 μl was infused into the dorsal hippocampus of mice, while in the studies using musci-

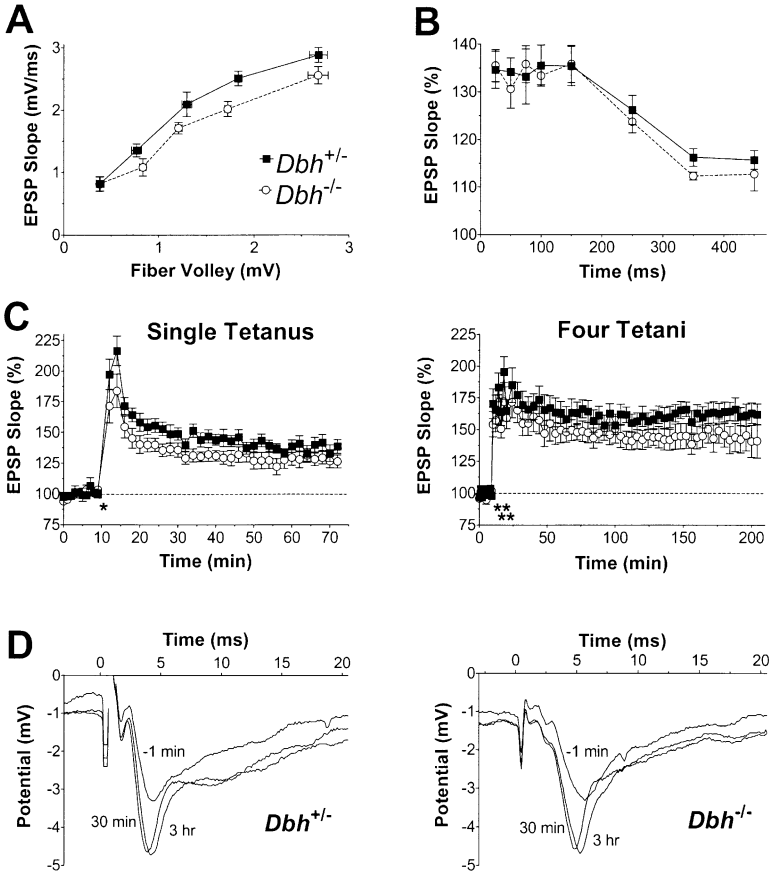

Figure 6. Hippocampal Synaptic Physiology and LTP in CA1

(A) Input-output. A generalized estimating equations model revealed a small but significant ($p < 0.01$) difference between the genotypes ($n = 11$–16 slices per genotype).

(B) Paired-pulse facilitation ($n = 10$–11 slices per genotype).

(C) LTP ($n = 10$–11 slices per genotype). For one tetanus, the last four time points were 138 ± 5.6% and 128 ± 5% for $Dbh^{+/-}$ and $Dbh^{-/-}$ ($p = 0.17$), and for four tetani, 162 ± 7.7% and 142 ± 12.5%, respectively ($p = 0.19$).

(D) Representative EPSPs for four tetani (C). Times are relative to the first tetanus.

mol, 0.5 μl was infused into the dorsal hippocampus of rats. Additional studies will be needed to address this issue.

Lesion studies suggest that the hippocampus plays a time-limited role in the retrieval of explicit memories, including contextual fear (Anagnostaras et al., 1999; Kim and Fanselow, 1992). Retrieval could become independent of NE because the hippocampus is no longer required ~4 days after acquisition. Alternatively, consolidation within the hippocampus could make retrieval independent of NE. The lesion studies favor the latter possibility because dependence on the hippocampus appears to last several weeks. However, future studies performing functional inactivation of the hippocampus could be enlightening because electrolytic lesions damage fibers of passage and excitotoxic lesions might affect regions receiving input from the hippocampus. Such collateral effects could impair retrieval beyond four days.

Studies examining intracellular signaling molecules suggest that some processes are important for the maintenance of memories one week or more after acquisition. Mutant mice lacking both type I and type VIII adenylyl cyclases and transgenic mice expressing a dominant-negative CaMKIV in the postnatal forebrain exhibit impaired contextual fear one week but not one day after training (Kang et al., 2001; Wong et al., 1999). α-*CaMKII*$^{+/-}$ mice are impaired in the Morris water maze ten but not three days after training, and in contextual fear ten but not one day after training, with a partial impairment at three days (Frankland et al., 2001). Interestingly, cortical but not hippocampal long-term potentiation is impaired in the α-*CaMKII*$^{+/-}$ mice. These and our results support the idea that there are phases of memory consolidation that have distinct molecular and anatomical requirements.

Adrenergic Signaling and Memory: a Hypothesis

We propose that release of NE in the hippocampus alters information processing via β_1 receptors to promote memory retrieval. A potential mechanism would be through enhancement of pattern completion (Marr, 1971; Rudy and O'Reilly, 1999). However, mutant mice lacking NR1 expression specifically in CA3 pyramidal neurons are deficient in pattern completion, yet these mice perform normally in a fully cued version of the Morris water maze (Nakazawa et al., 2002), in contrast to rats treated with propranolol. Instead, we hypothesize that information flow from the dentate gyrus (DG)/CA3 regions to CA1 depends on NE, while flow from entorhinal cortex does not (Figure 7). The latter is thought to mediate processing needed to activate place cells in CA1, while the former is likely involved in spatial and contextual memory storage and retrieval (Lisman, 1999; Moser and Paulsen, 2001). In the absence of NE, contextual and spatial information would still be stored in DG/CA3 but could not be transmitted to CA1 (Figure 7). Interestingly, the phenotype of propranolol-treated rats is similar to that observed in rats following excitotoxic lesions of CA3 (Brun et al., 2002). In the absence of NE,

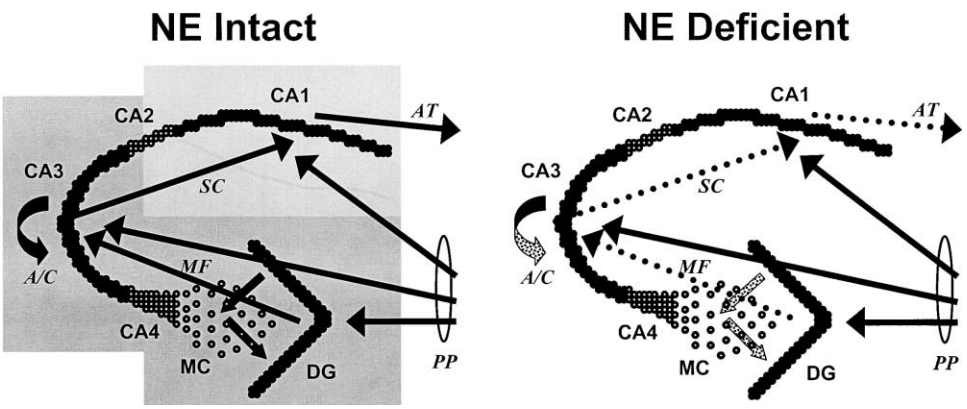

Figure 7. Excitatory Pathways in the Hippocampus and How They Might Be Affected in the Absence of NE

Sensory input to the hippocampus occurs via the perforant path (*PP*) from the entorhinal cortex. When adrenergic signaling is active, information thought to be critical for spatial and contextual memory retrieval (Brun et al., 2002) is efficiently transmitted from the dentate gyrus (DG)/CA3 regions to CA1 along the Schäffer collateral pathway (*SC*). When adrenergic signaling is absent, DG/CA3 processing is disrupted (as indicated by the dotted arrows), resulting in impaired matching within CA1 of current input from *PP* to memory. Gray shading indicates density of adrenergic innervation. *AT*, ammonic to temporal cortical pathway; MC, mossy cells; *MF*, mossy fiber pathway; *A/C*, associational/commissural pathway.

the point(s) at which information flow might be impaired is unknown; however, in the hippocampus, β_1 receptors are expressed mainly by the dentate granule cells and the CA1 pyramidal neurons (Nicholas et al., 1993).

It is known that salient cues activate the LC (Aston-Jones et al., 1997; Sara and Segal, 1991) and extracellular NE in the hypothalamus and NE turnover in the hippocampus increase if an animal is returned to an aversive context (Sara, 1985; Yokoo et al., 1990). Cue-activated release of NE in the hippocampus could facilitate retrieval of context. In our model, behavior that depends on salient cues (e.g., cued fear conditioning and possibly inhibitory avoidance) would be intact in the absence of NE. An alternate hypothesis is that tonic rather than phasic adrenergic signaling facilitates retrieval in the hippocampus. As a result, retrieval might be inhibited when extracellular levels of NE are minimal, such as during rapid eye movement sleep (Shouse et al., 2000).

Implications for Human Neurologic Disorders

Our results could be relevant when considering the etiology of symptoms associated with several CNS disorders. For example, memory retrieval difficulties associated with depression could be due in part to a functional impairment in adrenergic signaling (Ilsley et al., 1995; Ressler and Nemeroff, 1999). A hallmark of Korsakoff's psychosis is impaired memory retrieval, and loss of adrenergic signaling could play a role (Homewood and Bond, 1999; McEntee and Mair, 1978). Adrenergic neurons are known to degenerate in patients with Alzheimer's disease, which impairs memory retrieval (Backman et al., 1999; Iversen et al., 1983). However, a role for NE is not clear because extracellular NE is not decreased at times when symptoms are present (Elrod et al., 1997; Hoogendijk et al., 1999).

On the other hand, recurrent intrusive memories in posttraumatic stress disorder (PTSD) could be due in part to greater activity of the adrenergic system (Geracioti et al., 2001; Southwick et al., 1999). Our results suggest that if β blockers were to be effective in decreasing unwanted memories, it may be when symptoms are present rather than shortly after the traumatic event, as others have suggested (Pitman et al., 2002; Southwick et al., 1999). If adrenergic signaling is only temporarily involved in explicit memory retrieval, then blocking central β_1-adrenergic signaling may not be effective. However, it is possible that a hyperadrenergic state could enhance retrieval even if NE is no longer required, as has been suggested by animal studies (Devauges and Sara, 1991; Sara, 1985). In that case, reducing β_1 signaling might provide some relief from intrusive memories, analogous to a reduction in other PTSD symptoms by α_1-adrenergic blockade (Famularo et al., 1988; Kolb et al., 1984; Raskind et al., 2003).

Finally, there are reports suggesting that CNS-penetrant β blockers may impair memory in humans (Solomon et al., 1983), and some data suggest that NE may play a role in human memory consolidation (Cahill et al., 1994), although those results have proven difficult to reproduce (O'Carroll et al., 1999a, 1999b). These reports and our results suggest that CNS-impenetrant β blockers may be desirable when treating peripheral diseases such as cardiac failure (Sallach and Goldstein, 2003).

Experimental Procedures

Subjects

Dbh$^{-/-}$ mice were rescued prenatally as previously described (Thomas and Palmiter, 1997b). No significant differences were found by gender so data were combined. Where indicated, $\beta_1AR^{-/-}$ mice backcrossed to C57BL/6 ($N > 10$) and C57BL/6 control mice were used. Female Fisher 344 rats (Harlan, Indianapolis, IN) were 3–4 weeks old upon arrival. Mice were 3–6 months when tested and rats were 7–8 weeks for fear conditioning and 10–14 weeks for water maze. Studies were performed during the light phase, were in accordance with NIH guidelines and had the approval of IACUC at University of Pennsylvania.

Fear Conditioning

Subjects were given two 3 min preconditioning handling sessions. Training consisted of placing the animal in the apparatus (ENV-010MC with ENV-414S, Med Associates, St. Albans, VT) for two min,

after which an 84 dB, 2.8 kHz tone was activated for 30 s (for Figure 1A, a 83 dB white-noise generator was used, resulting in higher levels of cued freezing than the tone). Two seconds before the end of the tone a 2 s foot shock was delivered (1 mA for mice, 2 mA for rats). The mouse was returned to its home cage 30 s after shock. Contextual fear was tested for five min in the absence of the tone. Cued fear was tested by placing the mouse in a context containing distinct visual, tactile, and olfactory cues. After two min, the training tone was turned on for three min. Evaluation of nonspecific contextual fear was performed by exposing mice to this distinct context for three min on the day of training, several hours before training with shock. For testing one day later, mice were placed in this context for five min, several hr before testing in the shock context. Percent freezing was estimated by scoring the presence or absence of nonrespiratory movement every five seconds.

Water Maze

Rats were handled as described above. One day before training, a four trial shaping procedure was given in a one min pool. Each rat was placed on a visible platform for 10 s and then in the water at three progressively farther distances and given 20 s to escape onto the platform. The rat was left on the platform for 10 s after each. For training and testing, a two min pool located in a different room was used. The hidden platform (10 cm, clear) top was 2 cm below the surface. On the day of training, rats were allowed to find the platform located in the middle of one quadrant. Each rat was given 16 trials (one min each) partitioned into four blocks of four trials. Start positions at the edge between each quadrant varied in a pseudorandom fashion. If the rat did not escape in one min, it was placed on the platform for 10 s. The next trial began immediately afterward with an interblock interval of 30 min. For testing, a one min probe trial without the platform was given. Rats were divided randomly into two groups of ten and administered saline or (−)-propranolol. Training for the cued task was the same except that no shaping was given, three blocks of four trials were used, and the top of the visible platform was 1 cm above the surface. For cued testing, rats were given four trials with an intertrial interval of five min one day after training. For each cued training and testing trial, the platform was in the center of a different quadrant (pseudorandom). Data were collected using a tracking system (AccuScan Instruments, Columbus, OH).

Drugs

An injection of vehicle was given before each handling session and before either training or testing, whichever did not call for drug. L-DOPS was given at 1 g/kg (50 μl/g) subcutaneously 4–6 hr before training or testing (Thomas et al., 1998). When given, benserazide (0.25 g/kg, Sigma, St. Louis, MO) was coinjected with L-DOPS or injected alone. Other drugs were given subcutaneously using 10 μl/g of 0.9% saline. (−)-Propranolol HCl, (+)-propranolol HCl, nadolol, sotalol HCl, prazosin HCl, CGP 20712A (all from Sigma); (−)-atenolol, betaxolol HCl, ICI 118,551 HCl (all from Tocris, Ellisville, MO); and atipamezole HCl (Orion Pharma, Turku, Finland) were given 30 min before a session. Xamoterol hemifumarate and procaterol HCl (Tocris) were given one hr before testing.

NE and cAMP

NE and cAMP were analyzed as described (Robinson et al., 1992; Zhang et al., 2004).

CNS Infusion

A double guide cannula (C235 system, Plastics One, Roanoke, VA) was implanted under pentobarbitol anesthesia (65–70 mg/kg ip) using a stereotax (SAS75/EM40M, Cartesian Research, Sandy, OR). The guide was placed −0.8 or −1.7 mm AP and 1.5 mm bilateral for intracerebroventricular (ICV) or intracerebral infusions, respectively (Frankland and Paxinos, 1997). The guide extended 0.5 mm from the base for intracortical and 1.5 mm for intrahippocampal and ICV infusions, and the dummy extended 0.5 mm below the guide. One week after recovery, bilateral infusions were made into conscious mice while gently holding the nape of the neck. The dual injection cannula extended 0.9 mm below the guide. Propranolol, betaxolol, and (±)-isoproterenol (Sigma) were dissolved in aCSF (in mM: 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1.2 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, and 10 dextrose); nadolol and atenolol were dissolved in saline. Infusions were 1 μl/side, 0.4 μl/min. The injection cannula was left in place for 30 s before the mouse was returned to its home cage (15 min for $Dbh^{+/-}$ and 30 min for $Dbh^{-/-}$ mice) prior to testing. For $Dbh^{-/-}$ mice, infusions 15 min before testing resulted in nonspecific freezing. Infusion location was assessed later using 1 μl of 1% methylene blue.

Hippocampal Slice Recording

Transverse hippocampal slices (400 um) were prepared from 10–14 week-old mice. Brains were rapidly removed, chilled, cut in ice-cold, oxygenated sucrose aCSF (in mM: 220 sucrose, 3 KCl, 1.25 NaH_2PO_4, 1.2 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, and 10 dextrose) and transferred to a holding chamber (BSC-PC, Harvard Apparatus, Holliston, MA) containing oxygenated aCSF. Slices recovered for at least 1.5 hr at 30°C. Individual slices were then transferred to an interface-recording chamber (BSC-BU, Harvard Apparatus) at 30°C and continuously perfused (1–2 ml/min). Recording began at least 30 min later using a CyberAmp preamplifier and an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA) in bridge mode. Data were digitized at a sampling rate of 10 kHz. Extracellular recordings were obtained from strata radiatum of CA1 using 5–10 MΩ glass microelectrodes filled with 2 M NaCl. A bipolar stimulating electrode (MCE-100, Rhodes Medical Instruments, Summerland, CA) was placed in stratum radiatum to stimulate the SC (0.1 ms, 1/min). Intensity was set to evoke 30%–40% of maximum. Tetanic stimulation consisted of 1 s trains at 100 Hz (4 min intertrain interval for 4 trains). Data were acquired and analyzed using pCLAMP 7 (Axon Instruments).

Statistics

Data were analyzed with *Statistica* 6.0 (StatSoft) using factorial two-way ANOVA, repeated measures two-way ANOVA or one-way ANOVA. For water maze, groups were first tested as to whether their quadrant distribution was significantly different from random (25%) with Hotelling's *T*-squared generalized means test using the log of the ratios of percentage quadrant time. All groups were found to be significantly nonrandom. Post hoc comparisons were made using Duncan's range test.

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