Published in final edited form as:

Brain Res Bull. 2008 December 16; 77(6): 356–360. doi:10.1016/j.brainresbull.2008.08.016.

Selective Lesion of Septal Cholinergic Neurons in Rats Impairs Acquisition of a Delayed Matching to Position T-maze Task by Delaying the Shift from a Response to a Place Strategy

Nicholas F. Fitz¹, Robert B. Gibbs², and David A. Johnson^{1,*}

¹Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA 15282, USA

²Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, Pittsburgh, PA 15261, USA

Abstract

This study tested the hypothesis that septal cholinergic lesions impair acquisition of a delayed matching to position (DMP) T-maze task in male rats by affecting learning strategy. Rats received either the selective cholinergic immunotoxin, 192 IgG-saporin (SAP) or artificial cerebrospinal fluid directly into the medial septum. Two weeks later, animals were trained to acquire the DMP task. SAP treated rats took significantly longer to acquire the task than corresponding controls. Both SAP-treated and control rats adopted a persistent turn and utilized a response strategy during early periods of training. By the time rats reached criterion the persistent turn was no longer evident, and all rats had shifted to an allocentric strategy, i.e., were relying on extra maze cues to a significant degree. During the acquisition period, SAP-treated rats spent significantly more days showing a persistent turn and using a response strategy than corresponding controls. The added time spent using a response strategy accounted entirely for the added days required to reach criterion among the SAP-treated rats. This suggests that the principal mechanism by which septal cholinergic lesions impair DMP acquisition in male rats is by increasing the predisposition to use a response vs. a place strategy, thereby affecting the ability to switch from one strategy to another.

Keywords

hippocampus; cholinergic denervation; place strategy; response strategy; spatial orientation

INTRODUCTION

Cholinergic neurons of the medial septum (MS) that project to the hippocampus play an important role in learning, memory, and attentional processes, and have long been associated with the acquisition of working memory tasks [1,5,6,19]. More recently, investigations utilizing the selective cholinergic immunotoxin 192-IgG Saporin (SAP) have found that lesioning cholinergic neurons of the MS has little or no effect on working memory in tests such as the Morris water maze [4,13,17, for a review see 32]. However, this same lesion significantly

*Corresponding Author: David A. Johnson, Ph.D., Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA 15282, Phone: 412-396-5952, FAX: 412-396-4660, E-mail: johnsond@duq.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

delays acquisition of a delayed matching-to-position (DMP) T-maze task, which also requires intact working memory [20,21,25]. We previously reported that introduction of a mild aversive stimulus each day prior to training completely abolished the impairment in DMP acquisition produced by the cholinergic lesion in male rats [20]. This suggests that the effects of the cholinergic lesions on learning are, in part, dependent on the aversive or stressful nature of the task.

Rats can utilize a number of different strategies to solve spatial tasks [for a review see 18]. For example, an animal may utilize a consistent directional body turn to navigate a maze using either egocentric or allocentric cues, or a spatial strategy that relies on the location of extra maze visual cues [15,30,31]. Evidence suggests that turning strategies rely primarily on striatal circuits whereas spatial strategies rely more on hippocampal circuits [10,16,31,36]. As an example, when the dorsal hippocampus was inactivated via an infusion of lidocaine, use of a place strategy was inhibited [30]. Conversely, following stimulation of the dorsal hippocampus with glutamate, rodents preferentially chose a place learning strategy [30]. Therefore, another mechanism by which MS cholinergic lesions could affect DMP acquisition independent of working memory is by affecting the use and/or preservation of a place or response strategy. The purpose of this study was to test the hypothesis that MS cholinergic lesions impair DMP acquisition in male rats by causing alterations in the adoption and/or preservation of the learning strategies used within the DMP task.

MATERIALS AND METHODS

1. Intraseptal infusions

All experiments followed NIH guidelines for the care and use of laboratory animals and were approved by the Duquesne University Institutional Animal Care and Use Committee. All chemicals were purchased through Sigma Inc., St. Louis, MO unless stated otherwise. Male Sprague–Dawley rats (275–300 g) were purchased from Hilltop Lab Animals (Scottsdale, PA) and housed individually in a temperature and humidity controlled facility on a 12:12 h light: dark cycle and *ad libitum* access to food and water. For intraseptal infusion of SAP, animals were anesthetized with pentobarbital (50 mg/kg; IP; Ovation Pharmaceuticals, Deerfield, IL) and placed into a stereotaxic frame (Stoeling Co., Wooddale, IL). An incision was made exposing the dorsal aspect of the skull and a hole drilled over the MS (+0.2 bregma, 0.0 lateral). A stainless steel cannula (28 gauge, Plastics One Inc., Roanoke, VA) was lowered 5.6 mm from dura into the MS. Animals were infused with either 1 μ l of vehicle (artificial cerebrospinal fluid; CMA Inc., North Chelmsford, MA) or SAP (0.20 μ g in 1 μ l of artificial cerebrospinal fluid; Advanced Targeting Systems, San Diego, CA, Lot # 24–87) over 5 min at 0.2 μ l/min using a syringe pump (Harvard Apparatus, Holliston, MA). The dose of SAP was selected based on previous studies that demonstrated a substantial loss of cholinergic neurons in the MS with little non-selective damage to GABAergic neurons [25]. Following infusion, the cannula remained in place for 5 min to allow diffusion of the solution into the tissue. The incision was closed and animals were allowed to recover for 2 weeks before behavioral testing.

2. Behavioral testing

a. Delayed Matching to Position—Fourteen days following surgery, rats were trained to acquire the DMP task as recently described [20,21]. Rats were food deprived to 85% of their normal body weight and then adapted to the T-maze by placing them into the maze with sweetened reward pellets (45 mg Noyes pellets; Research Diets; New Brunswick, NJ) once per day for 5 days. Rats were then trained to run to the ends of each goal arm by using a series of six forced choices per day for 3 days. To avoid the introduction of side bias, right and left goal arms were alternated in a random and balanced fashion. Animals then began DMP training. Each rat received eight trial pairs per day. The first trial consisted of a forced choice in which

one of the goal arms was closed, forcing the animal to enter the open goal arm to receive a reward (two pellets). The rat was immediately returned to the approach alley for an open choice trial during which both goal arms could be entered. Returning to the same goal arm entered during the forced trial resulted in a reward (four pellets). Entering the opposite arm resulted in no reward and confinement in the goal arm for 10 s. Rats were run in groups of three or four. After each trial pair, rats were returned to their cages for 5–10 min, while training proceeded on the remaining animals of the group. Goal arms were varied in a random balanced fashion. Rats continued to receive eight trial pairs per day until a criterion of 15 correct choices out of 16 consecutive open choice trials was met. The number of days required to reach criterion was recorded.

b. Strategy Testing—Four additional groups of 8 animals each (two SAP treated and two control) were trained as above until one of two criteria was reached: one SAP and one control group was trained until the rats adopted a persistent turn during the open choice trial; defined as 15 out of 16 repeated entries into the right or left goal arm. The other SAP and control groups were trained until they acquired the DMP criterion defined as 15 correct choices out of 16 consecutive trial pairs.

For each group, once the criterion was reached, rats then received an initial training session consisting of eight trial pairs as described above. Four to six hours later, a second training session was performed consisting of eight trial pairs in which the maze was rotated 180° between the forced and open choice. This was done to evaluate the use of place vs. response strategies. Use of a response strategy was defined as re-entering the same physical arm of the maze that was visited on the preceding forced trial regardless of position in the room on at least 7 out of 8 consecutive trials. Use of a place strategy was defined as entering the arm located in the same position of the room that was visited on the preceding forced trial in at least 7 out of 8 consecutive trials. If neither of these criteria were met, the rat was categorized as having been disrupted in its behavioral strategy. So as not to disrupt the established learning strategy for animals that had acquired the DMP task, rats were rewarded no matter which arm was entered during the open choice trial. However, animals that displayed a persistent turning strategy were rewarded only if they entered the correct goal arm. Therefore, if the rat utilized a persistent turning strategy it would be rewarded only 50% of the time.

3. Choline acetyltransferase activity

Following the completion of behavioral training, animals were killed and ChAT activity in the hippocampus and frontal cortex was measured as previously described [22]. Briefly, animals were anesthetized with pentobarbital (100 mg/kg; IP), the brains removed, and tissues from the hippocampus and frontal cortex dissected, frozen, and processed. Tissues were not pooled. Frozen tissues were thawed and dissociated by sonication in a medium containing EDTA (10 mM) and Triton X-100 (0.5%) and diluted to a concentration of 10 mg tissue/ml. An aliquot of each sample was used for the determination of total protein [9]. Three 5 µl aliquots of each sample were incubated for 30 min at 37 °C in a medium containing ³[H] acetyl-CoA (50,000–60,000 d.p.m./tube, final concentration 0.25 mM acetyl-CoA), choline chloride (10.0 mM), physostigmine sulfate (0.2 mM), NaCl (300 mM), sodium phosphate buffer (pH 7.4, 50 mM), and EDTA (10 mM). The reaction was terminated with 4 ml sodium phosphate buffer (10 mM) followed by the addition of 1.6 ml of acetonitrile containing 5 mg/ml tetraphenylboron. The amount of ³[H] acetylcholine produced was determined by adding 8 ml of EconoFluor scintillation cocktail (Packard Instruments, Meriden, CT) and counting total cpm in the organic phase using an LKB beta-counter. Background was determined using identical tubes to which no sample was added. For each sample, the three reaction tubes containing sample were averaged and the difference between total cpm and background cpm was used to estimate the

total amount of ACh produced per sample. ChAT activity was then calculated for each sample as pmol ACh manufactured/h/ μ g protein.

4. Statistical analysis

The number of days required to reach criterion, and the numbers of days rats used a response strategy, were compared between SAP-treated rats and controls using a Student's T-test. To compare SAP treatment effects during different stages of acquisition, performance data were blocked into training periods of 5 days each (a total of 6 periods). For blocked data, once an animal achieved criterion based on previous studies, a performance value of 93.8% was assigned for the following days until day 30 at the end of block 6. Performance during acquisition testing was analyzed using two-way ANOVA with repeated measures on training period, and Student's T-test for treatment effects within training periods. A Fisher's Exact test was used to evaluate disruptions of response strategy following spatial manipulation. Statistical significance was defined as $p < 0.05$. GraphPad Prism 3.0 was utilized for all statistical analyses.

RESULTS

ChAT Activity

SAP infusion into the MS produced significant reductions in hippocampal ChAT activity relative to controls (5.49 ± 0.43 vs. 37.79 ± 1.56 pmol ACh/hr/ μ g protein; $p < 0.001$). There was non-significant decrease in ChAT activity in the frontal cortex of animals that received SAP infusion (31.07 ± 2.25 control vs. 25.74 ± 2.94 SAP; $p = 0.06$).

Acquisition of the DMP T-maze task

Two-way ANOVA of acquisition data blocked over 5 day training periods (Fig 1), showed significant effects by training period [$F(16,1,5) = 18.97$, $p < 0.0001$], treatment [$F = 86.73$, $p < 0.0001$] and interaction [$F = 4.14$, $p = 0.002$]. Student's T-test for training periods 3 (days 11–15) and 4 (days 16–20) revealed significantly decreased performance in the SAP treated group compared to controls (period 3, $p = 0.004$; period 4, $p = 0.003$).

SAP-treated rats took significantly longer to reach criterion on the DMP task than corresponding controls (21.6 ± 1.3 days vs. 16.0 ± 0.4 days; $p = 0.001$; Fig 2). Analysis showed that the total number of days that rats adopted a persistent turn was significantly greater for SAP treated rats than for controls (14.78 ± 1.76 days vs. 8.13 ± 1.66 days; $p = 0.001$; Fig 3). There was no significant difference between groups in the total number of days that a persistent turn was not evident. Subtracting the number of days during which a persistent turn was evident from the number of days required to reach criterion eliminated the difference between the SAP-treated rats and controls on this measure (7.44 ± 1.25 vs. 8.25 ± 1.41 days; $p = 0.63$).

Strategy Testing

For strategy testing, four additional groups of 8 rats each were trained. Two groups were treated with SAP and two with artificial CSF. One SAP treated animal failed to run the maze. At the time that rats adopted a persistent turn, rotating the maze 180° between the forced and open choice resulted in the majority of rats meeting the criterion for using a response strategy, while no rats met the criterion for using a place strategy. Two of eight rats in the control group, and 3 of seven rats in the SAP-treated group showed a disruption of performance as defined above (Fig 4a and 4b). In the set of control and SAP-treated rats that were trained until the DMP task was acquired, rotating the maze 180° resulted in no rats meeting the criterion for using a response strategy. Rather, 7 out of 8 controls, and in 8 out of 8 SAP-treated rats demonstrated a disruption in performance, indicating the use of a place strategy (Figure 4c and 4d).

DISCUSSION

This study replicates our previous work [21,22,25] demonstrating that septal cholinergic lesion delays DMP acquisition in rats. The number of days needed to acquire the task in the SAP group in this study was nearly identical to the SAP group in our initial investigation [25] confirming the efficacy and selectivity of the SAP dose.

This study extends our previous work by showing that the delay in acquisition of the DMP task by SAP treated animals was the result of the increased number of days SAP animals utilized a persistent turning strategy. The time it took the rats to reach criterion in the DMP task was, in part, a function of perseveration in maintaining the egocentric response strategy before adopting the more efficient place strategy. The enhanced perseveration of SAP treated animals for utilizing the response strategy in the DMP T-maze task is consistent with other studies demonstrating behavioral perseverations in rats with impaired hippocampal function [2,27, 35]. However, in those studies the hippocampal impairments were not the result of selective loss of cholinergic neurons. It is notable that the degree of perseveration in both control and SAP treatment groups was evident over 8 trial pairs even when the “correct” choice resulted in reward only 50% of the time.

Since cholinergic neurons of the basal ganglia are not affected by SAP [8], the mechanism for perseveration of the response strategy in SAP animals in this study is likely the result of a decrease in the ratio of ACh release in the hippocampus relative to the basal ganglia. Work by Gold and co-workers showed that the release of ACh in the hippocampus and caudate nucleus changes during training, and that the *ratio* of ACh release in the hippocampus and caudate could accurately reflect the use of place and response strategies (see [23] for review). For example, Pynch *et al.* [33] demonstrated that cholinergic tone is proportionally greater in the hippocampus than in the basal ganglia of rats utilizing a place strategy. Conversely, relative cholinergic tone was elevated in the basal ganglia compared to the hippocampus when rats were utilizing a response strategy. These findings are consistent with our results that show that cholinergic deafferentation of the hippocampus resulted in an increased predisposition to use a response vs. a place strategy during DMP acquisition. Even so, rats with depletion of hippocampal ACh were able to adopt a spatial strategy to reach criterion on the task, indicating that cholinergic projections to the hippocampus facilitate, but are not required, to adopt such a strategy.

A study by Cahill and Baxter tested strategy selection in rats using a cross-maze test. In contrast to the current study, they found that rats trained to approach a baited arm initially used a place strategy, and then shifted to a response strategy [12,31]. However, the two paradigms differ in that in the DMP task, unlike the cross-maze, the reward arm is changed in a semi-random fashion from the beginning of training. Therefore, for both treatment groups, the use of a response strategy early in training would enhance the rate of reward beyond that of the alternation strategy initially adopted by the animals.

In another study, Bizon *et al.* [7] found that hippocampal ACh was not necessary for spatial learning in rats in the Morris Water maze (MWM), and demonstrated that depletion of hippocampal ACh by SAP resulted in animals being more likely to use a place strategy. The use of a place strategy in rats with depleted hippocampal ACh is consistent with the results of the current investigation. However, the difference in the results between the studies relates more to perseveration in the use of a response strategy in the DMP task than the capacity of rats with hippocampal ACh depletion to use a place strategy *per se*. As with Cahill and Baxter study, differences in the use of strategies by the rats in the two studies were likely a function of the particular testing paradigms. In particular, the environment of the MWM is aversive while the DMP T-maze is not. Previous work in our laboratory demonstrated that the

introduction of a mild aversive stimulus into the DMP T-maze task paradigm abolished the delay in acquisition of the task in rats with septal cholinergic lesion [20]. To the extent that arousal decreases perseveration in rats with hippocampal depletion of ACh suggests that the role of ACh in the hippocampus may be related to an overall modulation of hippocampal activity than absolute function, and that activation of the hippocampus via other neuronal afferents could compensate for the loss stimulatory tone mediated by cholinergic enervation

The results of this study are similar to effects observed in ovariectomized female rats [21], with a couple of important differences. For example, in the present study, all rats displayed a persistent turn early on during acquisition, and cholinergic lesions affected only the number of days that a persistent turn was evident. In ovariectomized females, the majority of non-lesioned rats did not display a persistent turn during acquisition. In this case, cholinergic lesions increased both the percentage of rats that engaged in persistent turning as well as the number of days that a persistent turn was evident [21]. Notably, estradiol treatment did not appear to affect the likelihood of developing a persistent turn in ovariectomized females [22]. This suggests that males may have a greater predisposition towards using a response strategy during DMP training than females. Why males initially show a strong predisposition towards using an egocentric (e.g., response) learning strategy on the DMP task is not known. Rodents have a natural tendency not to re-enter a recently visited location, which manifests as spontaneous alternation behavior. The DMP task requires rats to counter this innate response. This is evident by the fact that rats performed at levels below chance at the start of training. The adoption of a persistent turn produces an immediate gain by enabling rats to improve their performance to chance levels. This egocentric approach may be easier for rats than one which relies on extra maze cues. This may be particularly true of males, which prefer to rely on geometric cues, as opposed to visual cues, to solve spatial tasks [3,26,34].

In summary, our findings show that male rats have a strong initial tendency to use an egocentric response strategy during DMP acquisition, and that septal cholinergic lesions impair DMP acquisition in males by increasing the number of days that the response strategy persists. This effect likely reflects a greater influence of striatal circuits on learning strategy in rats in which cholinergic enervation of the hippocampus has been compromised.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the excellent technical skills of Mrs. Christine Close and Mr. Douglas Nelson. This study was supported by NIH grants: AG16261(DAJ) and AG021471 (RBG)

REFERENCES

1. Araujo DM, Lapchak PA, Robitaille Y, Gauthier S, Quirion R. Differential alteration of various cholinergic markers in cortical and sub-cortical regions of the human brain in Alzheimer's disease. *J. Neurochem* 1988;50:1914–1923. [PubMed: 3373218]
2. Arkhipov V, Kuleshkaja N, Lebedev D. Behavioral perseveration and impairment of long-term memory in rats after intrahippocampal injection of kainic acid in subconvulsive dose, *Pharmacology. Biochemistry and Behavior* 2008;88:299–305.
3. Astur RS, Tropp J, Sava S, Constable RT, Markus EJ. Sex differences and correlations in a virtual Morris water task, a virtual radial arm maze, and mental rotation. *Behav. Brain Res* 2004;151:103–115. [PubMed: 15084426]
4. Baxter MG, Bucci DJ, Gorman LK, Wiley RG, Gallagher M. Selective immunotoxic lesions of basal forebrain cholinergic cells: effects on learning and memory in rats. *Behav. Neurol* 1995;109:714–722.
5. Bartus RT, Dean RL, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. *Science* 1982;217:408–417. [PubMed: 7046051]

6. Bartus RT, Flicker C, Dean RL, Pontecorvo M, Figueiredo JC, Fisher SK. Selective memory loss following nucleus basalis lesions: long term behavioral recovery despite persistent cholinergic deficiencies. *Pharmacol. Biochem. Behav* 1985;3:125–135. [PubMed: 4041042]
7. Bizon JL, Han JS, Hudon C, Gallager M. Effects of hippocampal cholinergic differentiation on learning strategy selection in a visible platform version of the water maze. *Hippocampus* 2003;13:676–684. [PubMed: 12962313]
8. Book AA, Wiley RG, Schweitzer JB. Specificity of 192 IgG-saporin for NGF receptor-positive cholinergic basal forebrain neurons in the rat. *Brain Res* 1992;590:350–355. [PubMed: 1358406]
9. Bradford M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principal of protein dye binding. *Anal. Biochem* 1976;72:248–253. [PubMed: 942051]
10. Brightwell JJ, Smith CA, Neve RL, Colombo PJ. Long-term memory for place learning is facilitated by expression of camp response element-binding protein in the dorsal hippocampus. *Learning and Memory* 2007;14:195–199. [PubMed: 17351144]
11. Bures J, Fenton AA, Kaminsky Yu, Wesierska M, Zahalka A. Rodent navigation after dissociation of the allocentric and idiothetic representations of space. *Neuropharmacology* 1998;37:689–699. [PubMed: 9705006]
12. Cahill JF, Baxter MG. Cholinergic and noncholinergic septal neurons modulate strategy selection in spatial learning. *European Journal of Neuroscience* 2001;14:1856–1864. [PubMed: 11860481]
13. Chappell J, McMahan R, Chiba A, Gallagher M. A re-examination of the role of basal forebrain cholinergic neurons in spatial working memory. *Neuropharmacology* 1998;37:481–487. [PubMed: 9704989]
14. Cimadevilla JM, Fenton AA, Bures J. Functional inactivation of dorsal hippocampus impairs active place avoidance in rats. *Neuroscience Letters* 2000;285:53–56. [PubMed: 10788706]
15. Clements KM, Saunders AJ, Robertson B-A, Wainwright PE. Spontaneously hypertensive, Wistar Kyoto and Sprague-Dawley rats differ in their use of place and response strategies in the water radial arm maze. *Neurobiology of Learning and Memory* 2007;87:285–294. [PubMed: 17056285]
16. Colombo PJ, Brightwell JJ, Countryman RA. Cognitive strategy-specific increases in phosphorylated camp response element-binding protein and c-Fos in the hippocampus and dorsal striatum. *J. Neurosci* 2003;23:3547–3554. [PubMed: 12716964]
17. Dornan WA, McCampbell AR, Tinkler GP, Hickman LJ, Bannon AW, Decker MW, Gunther KL. Comparison of site-specific injections into the basal forebrain on water maze and radial arm performance in the male rat after immunolesioning with 192 IgG-saporin. *Behav. Brain Res* 1996;82:93–101. [PubMed: 9021074]
18. Dudchenko PA. An overview of the tasks used to test working memory in rodents. *Neuroscience and Biobehavioral Reviews* 2004;28:699–709. [PubMed: 15555679]
19. Everitt BJ, Robins TW. Central cholinergic systems and cognition. *Ann. Rev. Psychol* 1997;48:649–684. [PubMed: 9046571]
20. Fitz NF, Gibbs RB, Johnson DA. Aversive stimulus attenuates impairment of acquisition in a delayed match to position T-maze task caused by a selective lesion of septo-hippocampal cholinergic projections. *Brain Res. Bull* 2006;69:660–665. [PubMed: 16716835]
21. Gibbs RB, Johnson DA. Cholinergic lesions produce task-selective effects on delayed matching to position and configural association learning related to response pattern and strategy. *Neurobiol. Learn. Mem* 2007;88:19–32. [PubMed: 17449284]
22. Gibbs RB. Estradiol enhances DMP acquisition via a mechanism not mediated by turning strategy but which requires intact basal forebrain cholinergic projections. *Horm Behav* 2007;52:352–359. [PubMed: 17583704]
23. Gold PE. Acetylcholine modulation of neural systems involved in learning and memory. *Neurobiol Learn Mem* 2003;80:194–210. [PubMed: 14521863]
24. Herrero AI, Sandi C, Venero C. Individual differences in anxiety trait are related to spatial learning abilities and hippocampal expression of mineralocorticoid receptors. *Neurobiology of Learning and Memory* 2006;86:150–159. [PubMed: 16580234]
25. Johnson DA, Zamboni NJ, Gibbs RB. Selective lesion of cholinergic neurons in the medial septum by 192 IgG-saporin impairs learning in a delayed matching to position T-maze paradigm. *Brain Res* 2002;943:132–141. [PubMed: 12088847]

26. Lawton CA. Gender differences in way-finding strategies: Relationship to spatial ability and spatial anxiety. *Sex Roles* 1994;30:765–779.
27. Maruki K, Izaki Y, Hori K, Nomura M, Yamauchi T. Effects of rat ventral and dorsal hippocampus temporal inactivation on delayed alternation task. *Brain Res* 2001;895:273–276. [PubMed: 11259790]
28. Morris RGM, Garrud P, Rawlins JNP, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. 1982;297:681–683.
29. O'Keefe J, Burgess N. Geometric determinants of the place fields of hippocampal neurons. *Nature* 1996;381:425–428. [PubMed: 8632799]
30. Packard M. Glutamate infused posttraining into the hippocampus or caudate-putamen differentially strengthens place and response learning. *PNAS* 1999;96:12881–12886. [PubMed: 10536017]
31. Packard MG, McGaugh JL. Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiology of Learning and Memory* 1996;65:65–72. [PubMed: 8673408]
32. Parent MB, Baxter MG. Septohippocampal acetylcholine: involved in but not necessary for learning and memory? *Learn Mem* 2004;11:9–20. [PubMed: 14747512]
33. Pych JC, Chang Q, Colon-Rivera C, Haag R. Acetylcholine release in the hippocampus and striatum during place and response training. *Learning and Memory* 2005;12:564–572. [PubMed: 16322358]
34. Sandstrom NJ, Kaufman J, Huettel SA. Males and females use different distal cues in a virtual environment navigation task. *Brain Res. Cogn. Brain Res* 1998;6:351–360. [PubMed: 9593991]
35. Whishaw IQ, Tomie J-A. Perseveration on place reversals in spatial swimming pool tasks: evidence for place learning in hippocampal rats. *Hippocampus* 1997;7:361–370. [PubMed: 9287076]
36. Yin HL, Knowlton BJ. Contributions of striatal subregions to place and response learning. *Learning and Memory* 2004;11:459–463. [PubMed: 15286184]

Figure 1.

Acquisition of the DMP task. Training was blocked into 5 day periods with each point representing t. The performance of both treatment groups (Control $n = 9$ and SAP $n = 8$) improved over time. During periods 3 and 4 (days 11–15 and 16–20 respectively) the performance of control was significantly better than the SAP group ** $p < 0.01$).

Figure 2. Comparison of the mean $\pm$ S.E.M., numbers of days to reach criterion for control and SAP treated rats. SAP-treated rats took significantly longer to reach criterion on the DMP task than corresponding controls (21.6 ± 1.3 days vs. 16.0 ± 0.4 days; ** $p < 0.01$)

Figure 3.

Comparison of the mean $\pm$ S.E.M., total number of days that rats adopted a persistent turn was significantly greater for SAP treated rats than for controls (14.78 ± 1.76 days vs. 8.13 ± 1.66 days ** $p < 0.01$)

Figure 4.

Figures: 4A and 4B. Disruption of persistent turning strategy (failure to turn in the same direction at least 7 of 8 trials during the open choice trials). There was no significant effect of rotating the T-maze 180° in either control or SAP treatment groups ($p > 0.05$; Fisher's Exact test) indicating that spatial cues were not a significant factor for either control or SAP treated animals when using a response strategy to navigate the T-maze. 4C and 4D. Disruption of place strategy (failure to return to goal arm during open choice trial for at least 7 of 8 trial pairs). There was a disruption in navigation by rotating the T-maze 180°, in both control and SAP treated groups ($p < 0.002$; Fisher's Exact test) This result indicates that visual spatial cues played a significant role in navigation for both treatment groups once the rats achieved criterion in the DMP T-maze task.