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# The role of norepinephrine in spatial reference and spatial working memory

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**Abstract**

The adrenergic system (utilizing norepinephrine, NE, as a neurotransmitter) is implicated in hippocampus-based learning and memory, in addition to its well known peripheral actions mediated by the sympathetic nervous system. We have produced a strain of mice in which the gene coding for the enzyme dopamine beta-hydroxylase (Dbh), which catalyzes the synthesis of NE from dopamine, has been disrupted. Mice recessive (Dbh-/-) for the Dbh gene mutation lack endogenous NE and epinephrine, while heterozygous mice (Dbh+/-) have normal levels of NE and epinephrine and display normal phenotype.

Previous studies have indicated that NE is necessary and sufficient for the retrieval of intermediate-term contextual and spatial memories, but is not necessary for the retrieval or consolidation of emotional memories in general (Thomas et al. 1996). We tested whether this relationship would stand for memories that were appetitive rather than aversive. We tested 20 Dbh-/- and 20 Dbh+/- mice in an eight-arm radial maze. We found no difference between KOs and controls in ability to recall spatial cues 24 hours after training. This negative result indicated that NE may not be critical for retrieval of all hippocampus-dependent memories but specifically those that are aversive.

Using a more standard variation of the above protocol on the radial arm maze, we used this apparatus to test the role of NE in spatial working memory. We found significant, robust differences between Dbh-/- and Dbh+/- mice after a training period of approximately 14 days. To test whether this difference was due to a potential deficit in acquisition or performance, we restored NE in Dbh-/- mice by administering the synthetic precursor L-DOPS after four days of stable behavioral differences between genotypes. In a separate trial, we also restored NE signaling with dexmedetomidine, a selective alpha-2 receptor agonist. A gradual improvement by Dbh-/- mice to levels comparable to Dbh+/- mice indicated that NE is critical for the acquisition of spatial working memory, and suggested a role for the alpha-2 adrenergic receptor in the processing of spatial working memory.

# The role of norepinephrine in spatial reference and spatial working memory

**Gertner, M.J. & Thomas, S.A. (2006)**

## Introduction

For the last year we have been exploring the effects of adrenergic signaling, concentrating on the neurotransmitter norepinephrine (NE), and its effects on hippocampal-based learning and memory. Previous rodent studies suggested that adrenergic signaling had a time-specific effect in consolidation and retrieval of spatial memories, but only in paradigms that produced an aversive stimulus. We sought to examine whether this same effect would be produced in a behavioral paradigm that was appetitive (involving a reward-based stimulus) but not aversive. When we found it did not reproduce, we used the same paradigm to test acquisition of short-term working memory, which may or may not be hippocampus-based. We found a critical role for adrenergic signaling in working memory, confirmed by both behavioral genetic tests as well as pharmacological manipulations that isolate the activity of norepinephrine.

## Background

Studies have already shown that the hippocampus is an important region for the acquisition and consolidation of explicit forms of declarative memories. Researchers have used animal paradigms that measure contextual learning (Anagnostaras et al., 2001) as well as spatial learning (Morris et al., 2003). In both cases, laboratory animals are forced to learn about their surroundings and relate them to a specific stimulus or group of stimuli. In both cases, however, the paradigms presented the animals with stimuli that evoked particularly emotional responses; Anagnostaras et al., for example, elicited fear conditioning responses via pairings of a tone and foot shock. These particularly emotional forms of memory were dependent on hippocampus function. Other paradigms that presented less emotional or aversive stimuli, such as the Morris water maze, were
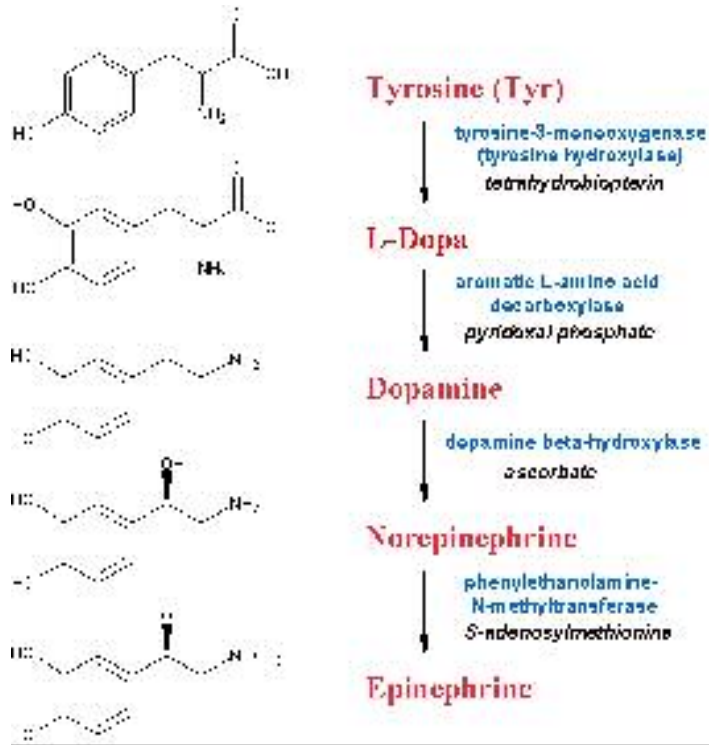
also shown to be dependent on hippocampus functioning in the acquisition of spatial memory (Morris et al., 1982). Yet the Morris water maze can also be considered somewhat aversive; laboratory animals are inherently afraid of water and will seek to escape it to dryer territory. With these two paradigms in place, researchers began to question the neurochemical mechanisms underlying hippocampal function in the formation of these emotionally-laden memories.

With many new studies focus on theories of learning and memory and different signaling systems that are activated during memory formation, the role for the adrenergic system in learning and memory remains controversial. Schroeter et al. (2000) showed that the hippocampus has one of the densest inputs of adrenergic terminals in the CNS through immunolocalization of the I-norepinephrine transporter. This supports hypothesis that norepinephrine and epinephrine (E) play a role in learning and memory. Most of the hypotheses have focused around adrenergic enhancement of memories that result from emotional events (Izquierdo and Medina, 1997). This enhancement could take place during memory acquisition, consolidation or retrieval, and several tests tried to determine which part of memory formation contains an adrenergic-dependent mechanism.

In order to target the adrenergic system as the specific variable for memory-dependent processes, we used mice that lack the gene coding for the enzyme dopamine Beta-hydroxylase (Thomas et al., 1995). These mice, which are homozygous recessive for the Dbh gene (-/-), lack the ability to synthesize norepinephrine from dopamine in noradrenergic terminals. Since epinephrine is endogenously synthesized from epinephrine, Dbh deficient mice lack both neurochemicals in all of its structures of

localization. The flow chart on the next page shows the synthesis of norepinephrine from dopamine, and illustrates how deletion of the Dbh gene can result in the elimination of all adrenergic chemicals from an animal.

Prior studies using Dbh knockout mice in an inhibitory avoidance paradigm suggest that NE and E may not be necessary for emotional memory consolidation (Thomas and Palmiter, 1997). Other studies using more aversive stimuli, however, seem to show that memory consolidation but not acquisition depends on adrenergic signaling. Knockout mice were tested for spatial navigation in a Morris water maze and were found to exhibit a deficit in retaining spatial memory two days after the last training session. However, when Dbh KO mice were tested two hours after the last training session, they were able to find the hidden platform just as well as controls (Thomas and Palmiter, 1997). These two studies seem to contradict each other, and more work needed to be done to identify which mechanisms of memory formation were actually dependent on NE/E.

Dbh KO mice were subjected to Pavlovian fear conditioning, which allowed the paradigm to study the mechanisms of acquisition, consolidation, and retrieval over time

(Murchison et al., 2004). The authors found that mice lacking NE/E exhibited impaired contextual but not cued fear memory one day after training. These results suggested that adrenergic signaling is critical for the retrieval of intermediate-term contextual and spatial memories, but not for the retrieval of emotional memories in general (Thomas et al., 2004). By injecting wild-type mice with antagonists to the beta-1 adrenergic receptor, we found that the role of norepinephrine in retrieval is mediated by a Beta-1 receptor-dependent mechanism in the hippocampus (Thomas et al. 2002). In 1999, Przybyslawski et al. found that the Beta-adrenergic antagonist propranolol impaired responses in a footshock-reinforced conditioned emotional response task, but only when the drug was administered after a reactivation trial. Other studies have found that Beta-adrenergic receptors can control hippocampal responses during recognition of emotional verbal responses in humans (Strange and Dolan, 2004). Overall, these studies indicated that adrenergic signaling was associated with the retrieval of emotion-related memory formation. The results currently provide a base for pharmacotherapies against syndromes such as Post Traumatic Stress Disorder. Beta-receptor antagonists might be promising pharmaceutical agents for attenuating debilitating emotional memories at the time of their reactivation, or retrieval (Przybylawski, et al., 1999). However, these adrenergic mechanisms might have more general functions with memory retrieval that do not necessarily involve emotional memories. In the case of the fear conditioning and the Morris water maze, memory formation occurred due to the presence of aversive stimuli. We sought to examine whether the same deficits in memory retrieval in Dbh -/- mice would be exhibited in paradigms that were appetitive rather than aversive.

We selected the radial arm maze as an appetitive behavioral task that would measure memory retrieval in Dbh -/- mice. Previous studies indicated that the radial arm maze can be used to assess two types of spatial memory: spatial working memory, measured by reentries into unbaited arms, and spatial reference memory, measured by first entries into unbaited arms (Olton and Honig, 1978). Formation of these memories may involve the hippocampus, but also involve different neuronal mechanisms (Bannerman et al., 2003). An agonist to the NMDA receptor, which functions in the formation of LTP and LTD in the hippocampus, decreases working memory errors but not reference memory errors (Pussinen and Sirvio, 1999). A more recent study showed that lesions to the dorsal hippocampus can disrupt both reference and working spatial memory, but lesions to the ventral side do not (Feldon, et al., 2004). These results confirmed that the radial arm maze induces activation of memory-formation processes involved in the hippocampus, the structure we wanted to target.

Before initiating our experiment, we checked to see if any prior studies had linked adrenergic signaling in the hippocampus with rodent models of spatial working or reference memory. Results proved to be inconclusive. We found that propranolol increased the amount of working memory errors in a three-panel runway task, but only when combined with the muscarinic antagonist scopolamine (Kobayashi et al., 1995). Mice lacking the alpha-2c adrenergic receptor made more working memory errors in a radial arm maze task, but only immediately after the baited arm was switched. This deficit in spatial working memory was alleviated by administration of an alpha-2c agonist (Bjorklund et al., 2001). Neither study was able to induce spatial reference memory errors.

Last year, we ran a pilot experiment that determined a 4-minute trial period was enough time for wild-type Dbh +/- mice to learn the radial arm maze paradigm and correctly recall 24 hours later.  The pilot experiment also demonstrated that mice should not be forced or "ushered" into the correct arm during training if they did not find it within 4 minutes.  The pilot experiment found no significant differences in acquisition rate between those mice that were ushered and those that were not.  Finally, the pilot concluded no sex differences in acquisition or retrieval within groups of Dbh +/- and Dbh -/- mice.

**Methods**

We assessed spatial reference memory and working memory in Dbh knockout mice by measuring their performance in an eight-arm radial arm maze (Olton and Honig, 1978).  The maze was created out of plexiglass with wells drilled at the ends of eight arms 22.7 cm in length attached to a center platform with a diameter of 17.7 cm. Attached to the sides of each arm were clear plexiglass walls measuring 10.5 cm high and about a quarter of an inch thick. A smaller wall also 10.5 cm high but measuring 6.4 cm length was placed at the end of each arm to prevent the mice from getting out.  No roof component was added.  The wells were drilled about 2.5 cm from the edge of each arm, and were drilled completely through the arm so that the bottom platform could be observed.  The maze rested on this bottom platform to facilitate its maneuverability.

The deep wells allowed food deposits (in the form of Coco Krispies) to be placed in each well, which signified a baited arm.  In addition, the walls at the end of each arm contained a small hole about 1/3 cm in diameter.  Taped across the hole was an additional food deposit, which was used to eliminate olfactory cues between arms.  Elimination of

olfactory cues provides one less variable to account for performance in a radial arm maze (Olton & Honig, 1978).

We instituted a 5-day habituation procedure during which mice were subjected to the novel food reward in their home environment, the radial arm maze without food, and the radial arm maze with food distributed evenly but sparsely. All mice were kept without food for the 23.5 hours before each experiment day. In order to ensure that mice retained 80% of their original body weight, mice were allowed to eat freely for 30 minutes after handling.

**Reference Memory test:** Training and acquisition occurred on the sixth day. Food-deprived mice were run in a series of four blocks of four trials each. During the trials, one of the arms was baited (females, arm #2, males, arm #6) with the food reward inside the well. 20 Dbh +/- and 20 Dbh -/- mice were placed at the end of one of the unbaited arms facing toward the center one at a time, and were allowed to explore to find the food reward. The pattern of arm placement was pseudorandomized, as the same start pattern was used for each mouse. Passage throughout the maze was recorded each time a mouse encountered a food well; the number of the well (1-8) and the time was recorded. Partial entries into arms that did not involve the mouse encountering the food well were not recorded. Trials were stopped when a mouse found the correctly baited arm and consumed reward or when 4 minutes passed. The same well remained baited throughout all of the trials. The maze was thoroughly cleaned between each trial to eliminate olfactory cues from other mice.

After the training/acquisition stage, mice were returned to home cage and food-deprived for 24 hours. Testing trials for extinction took place on day seven. Mice were

placed individually in a different pseudorandomized arm and allowed to explore. This time, none of the arms were baited, and time limitation for each trial was not necessary. The amount of time it took for the mouse to revisit the food well that was previously baited the day before was measured. Reference memory errors were marked as entries into incorrect arms preceding the correct arm. The extinction trials stopped when the mouse visited the previously baited food well. Each mouse ran six extinction trials. Once again, the maze was extensively cleaned between trials.

**Working Memory test:** After five days of habituation, mice were introduced to maze with four out of eight arms baited. Trials began with mice placed in the center platform. Trials ended when mice successfully retrieved all of the baits. Working memory errors were scored when a mouse entered an arm that it previously visited. No time limit was instituted during the trials.

One group of mice n=16 received one working memory trial per day, while another group (n=16) received 2 working memory trials per day, spaced 5 hours apart. Both groups contained an equal number of KO and wild type. Proximal and distal spatial cues were kept constant throughout all trials. Working memory errors were measured in both groups for 30 consecutive days.

**L-DOPS recovery:** This test was run after the working memory experiment and involved 20 Dbh +/- and 20 Dbh -/- mice. Mice were involved in the same procedure as working memory trials above. By day 20, acquisition rates were stabilized at significantly different levels between KOs and wild-type mice. At day 22, all mice received subcutaneous injections of 1mg/g L-DOPS, mixed with a peripheral decarboxylase inhibitor benserazide. Injections were given 5 hours before trials. At day 31, all mice

received injections of vehicle solution (saline).  At day 40, all mice were restored with L-DOPS.  Because of the observed 24-hour delay before L-DOPS recovery, we gave the mice no testing at day 46, followed by vehicle injection and testing on day 47 and L-DOPS injection and testing at day 48.   The period of no testing was so the mice would not shift maze strategies depending on whether their working memory was intact under L-DOPS or deficient under vehicle.   Alternating vehicle and L-DOPS injections examines whether acute changes in adrenergic signaling will affect working memory errors in Dbh -/- mice.

**Dexmedetomidine recovery:** At day 49, all mice were injected with L-DOPS and tested.  On day 50, all mice involved in the previous L-DOPS experiment were given a day of no testing.  This delay was to eliminate all L-DOPS that was injected 24 hours earlier.  On day 51, all mice were given a vehicle solution five hours before testing.  On day 52, the selective alpha-2 agonist, dexmedetomidine, was administered subcutaneously to all mice 30 minutes prior to testing.  We used a 5 ug/kg dose that was consistent with previous studies that observed dexmedetomidine-induced changes in working memory without any of its sedative effects (Tanila, et al. 1999; Thomas et al. unpublished).  On day 53, we administered vehicle solution to all mice 30 minutes prior to testing.

**Results**

**No significant differences in spatial reference memory errors were observed between Dbh +/- and Dbh -/- mice.**

We measured the number of reference memory errors the mice made during acquisition and extinction by marking each visit into an incorrect arm

Figure 6 shows the mean number of errors made during the acquisition stage for both KOs and controls. Mean errors for KO (32.6667 +/- 6.8605) were only slightly above mean errors for controls (29.8333 +/- 8.7730). Therefore the difference was not statistically significant (p>.05). . These results suggest no difference in learning behavior between KO and controls. Instead, they show that the two groups made the same amount of mistakes before they learned the correct arm.
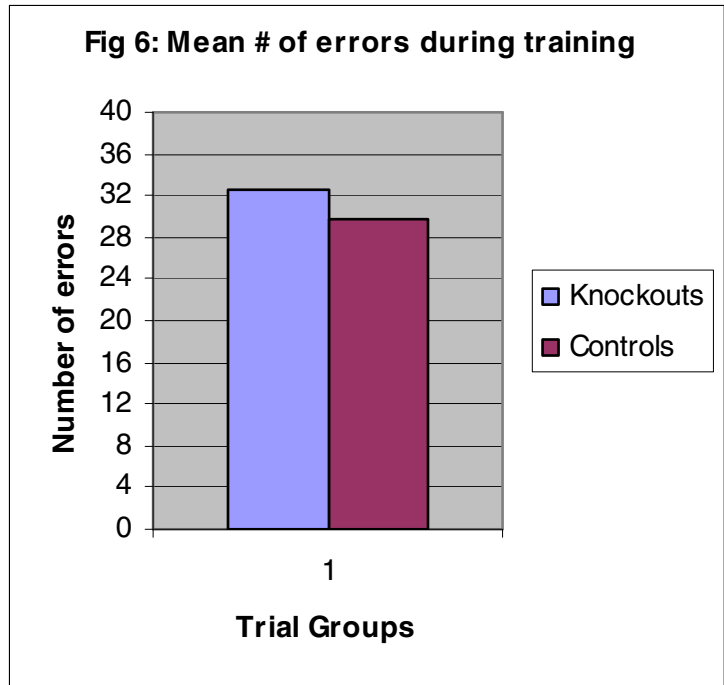
**Fig 6: Mean # of errors during training**

Figure 7 shows that the difference in mean number of errors was even less significant during the retrieval stage. KOs made slightly more errors (7 +/- 1.0954) than controls (6.6667 +/- 3.0768). The consistency between acquisition and retrieval remains apparent through measurements of mean number of errors, as it did for the measurement of mean latency. However, the almost similar amount of errors made by the KOs and the controls during the retrieval stage provides evidence against the theory that Dbh knockouts experience deficits

**Fig 7. Reference Memory errors during extinction**

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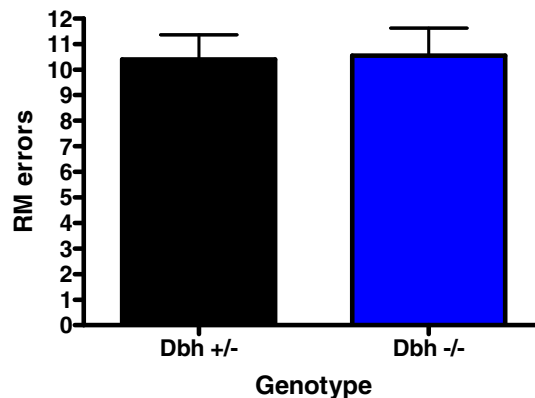
in memory retention in this task.

We also analyzed mouse performance over time by measuring acquisition rate on day 6 and extinction rate on day 7. On day 6, both Dbh +/- and Dbh -/- mice improved in performance across four blocks of four trials (Figure 8). By the fourth block, both KOs and wild-type were making

**Fig 8. Reference Memory acquisition**
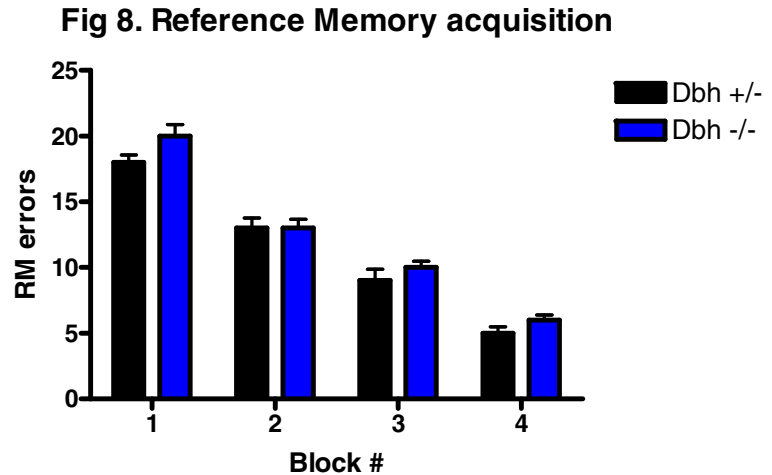
Dbh +/-
Dbh -/-

significantly less reference memory errors than the first block. However, at none of the four blocks were the differences in reference memory errors between Dbh +/- and Dbh -/- statistically significant (P>.05).

On day 7, controls and KO displayed a strong preference for the arm that had been baited the previous day. This preference remained strong for the first 3 trials, after which robust extinction occurred. As the mice

**Fig 9. Reference Memory extinction**
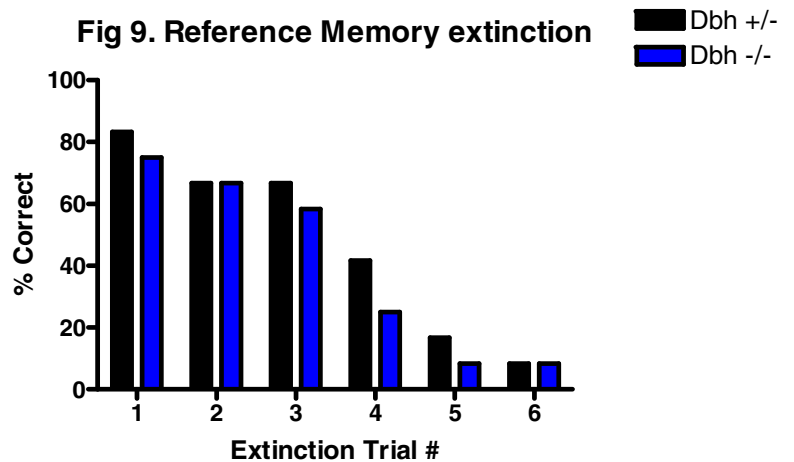
Dbh +/-
Dbh -/-

realized that the previously baited arm wasn't baited anymore, they began to search other arms. Yet the differences in performance between Dbh +/- and Dbh -/- mice were not significant (2-way ANOVA, p>.05). For example, 83% of Dbh +/- mice visited the correct arm on the first extinction trials, while 75% of Dbh -/- did so. None of the other extinction trials exhibited differences. By the sixth extinction trial less than 10 percent of

both KOs and wild-type were visiting the previously baited arm first. The results demonstrated that both mice were able to use spatial cues with relatively the same efficiency to learn the task and then retrieve and extinguish memory for this experience the following day. This suggested that norepinephrine does not play a role in the acquisition or 24-hour retention of an appetitively motivated spatial reference memory.

**Dbh +/- mice and Dbh -/- mice did show significant differences in acquisition of spatial working memory.**

Dbh +/- mice made significantly fewer working memory errors than Dbh -/- mice after 14 days, regardless whether the mice were given one (Figure 10) or two (Figure 11) acquisition trials per day. Both controls and wild-type improved over time. Data was combined for the 1trial/day mice so that each bin was the average number of errors over every 2 trials (Figure 10). Data was smoothed for the 2trial/day mice so that each bin was the average errors over 4 trials (Figure 11). The 2 trial/day mice were given 60 trials overall while the 1trial/day mice were given 30 trials overall. Differences were not apparent until 14



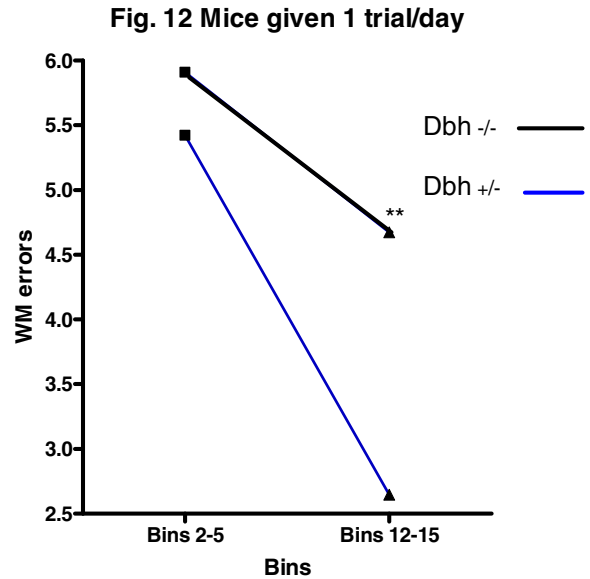**Fig. 10 Working Memory acquisition**
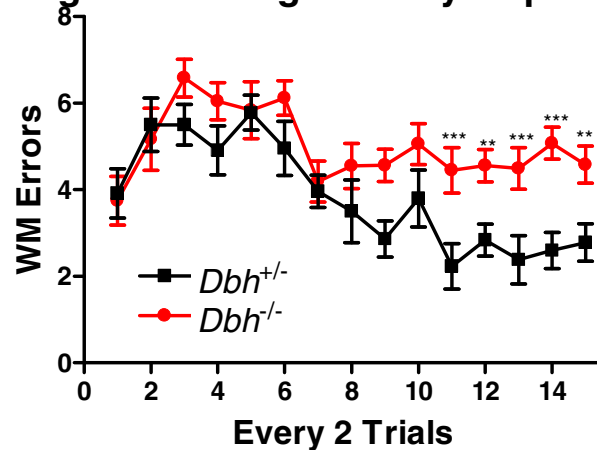


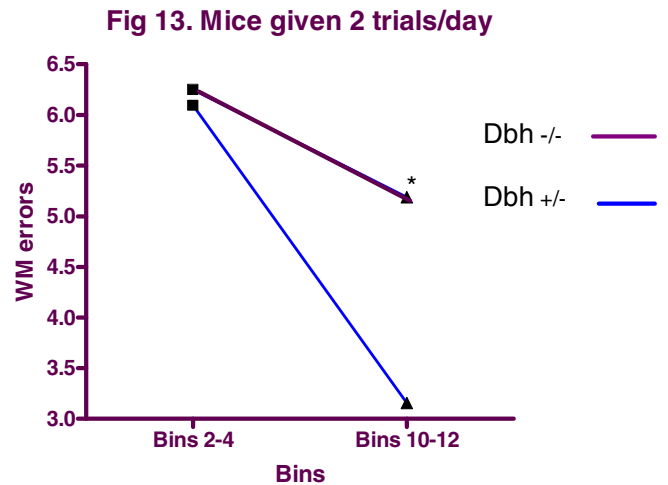**Fig 11. Working Memory acquisition**
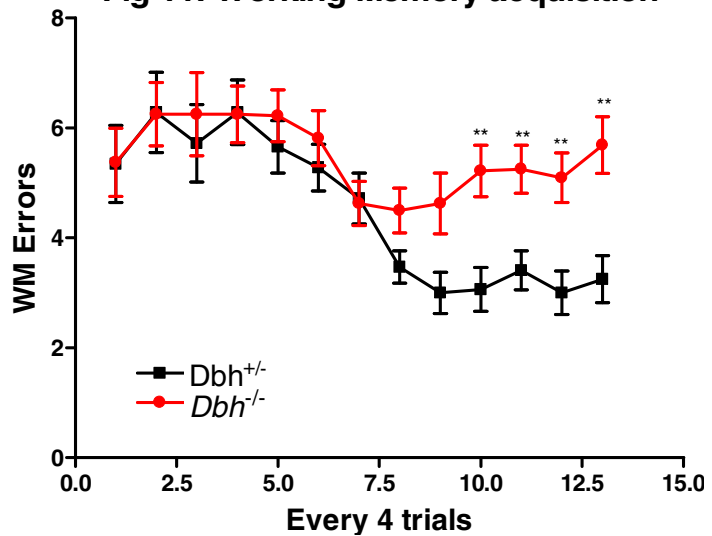
days of acquisition (28 trials for the 2 trial per day mice). These results indicate that the 2 trial per day mice did not learn any faster than the 1 trial per day mice. It may be that the time between trials is in the critical variable for acquisition.

Performance improved for Dbh +/- and Dbh -/- mice over time, but clearly demonstrated a greater improvement for wild-type mice. For Dbh +/- mice given one trial per day, working memory errors declined from an average of 5.5 across four early bins to just over 2.5 across the last four bins (bins consisted of 2 trials). Dbh -/- improved from an average of 6 WM errors to just under 5 WM errors across the same time interval (Figure 12). The difference in WM errors at the end of the 30 trials was significant at the .05 level.

**Fig. 12 Mice given 1 trial/day**

For the mice given 2 trials per day, wild-type mice made improved from an average just over 6.0 WM errors per trial to an average just over 3.0 WM errors per trial. Knockout mice started at almost exactly the same performance but only improved to an average of approximately 5.25 WM errors per trial. These averages were across three early bins (trials 5-16) and three later bins (trials 37-48). The difference in final stabilized

**Fig 13. Mice given 2 trials/day**

performance between Dbh +/- and Dbh -/- mice after 60 total trials (30 days, 2 trials per day) was significant at the .05 level.

**The differences in working memory acquisition between Dbh -/- and Dbh +/- mice were eliminated with injection of L-DOPS 5 hours before testing.**

After robust differences in working memory acquisition developed between Dbh +/- and Dbh -/- mice, L-DOPS injected at day 22 attenuated the deficits in Dbh -/- mice over a period of about six days. After WM acquisition levels stabilized for a few days, mice were taken off L-DOPS and injected with vehicle solution 5 hours before training starting on day 31. By day 35, deficits in WM acquisition were restored in Dbh -/- mice. At day 40, L-DOPS was once again injected into both control and KO mice. This time, a sharp improvement was observed in Dbh -/- performance, as significant deficits were eliminated by day 42 (Total experiment shown in Figure 14).

These results showed that L-DOPS injection at day 22 produced a gradual attenuation of deficits in spatial working memory in Dbh -/- mice, to levels comparable



**Fig. 14 L-DOPS recovery of Working Memory acquisition**
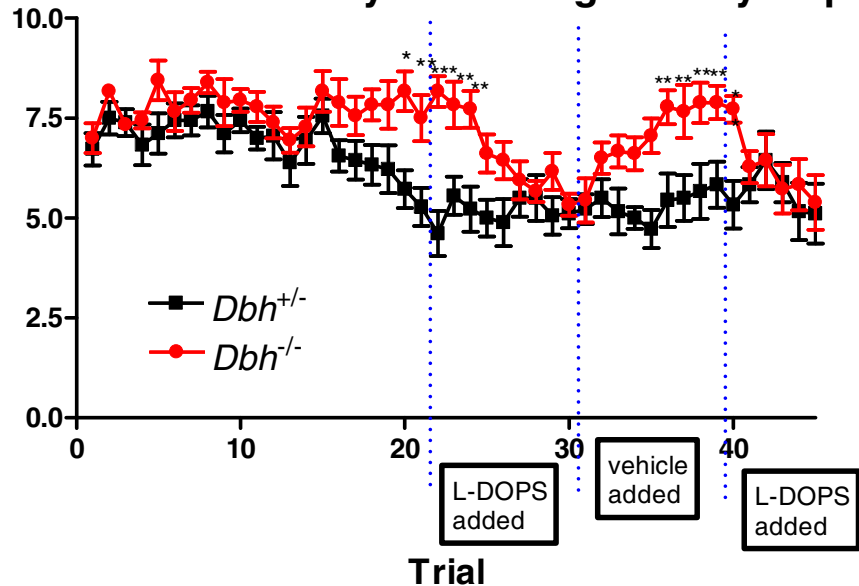
to Dbh +/- mice. These deficits were restored relatively quickly when mice were taken

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off L-DOPS and injected with vehicle. When L-DOPS was restored after 9 days of vehicle treatment, the deficits were attenuated again, but a delay period of 24 hours was observed before the deficits in Dbh -/- was eliminated. This delay suggests that the Dbh -/- mice were shifting maze strategies when they were under L-DOPS and when they were under vehicle. There was also no observed improvement in the control mice after L-DOPS injection, indicating that the Dbh +/- mice acquired the task at their maximal level of performance.

In an attempt to eliminate the shifting of strategies, we subjected the mice to a day of no testing (day 46) followed by alternating days of vehicle and L-DOPS. We found that the delays between injections and observed effects were eliminated when the mice were given a day of no testing. Dbh -/- mice were severely impaired after injection of vehicle on day 47, but were restored to normal WM levels after L-DOPS injection on day 48. This suggests that the mice were operating under an L-DOPS-induced strategy, since they had received L-DOPS prior to the day of no testing.

**Dexmedetomidine, a selective alpha-2 agonist, attenuates deficits in spatial working memory in Dbh -/- mice after a 5ug/kg dose was injected 30 minutes before testing.**

A subcutaneous injection of 5ug/kg dose of a selective alpha-2 agonist, dexmedetomidine, attenuated the WM deficits in Dbh -/- mice (Figure 15). Injection of vehicle on day 51 after a period of no testing (day 52) produced an increase in WM errors



Fig. 15 Dexmedetomidine Recovery

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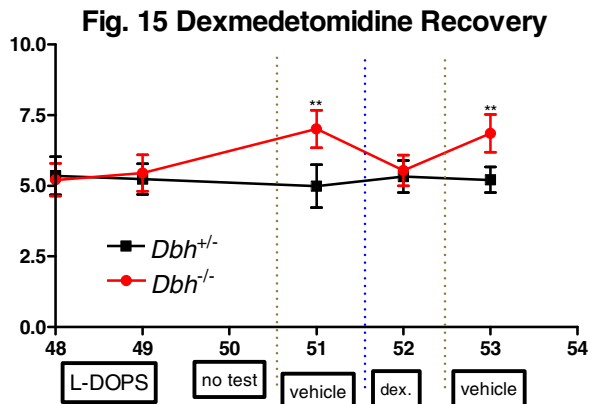
in Dbh -/- mice to a level comparable to previous performance. The day of no testing showed that the mice did not shift maze strategies from when they were under L-DOPS to when they were under vehicle treatment. On day 52, dexmedetomidine brought back WM performance in Dbh -/- mice to levels similar to Dbh +/- mice when injected 30 minutes before testing. On day 53, all mice were administered vehicle, this time 30 minutes before testing instead of five hours before testing. Deficits in WM were once again observed in Dbh -/- mice on day 53.

**Conclusions**

**Reference Memory**

The lack of differences between Dbh +/- and Dbh -/- mice in the appetitive spatial reference memory paradigm suggests a more specific role for norepinephrine in the retrieval of aversive spatial reference memory. Previous deficits in the retrieval of contextual and spatial memories involved aversive paradigms such as Morris water maze and fear conditioning. The radial arm maze involves learning that is appetitive and minimally aversive. Therefore, our results are negative but interesting, that norepinephrine's role in retrieval of spatial and contextual memories may be limited to memories that are aversively motivated.

The role of norepinephrine in the "fight or flight response" of the sympathetic nervous system would support the idea that norepinephrine is linked to retrieval of aversive spatial and contextual memories. We also know that this role for norepinephrine is even more defined, since it is critical for the retrieval of contextual but not cued fear conditioning (Murchison et al. 2004). Therefore, our results suggest that norepinephrine plays a role in the retrieval of spatial or contextual memories that are aversively

motivated, but does not play a role in the retrieval of emotional memories in general. The peripheral actions of norepinephrine, which involve the adrenal gland and stress response, seem to corroborate this idea. Norepinephrine and its receptor family are necessary and sufficient for the immediate-term retrieval of aversive stimuli, but are not needed for the learning and retrieval of other reward-based stimuli. The findings suggest that another mechanism in the hippocampus modulates the retrieval of appetitively motivated contextual and spatial memories. Alternatively, appetitive and aversive memories utilize the same mechanisms for retrieval, except that aversive hippocampus-dependent memory retrieval additionally requires adrenergic signaling.

**Working memory**

Our studies found a critical role for norepinephrine in the acquisition of working memory using a more standard protocol of the radial arm maze. This task was not considered aversive, yet still produced robust deficits between Dbh +/- and Dbh -/- mice after 14 days of training. The measurement of working memory errors involved a shorter time component than the test involving reference memory retrieval, but still measured acquisition as a change over time. Dbh +/- mice were able to perform the task better after 14 days while Dbh -/- mice were still making the same number of errors, indicating that something was not allowing them to fully acquire the task and reduce errors to the same level as the Dbh +/- mice.

An attenuation of the working memory deficits after injection of the NE synthetic precursor L-DOPS provided further evidence that NE has a critical role in the acquisition of spatial memory. A gradual improvement by the Dbh -/- mice to levels attained by Dbh +/- mice suggested that NE is necessary for the acquisition of the spatial working

memory task  Trial-by trial observations (Figure 14) showed that Dbh -/- mice showed roughly the same acquisition curve after L-DOPS injection as Dbh +/- showed after 14 days but before L-DOPS injection.  This provided further evidence that NE, which is restored to the CNS by L-DOPS, is critical for the acquisition of the working memory version of the radial arm maze paradigm.

The role of NE in acquisition of working memory tasks was further supported when working memory deficits returned in Dbh -/- mice after vehicle injection 5 hours before testing.  L-DOPS provides an acute restoration of NE, but its levels wear off after 24-48 hours.  The vehicle, which consisted of simple saline, did not add any NE and therefore increased the number of WM errors by Dbh -/- to levels that were once again statistically significant.  Performance in Dbh +/- mice stayed relatively the same throughout the whole experiment, indicating that elevating NE beyond control levels does not affect acquisition of performance of this task.

When L-DOPS was reintroduced to the Dbh -/- mice at day 40, we saw a sharp attenuation of deficits to levels comparable to Dbh +/- mice.  This less gradual decline suggests that NE is critical for working memory performance.  No longer is acquisition needed for the Dbh -/- mice; the NE restored by L-DOPS allows them to function just as well as wild-types.  However, the 24-hour delay between L-DOPS injection and recovery suggests that the Dbh -/- mice may require a day to shift performance-based strategies from performance without NE to thatwhe n NE is present. The reintroduction of L-DOPS recovery further that the NE-dependent effects on working memory acquisition are not based on performance alone.  NE improves working memory in Dbh -/- mice after they have acquired aspects of the paradigm.

We were able to eliminate the shifting of strategies by giving the mice a day off in between testing (day 46), after which we alternated between vehicle and L-DOPS injection. The immediate reduction in performance by Dbh -/- mice after vehicle injection suggests that the mice were operating under a strategy induced by L-DOPS, and did not shift their strategy in the time after vehicle injection but preceding testing. When L-DOPS was administered at day 48, Dbh -/- performance immediately dropped back to normal the same day. This same effect was observed with administration of the alpha-2 agonist dexmedetomidine at day 52, after days of no testing and vehicle injection. By giving the mice a day without testing, we altered the effects of our pharmacologic methods from those of gradual change to an immediate yet robust change. The acute effects of both L-DOPS and dexmedetomidine suggests that the Dbh -/- mice might be able to compensate somewhat by assuming a performance-based strategy when they lack drugs necessary for proper functioning of WM. This compensation period could explain why our pharmacological methods done at days 22 and 31 resulted in gradual changes in performance, as opposed to the acute changes at days 48 and 52.

**Discussion**

This improvement over time begs the question of whether there are two memory-dependent processes at work here. It is well known that working memory, as well as some short term memory formation, is modulated by structures in the prefrontal cortical area and not necessarily by hippocampus, which has been the central focus of our memory studies discussed so far. However, the acquisition of our working memory procedure over time may involve hippocampal-based processes, since both groups of mice had to recall information learned from day to day. Dbh +/- mice displayed an

acquisition curve that plateaud at fewer errors, indicative of performance improvement. Dbh -/- mice exhibited the same acquisition curve only after L-DOPS injection. Performance then worsened in Dbh -/- mice when vehicle was administered. Interestingly, the Dbh -/- mice did not display the same gradual acquisition curve after reintroduction of L-DOPS. Instead, the attenuation of deficits was complete by the second day. This suggests that the Dbh -/- mice were recalling information that had been learned prior to the reintroduction of L-DOPS at day 40. Others have suggested that this retrieval process could involve the hippocampus as well as prefrontal cortex. Further studies in which one of the two areas are inactivated or ablated are needed to identify the role of each structure in spatial working memory in Dbh deficient mice.

The gradual changes in performance in Dbh -/- mice after introduction or removal of L-DOPS could be caused by a shifting of maze strategies between an L-DOPS-induced state and a state when no L-DOPS is on board. It is unclear how exactly this alternation of strategies could affect performance; however, we observed that Dbh -/- exhibited more immediate changes in performance after a day of no testing. The day of no testing could have affected performance by keeping the mice in an L-DOPS induced state even when vehicle was injected. We then saw a rapid increase in WM errors, followed by rapid improvement after L-DOPS was injected 24 hours later. This same effect, at a slightly lesser scale, was observed with the alpha-2 agonist dexmedetomidine. By alternating days of no testing, vehicle, and treatment, we were able to determine that our drugs do have acute effects on mice performance that may be compensated by an innate cognitive mechanism that has yet to be fully investigated.

Finally, our reference memory tests provided a negative result that further confirmed that Dbh deficient mice exhibit time-specific deficits in memory retrieval only in aversive paradigms.  The role of NE and E in the mammalian stress response suggests that the two neurotransmitters are critical in modulating emotional types of behavior.  Our results suggest that NE and E might not be critical in modulating reward-based spatial reference memory retrieval.  The role of NE in modulating aversive memory retrieval could have implications for the treatment of stress-related memory disorders, such as Post Traumatic Stress Disorder.  But NE and E do not appear to be required for all emotionally laden memories, such as appetitive memories and aversive memories that do not depend on the hippocampus.

**Future studies**

The lack of improvement of Dbh +/- mice after L-DOPS treatment indicated that control mice were operating at close to maximum efficiency in learning the maze.  The radial arm maze allowed for the test of separate memory pathways through modifications of protocol.  Now that hypothesized deficits in working memory have been identified, we could test whether these deficits would still be apparent in an aversive paradigm.  In a procedure opposite to our reference memory tests, we could analyze the extent of working memory deficits in aversive test, such as Morris water maze.  However, studies have shown that NE agonists attenuate working memory deficits in other appetitive tasks such as spatial delayed alternation tasks or spatial discrimination control tasks (Birnbaum et al. 2000).  These studies suggest that NE might have a role in spatial working memory regardless of the nature of the behavioral task.

Further pharmacological tests could involve injections of an alpha-2 receptor agonist, which is thought to modulate spatial working memory (Franowicz et al. 2002). This test will elucidate the mechanisms of working memory at the receptor level. Other studies specifically point to the alpha-2 receptor as a modulator of working memory in the prefrontal area of the cortex (Mao et al. 1999). While adrenergic input into the hippocampus has been well documented, noradrenergic signaling in the prefrontal cortex has also been demonstrated (Mao et al. 1999). Histology or immunocytochemistry analysis could be used to isolate the pathway of noradrenergic input from locus coeruleus into the prefrontal cortex and whether these pathways share connections with the hippocampus (Aston-Jones & Cohen, 2005). One could attempt to isolate specific pathways that may be functioning during the acquisition of reference memory and during the acquisition of working memory, since they seem to be distinct in our behavioral and pharmacological assays.

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