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Oxotremorine infusions into the medial septal area of middle-aged rats affect spatial reference memory and ChAT activity

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Abstract

Age-related spatial memory deficits are correlated with septohippocampal cholinergic system degeneration. The present study examined the effect of intraseptal infusions of the cholinergic agonist, oxotremorine, on spatial reference memory in middle-aged rats using place discrimination in the water maze, and on cholinergic activity using choline acetyltransferase (ChAT) activity. Oxotremorine mildly improved the rate of place discrimination acquisition of middle-aged rats during initial sessions only, but did not affect asymptotic levels of performance achieved. Of the brain regions assayed, ChAT activity increased with age in the temporal cortex and dorsal CA2/3 region of the hippocampus. Oxotremorine significantly decreased ChAT activity in the dorsal hippocampus. In contrast to our previous results in aged rats indicating a more robust effect of oxotremorine on spatial working memory, the present results suggest a modest effect of intraseptal oxotremorine on the acquisition of a spatial reference memory task.

Keywords: Oxotremorine; Intraseptal infusion; Spatial reference memory; ChAT activity; Middle-aged; Fischer-344 rat; Medial septal area

1. Introduction

Spatial memory deteriorates markedly with increasing age in rats. This deficit has been described using a variety of tasks including the circular platform [7,8], the radial arm maze [22–24,43,44], and the Morris water maze [19,29,34,37,38,40,63]. Two types of spatial memory, spatial reference memory and spatial working memory, have been studied extensively in the Morris water maze [29,30,34,37,38,40,60,63,79]. Reference memory refers to memory for information that remains constant over repeated trials and is therefore, trial independent [68]. Reference memory is required to learn the general rules of any task (e.g., run to the end of a maze or swim to a platform). In contrast, working memory refers to memory in which the information to be remembered changes in repeated trials [68]. Thus, working memory is trial dependent and is required in tasks involving changing stimulus-response relation-

ships. Relative to young (3–4 months) rats, aged (21–24 months) rats have impaired spatial reference and working memory, whereas middle-aged (16–18 months) rats have impaired spatial reference memory only [19,29,34,37,38].

The septohippocampal cholinergic system is involved in spatial memory function, and neurons in this system undergo age-related degeneration [2,21,31,69–71]. The relationship between septohippocampal cholinergic system deterioration and spatial memory deficits in aged rats has been studied extensively. Age-related spatial memory deficits have been correlated with decreased morphological [3,29,30,43,44], neurochemical [27,41,61], metabolic [39], and electrophysiological [7,23–25] markers in the basal forebrain and hippocampus. Because of the numerous correlations between septohippocampal degeneration and spatial memory deficits, it has been hypothesized that interventions designed to restore cholinergic function will improve spatial memory performance in aged rats [9,10].

The hippocampus receives its major cholinergic and GABAergic projections from the medial septal area

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(MSA) of the basal forebrain [33,35,51,58,59]. The two major populations of neurons in the MSA, cholinergic and GABAergic, have both cholinergic and GABAergic receptors on their dendrites and cell bodies [11,18,56,57,72]. Axons from cholinergic MSA neurons innervate hippocampal glutamatergic pyramidal cells in Ammon's horn and granule cells in the dentate gyrus, as well as hippocampal GABAergic interneurons [35,36,59,81]. The GABAergic projection from the MSA primarily innervates hippocampal GABAergic interneurons [4,33,51]. Both hippocampal pyramidal neurons and GABAergic interneurons project back to the MSA [56,78]. Thus, the septohippocampal system forms an interconnected loop in which several neurotransmitter systems interact and modulate each other's activity.

The activity of MSA neurons is increased by iontophoretic application of acetylcholine and cholinergic agonists, and decreased by cholinergic antagonists and GABAergic agonists [52–55]. Because of the extensive septohippocampal connectivity, manipulations of MSA activity can profoundly influence activity in the hippocampus. Studies using young rats have demonstrated that direct intraseptal infusions of cholinergic antagonists or GABAergic agonists disrupt hippocampal cholinergic functioning by decreasing the turnover and release of acetylcholine [15,20,50], decreasing high-affinity choline uptake [12,80], and suppressing the 4–12 Hz oscillatory theta rhythm [1,46–48]. Infusions of cholinergic antagonists and GABAergic agonists also significantly impair memory relative to control rats infused with saline, suggesting that disruption of hippocampal activity is related to a disruption of memory processes [12,15,26,46,48,76]. Conversely, intraseptal infusions of compounds that excite MSA neurons should enhance hippocampal activity. In young rats, cholinergic agonists increase the power of the hippocampal theta rhythm [46], but have been reported to both increase and decrease hippocampal acetylcholine release [20,50]. Infusions of cholinergic agonists do not significantly affect mnemonic processes in young rats, perhaps because their cholinergic system is functioning at an optimal level [46,65].

The responses of MSA neurons to pharmacological manipulations are not altered in aged rats [54]. As in young rats, infusions of cholinergic agonists in aged rats alter hippocampal electrophysiology. Intraseptal infusions of the cholinergic agonist oxotremorine into aged rats shifted the peak frequency of hippocampal theta, increased the power of hippocampal theta, increased the initial slope of the hippocampal population excitatory post-synaptic potential [5,45,62,65], and altered hippocampal long-term potentiation and depression [73]. However, in contrast to their effects in young rats, intraseptal infusions of cholinergic agonists can improve the spatial memory of aged rats. Several studies have

reported that oxotremorine improved the spatial working memory of aged rats, as measured by choice accuracy in a T-maze spatial alternation task [6,62,65]. Oxotremorine's effects on both hippocampal activity and one type of spatial memory raise the possibility that it may significantly improve other types of spatial memory in impaired aging rats.

The present study examined the ability of oxotremorine, a muscarinic agonist [28], to improve spatial reference memory and affect hippocampal cholinergic activity. Spatial reference memory, as measured by the Morris water maze, is impaired in both middle-aged (16–18 month old) and aged (22–24 month old) rats [19,29,34]. If septohippocampal cholinergic system deterioration contributes to the observed spatial reference memory deficit in middle-aged and aged rats, then restoration of septohippocampal cholinergic system function should improve spatial reference memory.

Whereas most studies of potential cognitive enhancers use aged rats as subjects, few studies examine the effects of pharmacological compounds to improve the memory of younger impaired rats [17]. Although it is a valuable goal to improve memory when both the brain and behavior are severely degenerated as is the case in aged rats, it may be more clinically relevant to treat memory deficits when they initially appear, at which point the memory impairment and neuronal degeneration are more mild compared to aged impaired subjects [34,49] and the brain may be more responsive to treatment. This approach to cognition enhancement is unique in that the effectiveness of novel cognitive enhancers can be assessed in younger impaired rats in which neuronal degeneration is in its preliminary stages. This study used middle-aged rats to examine the effects of intraseptal oxotremorine infusions on spatial reference memory as measured by place discrimination in the water maze, a task in which middle-aged rats are impaired [34].

Thus far, previous studies examining the effects of intracranial infusions on behavior have not measured the effect of the infusion on markers of the targeted neurotransmitter system. Therefore, in order to measure the effect of oxotremorine on hippocampal cholinergic activity, activity of the enzyme choline acetyltransferase (ChAT), which synthesizes acetylcholine, was measured after oxotremorine or saline infusion. ChAT activity was also measured in the caudate putamen and in the frontal and temporal cortices. Because decreased hippocampal ChAT activity has been significantly correlated with impaired spatial memory in aged rats [27], it was hypothesized that intraseptal oxotremorine infusion would increase hippocampal ChAT activity. No significant effect of oxotremorine was expected in the other brain areas measured.

It is important to discriminate whether oxotremorine-induced changes in place discrimination are mnemonic or sensorimotor in origin. Performance in the water

maze can be affected by changes in such non-mnemonic abilities as motor skills, motivation, and vision, and some measures of place discrimination (e.g., swim distance or swim time) may be affected if oxotremorine improves sensorimotor ability rather than mnemonic ability. Therefore, straight swim and visual discrimination procedures in the water maze were used to measure motor skills, motivation, and visual acuity.

2. Materials and methods

2.1. Subjects

Male Fischer-344 rats were obtained from the NIA colony at Harlan Sprague-Dawley (Indianapolis, IN). At the beginning of behavioral testing, the rats were 4 and 17 months of age. They were housed 1–2 per cage in a colony room with a 12:12 light/dark cycle and behavioral testing was performed during the light portion of the cycle. Food and water were provided ad libitum.

2.2. Surgery

Anesthesia was administered with a mixture of 33% O₂ (Puritan Bennett, Linthicum Heights, MD), 66% N₂O₂ (Puritan Bennett), and 1% ethrane (Ohmeda, Liberty Corner, NJ). Gas anesthetic was administered via a mask (modification of [74]) which fit a Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The scalp was shaved, swabbed with Betadine (Purdue Frederick, Norwalk, CT), and retracted. The landmarks bregma and lambda were placed in the same horizontal plane. One hole was drilled for the placement of a guide cannula, and 3 or 4 holes were drilled for the placement of jewelers screws which were screwed into the skull. A stainless steel guide cannula (15 mm long, 26 gauge hypodermic tubing; Small Parts, Inc., Miami Lakes, FL) was placed into the brain above the MSA, 0.7 mm anterior and 1.4 or 1.5 mm lateral to bregma, and 4.1 or 4.6 mm ventral to dura at a 15° angle toward the midline [75]. The cannula and screws were fixed to the skull with dental acrylic (Co-oral-ite Dental Mfg. Co., Diamond Springs, CA). A stainless steel stylet (14 mm long, 32-gauge hypodermic tubing; Small Parts, Inc.) was inserted into the guide cannula to prevent clogging. Each rat was given 1 week to recover before the start of behavioral testing.

2.3. Drug infusion

Each rat was assigned to one of five groups: (1) 4 months, saline-infused, (2) 4 months, unoperated control, (3) 17 months, unoperated control, (4) 17 months, saline-

infused, and (5) 17 months, oxotremorine (2 µg)-infused. A 2 µg dose was chosen because it improved spatial working memory and increased the power of hippocampal theta in 22-month-old rats without causing seizures [6,65]. Oxotremorine was obtained from Sigma Chemical (St. Louis, MO) and 0.9% sterile saline (NaCl) was obtained from Abbott Labs (North Chicago, IL). Oxotremorine was dissolved in saline at a concentration of 4 µg/µl and stored frozen (–20°C) in 30 µl aliquots. For 2 days prior to behavioral testing, each rat was handled for approx. 5 min.

Drugs were infused immediately before each behavioral test session, with the exception of shaping sessions. Drugs were delivered through a stainless steel injector (32 gauge hypodermic tubing; Small Parts, Inc.) bent at a 45° angle 17 mm from the tip and connected to polyethylene tubing (PE-10; Becton-Dickinson, Parsippany, NJ). The infuser and tubing were attached to a 10 µl Hamilton syringe mounted on a microinfusion pump (Orion Research Inc., Boston, MA or Carnegie Medicin AB, Stockholm, Sweden) calibrated to deliver fluid at a rate of 0.1 µl/min.

For each infusion, the stylet was removed and the injector was inserted into the guide cannula. Then either oxotremorine or saline was delivered for 5 min, during which time the rat was free to move. The injector was left in place for an additional minute, after which the injector was removed and the stylet replaced. Behavioral testing occurred immediately after infusion.

2.4. Apparatus

A Morris water maze was used for all behavioral procedures [66,67]. A galvanized metal tank, 1.8 m in diameter and 0.6 m high, and painted white on the inside, was located in the center of a small room and was surrounded by many extramaze cues on the walls of the room. Water filled the tank to a depth of 35 cm, and was maintained at 24 ± 2°C by two aquarium heaters which were removed prior to testing. Non-toxic white watercolor paint was added to the water to make it opaque.

A camera (Burle Security Products, Lancaster, PA), mounted 1.4 m above the surface of the water, was connected to an automated tracking system (HVS Image Analysis VP-112, Hampton, England) which recorded behavioral data. Light was provided by four 40-W bulbs, mounted in a square pattern, 1.2 m above the surface of the water. A small radio provided a constant background noise.

A transparent plastic escape platform was placed in the tank. The top was 10 × 10 cm, with nine holes, 1 cm in diameter, to provide a gripping surface. The platform was raised and lowered via a cable located on the bottom of the tank. In its raised position, the platform was 2 cm beneath the water level, and in its lowered

position, it was 10 cm beneath the water level. The rat could escape only by climbing onto the raised platform.

For the shaping and straight swim procedures (see below), no spatial discrimination was required. A black curtain surrounded the tank to minimize visual extramaze cues. Two pieces of transparent plastic, 128 cm long and 61 cm high, were placed parallel to each other in the water to form a 14 cm wide alley which extended 26 cm above the surface of the water. One end of the alley was placed adjacent to the tank wall. The other end extended into the middle of the tank and was closed by a third piece of transparent plastic, 14 cm wide and 61 cm high. The platform was placed 30 cm from this end of the alley.

2.5. Behavioral tasks

In order to minimize trauma to the brain from repeated infusions of oxotremorine, which may produce seizures (A. Markowska, unpublished observations), all testing sessions were separated by 2 or 3 days, rather than run on consecutive days.

2.5.1. Shaping

In the shaping procedure, the rats were trained to climb on the platform. One session consisting of 10 trials was conducted and no drug infusions were given prior to testing. During the first two trials, the rat was placed on the platform for 10 s. During trials 3–10, the rat was placed in the water at locations (two trials per location) progressively further from the platform and allowed to swim to the platform. The final location was halfway between the platform and the edge of the tank. If the platform was not located within 10 s, then the experimenter guided the rat to the platform, where he remained for 5 s. No data were recorded during this procedure. The intertrial interval was 2–3 min.

2.5.2. Straight swim

The straight swim procedure trained the rats to swim to the platform. One session consisting of six trials was conducted and drug infusions were given immediately before testing. Each rat was placed at the end of the alley and allowed to swim to the platform. The time to climb on the platform was recorded. If the platform was not located within 60 s, then the rat was guided there and remained on the platform for 5 s. The intertrial interval was 2–3 min.

2.5.3. Place discrimination

Spatial reference memory was measured using place discrimination. Seven sessions (two per week) were conducted and drugs were infused immediately before each test session. The tank was divided into four quadrants by two imaginary perpendicular lines intersecting in the center of the tank. The platform was located in the

middle of one quadrant, 40 cm from the wall of the tank, and it remained in this location throughout place discrimination testing. One start position was located at the edge of the tank in the middle of each of the three quadrants without the platform. The experimenter stood in the southwest corner of the room during all trials.

A trial began by placing the rat into the water facing the center of the tank at one of the three start positions. The start position used was always different from that in the previous trial and each of the three positions was used twice during the session. The same sequence of start positions was used for all rats within a session, but the sequence differed between sessions. The trial ended when the rat climbed onto the platform, or after 60 s, at which point the rat was guided there. The rat remained on the platform for 10 s, and was then placed in a holding cage for an intertrial interval of 3–4 min. During the first five trials (platform trials), the platform was raised and available for escape. The sixth trial of each session was a variable-interval probe trial in which the platform was lowered for a variable interval of time [64]. Intervals of 10, 20, 30 and 40 s were varied such that the interval in a given session was different from that in the previous session. The same interval was used for all rats within a session. At the end of a variable-interval, the platform was raised and available for escape.

Three measures were analyzed for the platform trials. Swim time (s) was the time to reach the platform. Swim distance was the distance (cm) of the path between the start location and the platform. Heading angle was the angle (deg) between the direction when leaving the start location and a straight line drawn from the start location to the platform. For all three measures, lower scores indicated better performance. Three measures were analyzed for the probe trials. Quadrant time was the percentage of time spent in the quadrant containing the platform. Annulus-40 time was the percentage of time spent within a circle 40 cm in diameter, centered on the location of the platform. Platform crossings was the number of times per 10 s that the rat crossed the 10 × 10 cm location of the submerged platform. In order to correct for variations in the duration of the probe trial between sessions, the number of crossings made during each probe trial was divided by the length of the trial and multiplied by 10 to yield the number of crossings per 10 s. For all three measures, higher scores indicated better performance.

2.5.4. Visual discrimination

The rats' ability to use visual cues to find the platform was measured using visual discrimination. One session consisting of six trials was conducted and drugs were infused immediately before the beginning of a session. The apparatus and experimenter position were the same as in place discrimination except that visual cues were present inside of the tank. A square (10 × 10 cm) styro-

foam block, 3.75 cm high, was attached to the top of the platform with two screws. A painted cup (9.5 cm in diameter and 7.5 cm high) hung from a string 24 cm over the platform and a white circular piece of paper, 18 cm in diameter, was placed on top of the cup. These two cues always hung directly over the platform.

The platform and hanging cues were moved to a different location in the tank for each trial. This location varied both in terms of the quadrant and the distance from the edge of the tank. Start positions and platform locations varied such that each location was used once per session and no location was used twice in a row. Each rat was allowed 60 s to climb onto the platform, after which time the rat was guided to it. Time to climb onto the platform was recorded. The intertrial interval was 1–2 min.

2.6. Procedure

All rats were handled for 15–20 min a day for 7 days before surgery. After the surgical recovery period, rats were tested in the water maze twice a week, on Mondays and Thursdays or Tuesdays and Fridays in the following order: shaping (1 session), straight swim (1 session), place discrimination (7 sessions), and visual discrimination (1 session).

2.7. ChAT activity

All rats were decapitated 2–3 days following the final test session. Drugs were infused into each of the cannulated rats prior to decapitation. Brains were cut into three blocks. The first block, containing the cannula track, was cut 1 mm anterior to the median eminence (Bregma = -1.8 mm) and the frontal cortex was dissected bilaterally from this block for neurochemical analysis. The rest of the block was placed in a 10% formalin solution for histological examination of the cannula track. Eight brain areas were dissected bilaterally from the remaining two blocks of brain to examine the effect of oxotremorine on ChAT activity: posterior caudate putamen, dorsal and ventral hippocampus (areas CA1, CA2/CA3, dentate gyrus), and temporal cortex.

ChAT activity was assayed by the method of Fonnum [32]. Briefly, tissue samples were homogenized in 0.32 M sucrose. ChAT activity was measured by the formation of [14 C]acetylcholine (ACh) from [14 C]acetyl coenzyme-A and choline. The product was separated from the labeled substrate by an extraction which yielded high efficiency, a low-blank, and ensured measurement of [14 C]ACh only. The incubation mixture (final volume, 100 μ l) contained: sodium chloride 200 mM; sodium phosphate (pH 7) 50 mM; eserine, 0.075 mM; choline chloride, 6 mM; [14 C]acetyl coenzyme-A 0.1 mM; and bovine serum albumin, 0.5 mg/ml. A 40 μ l aliquot of homogenized tissue was combined with 10 μ l

of a solution containing 2% Triton-X 100 and 50 mM ethylenediaminetetraacetic acid (pH 7.4), and this was added to the incubation mixture. Incubation was carried out for 15 min at 37°C. The newly formed [14 C]ACh was extracted into a hydrophobic mixture containing sodium tetraphenyl boron. The protein concentration of the tissue was measured using the method of Bradford [13] with bovine serum albumin as a standard.

2.8. Histology

The histological examination verified the location of the guide cannula and assessed inflammation in the vicinity of the infusion. Following submersion in formalin, the brain was placed in a 30% sucrose solution for 2–3 days. The brain was then frozen in dry ice and cut in 50 μ m coronal sections on a sliding microtome. Sections containing the cannula track were mounted and Nissl stained using neutral red. No rats with incorrectly placed cannulae or large inflammations around the infusion site were included in the data analyses.

2.9. Data analysis

Data analyses were performed with SYSTAT 5.03 (SYSTAT Inc., Evanston, IL). Because the behavior of rats infused with saline was not significantly different from that of rats receiving no infusions, the results are reported within an age group. Thus, the analyses are reported for three groups: (1) 4-month control (a combination of the 4-month saline-infused and 4-month unoperated groups); (2) 17-month control (a combination of the 17-month saline-infused and 17-month unoperated groups); and (3) 17-month oxotremorine. Analyses of variance (ANOVAs), one-way or repeated measures, including all three groups were performed on each measure. If, in the repeated measures analyses, the main effect of group or interaction was significant, then ANOVAs were performed on pairs of two of the three groups (4-month control vs. 17-month control, 4-month control vs. 17-month oxotremorine, and 17-month control vs. 17-month oxotremorine) to measure specific differences between groups. After each one-way ANOVA, planned contrasts were performed regardless of a significant main effect and were used to examine specific between group differences. An alpha level of 0.05 was used for all statistical tests.

2.9.1. Straight swim

Group differences in swim time across the session were measured using a repeated measures ANOVA with trials as the repeated measure.

2.9.2. Place discrimination

For platform trial measures, the mean \pm standard error of the mean (SEM) of 1–5 trials for each session was

calculated for each rat, yielding seven values (one per session) for each measure per rat. For probe trials, the individual probe trial measures obtained in each session were used in the analysis, yielding seven values for each measure per rat. A repeated measures ANOVA including all three groups was performed for each measure with sessions as the repeated measure. Separate repeated measures ANOVAs were performed on pairs of groups to measure specific differences between the three groups.

In addition to examining performance throughout acquisition, performance during initial and asymptotic sessions was analyzed to assess the possibility that oxotremorine differentially affected initial and asymptotic performance. Therefore, a three-way ANOVA (Groups \times Periods \times Sessions) with repeated measures on two factors (Periods and Sessions) was performed on each measure. The term 'period' represented initial and asymptotic sessions, with 'initial' including Sessions 1–3 and 'asymptotic' including Sessions 5–7. Planned contrasts within each period were performed to measure differences among the groups.

2.9.3. Visual discrimination

A group mean \pm SEM was calculated for each trial. Repeated measures ANOVAs (three-group and two-group) were performed using these means to measure differences in performance within the session.

2.9.4. ChAT activity

Mean ChAT activity \pm SEM was calculated for each brain area for each group. Separate one-way ANOVAs and planned contrasts were performed for each brain area. Correlations between behavioral measures and ChAT activity were measured using the Pearson product-moment correlation coefficient.

3. Results

The number of rats included in the analyses was as follows for straight swim and place discrimination: 4-month control ($n=9$), 17-month control ($n=11$), 17-month oxotremorine ($n=7$). After place discrimination testing, one 17-month rat irreversibly blocked his cannula which prevented further infusion. This rat was tested as an uninfused control in visual discrimination, yielding the following sample sizes: 4-month control ($n=9$), 17-month control ($n=12$), and 17-month oxotremorine ($n=6$). For ChAT activity, the number of rats included depended on the brain area: 4-month control ($n=7-8$), 17-month control ($n=8-9$), 17-month oxotremorine ($n=4-6$). Rats included in the analyses had cannula tracks ending in the medial septum or vertical limb of the diagonal band, and had minimal inflammation surrounding the cannula track. In one rat, the cannula track ended in the dorsal horizontal limb of the

diagonal band. No rats died during the course of the experiment.

3.1. Straight swim

Swim time was not significantly different among the groups, as indicated by a non-significant group effect and Group \times Session interaction, suggesting that neither age nor oxotremorine affected ability to swim to the platform. A significant session effect indicated that all groups improved during the session ($F(5,120)=3.25$, $P<0.01$).

3.2. Place discrimination

The results of repeated-measures analyses performed on the seven sessions of place discrimination are presented in Table 1. All F values from the three-group ANOVA are presented, but the F values for the two-group ANOVAs are summarized.

3.2.1. Three-group ANOVAs

As illustrated in Table 1, the main effect of group was significant for the swim time, quadrant time, annulus-40 time, and platform crossings measures across the seven sessions of place discrimination. The group effect was not significant for swim distance and heading angle. Significant session effects were obtained for all six measures. A significant Group \times Session interaction was present in heading angle, quadrant time, and platform crossings. Because neither the group effect nor the Group \times Session interaction was significant for swim distance, no further analyses were performed on this measure.

In the three-way ANOVAs, Period \times Group and Period \times Session \times Group interactions indicate differences in group performance between initial and asymptotic sessions. The Period \times Group interaction was significant for heading angle ($F(2,24)=4.3$, $P<0.05$) and platform crossings ($F(2,24)=3.53$, $P<0.05$). The Period \times Session \times Group interaction was significant for quadrant time ($F(4,48)=3.79$, $P<0.01$), and annulus-40 time ($F(4,48)=2.78$, $P<0.05$).

3.2.2. 4-month control vs. 17-month control

Age significantly impaired performance in several measures. Repeated-measures ANOVAs revealed a significant difference between the control groups in swim time, quadrant time, annulus-40 time, and platform crossings (Table 1), suggesting a detrimental effect of age on these measures. Both groups improved throughout testing, as indicated by significant session effects in swim time, heading angle, quadrant time, annulus-40 time, and platform crossings. The rate of improvement was significantly slower in the 17-month controls for quadrant time and annulus-40 time, as suggested by

Table 1
Summary of repeated measures ANOVA results for place discrimination measures

Measure	Group		Session		Group × Session		4MOCON vs. 17MOCON	17MOCON vs. 17MOOXO	4MOCON vs. 17MOOXO
	df	F	df	F	df	F			
Swim time	2,24	5.97**	6,144	49.27**	12,144	0.85	a,b	b	a,b
Swim distance	2,24	0.63	6,144	38.54**	12,144	1.00			
Heading angle	2,24	0.16	6,144	8.84**	12,144	2.54**	b	a,b,c	b,c
Quadrant time	2,24	6.97**	6,144	20.72**	12,144	2.05*	a,b,c	b	a,b,c
Annulus-40 time	2,24	7.39**	6,144	26.62**	12,144	0.2	a,b,c	b	a,b
Platform crossings	2,24	7.24**	6,144	5.82**	12,144	1.99*	a,b	b	a,b

Note: All ANOVA *F* and *P* values are presented for the three-group analysis. *P* values for the two-group ANOVAs are summarized above. 4MOCON, 4-month control; 17MOCON, 17-month control; 17MOOXO, 17-month oxotremorine.

a, significant group effect, $P \leq 0.05$; b, significant session effect, $P \leq 0.05$. c, significant interaction, $P \leq 0.05$. * $P \leq 0.05$; ** $P \leq 0.01$.

significant Group × Session interactions (Table 1). Planned contrasts performed for initial and asymptotic sessions revealed that the 17-month controls had significantly impaired swim time, quadrant time, and annulus-40 time during the initial three sessions relative to the 4-month controls (P values < 0.05). The 17-month controls also had significantly impaired swim time, quadrant time, and platform crossings during asymptotic sessions (P values < 0.05).

3.2.3. 17-month control vs. 17-month oxotremorine

Oxotremorine significantly improved the heading angle of the 17-month oxotremorine group relative to the 17-month control group (Table 1). During the initial three sessions, the heading angle of the 17-month oxotremorine group was significantly lower than that of the 17-month control group, but not significantly different from that of the 4-month control group (Fig. 1). However, the two 17-month groups were not significantly different during asymptotic sessions. The differential rate of improvement in heading angle across the seven place discrimination sessions indicated by the significant Group × Session interaction (Table 1) also suggests an improvement by oxotremorine. Oxotremorine also slightly improved quadrant time, annulus-40 time, and platform crossings of the 17-month oxotremorine group relative to the 17-month control group during initial sessions, but this difference did not reach significance.

3.2.4. 4-month control vs. 17-month oxotremorine

Oxotremorine improved heading angle to a level similar to controls, as indicated by a non-significant difference between the 4-month control and 17-month oxotremorine groups across the seven place discrimination sessions (Table 1) and in both initial and asymptotic periods. The 17-month oxotremorine group had significantly different swim time, quadrant time, annulus-40 time, and platform crossings relative to the 4-month controls across the seven place discrimination sessions

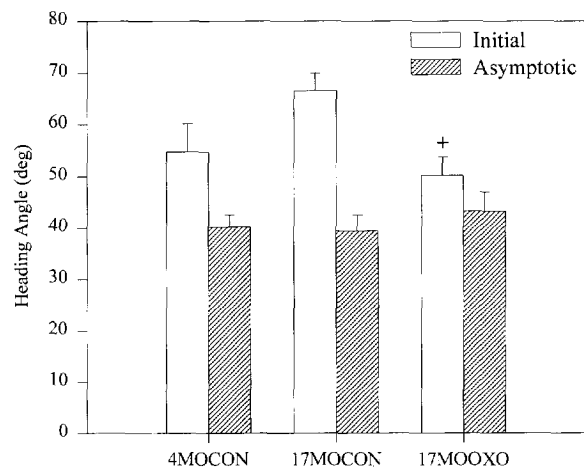


Fig. 1. Oxotremorine significantly decreased heading angle relative to 17-month controls during initial place discrimination sessions. The three groups were not significantly different during asymptotic sessions. Each bar represents the mean (+SEM) of each group for Sessions 1–4 (initial) or 5–7 (asymptotic). 4MOCON, 4-month control group; 17MOCON, 17-month control group; 17MOOXO, 17-month oxotremorine group. ⁺ $P < 0.05$ relative to the 17-month control group.

(Table 1). However, only platform crossings was significantly different between the two groups during initial sessions, suggesting an improvement by in the swim time, quadrant time, and annulus-40 time measures. This improvement was not observed in asymptotic sessions, as indicated by significant differences between the groups in swim time and quadrant time.

3.3. Visual discrimination

Swim time in the visual discrimination was not significantly affected by age or oxotremorine, as indicated by non-significant group effects in the three-group and two-group ANOVAs (Fig. 2). However, the Group × Trial interactions in the three-group ANOVA ($F(5,115) = 7.88$, $P < 0.01$), and the three two-group ANOVAs (P values < 0.05) were significant. This inter-

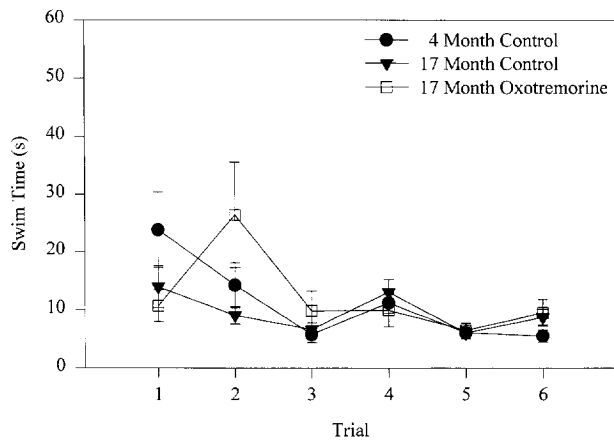


Fig. 2. Neither age nor oxotremorine significantly affected swim time in the visual discrimination. Each point represents the mean group performance (\pm SEM) during each trial of the visual discrimination session.

action likely results from the increased swim time of the 17-month oxotremorine group in Trial 2. The significant trial effects in the three-group ANOVA ($F(10,115)=3.02$, $P<0.01$), and two-group ANOVAs (P values <0.05) indicate that the groups improved throughout the session.

3.4. Neurochemistry

ChAT activity was significantly different among the three groups in only one brain area, the temporal cortex ($F(2,19)=11.46$, $P<0.01$). In this area, planned contrasts revealed that both the 17-month control and 17-month oxotremorine groups had higher ChAT activity relative to the 4-month controls ($F(1,19)=22.63$, $P<0.01$ and $F(1,19)=7.20$, $P<0.05$, respectively), suggesting an age-related increase in ChAT activity that was not affected by oxotremorine (Fig. 3B). Planned contrasts illustrated that ChAT activity differed between the groups in the CA2/3 region of the dorsal hippocampus. Although neither 17-month group had significantly different ChAT activity from the 4-month group in the dorsal hippocampus, the planned contrasts revealed that oxotremorine significantly decreased ChAT activity in the 17-month oxotremorine group relative to the 17-month control group in this area ($F(1,18)=5.10$, $P<0.05$; Fig. 3A). ChAT activity in the CA2/3 region of the dorsal hippocampus was significantly correlated with heading angle during the initial sessions of place discrimination ($r=0.47$, $P<0.05$).

4. Discussion

The results of this study suggest that direct intraseptal infusions of oxotremorine moderately improved spatial reference memory in middle-aged mnemonically

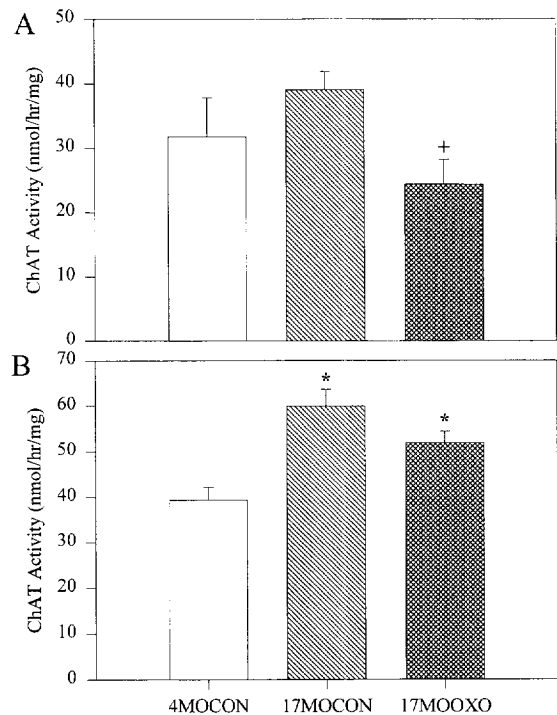


Fig. 3. ChAT activity in the CA2/3 region of the dorsal hippocampus (A) and temporal cortex (B). Each bar represents the mean (\pm SEM) ChAT activity for each group. In the dorsal hippocampus, oxotremorine significantly decreased ChAT activity relative to the 17-month control group. In the temporal cortex, both 17-month groups had a significantly higher ChAT activity than the 4-month controls. Oxotremorine did not significantly affect ChAT activity in the temporal cortex. * $P<0.05$ relative to the 4-month control group. + $P<0.05$ relative to the 17-month control group.

impaired rats. The modest improvement observed was reflected in the rate of acquisition of the task, rather than in the asymptotic level of performance achieved. The 17-month control rats had a larger heading angle than 17-month oxotremorine rats during the initial sessions of place discrimination, suggesting that the initial route to the platform of 17-month oxotremorine rats was more direct than that of 17-month controls. Oxotremorine also slightly improved performance in the place discrimination probe trial measures during initial, but not asymptotic, test sessions. Together with the observation that oxotremorine did not affect performance in asymptotic sessions, the behavioral data suggest that oxotremorine infusion in middle-aged rats did not increase asymptotic levels of performance but moderately accelerated the rate at which asymptotic levels were achieved.

The results of this study are consistent with those of previous studies using intraseptal infusions of oxotremorine in aged rats. In these studies, oxotremorine significantly reduced spatial working memory deficits in aged rats tested in a T-maze alternation task [6,45,62,65]. However, the effects of oxotremorine on spatial working memory were more robust, as indicated by the finding

that the working memory of 22-month-old rats was significantly improved after oxotremorine infusion relative to after saline infusion [6,45,62,65]. There are two possible explanations for the difference in the magnitude of oxotremorine's effect on these two types of spatial memory. One possibility is that the neuronal structures affected by oxotremorine infusion are more involved in spatial working memory than in spatial reference memory [47,48]. Another possibility is that the aged brain is more amenable to cognitive enhancement than the middle-aged brain. Further research will be needed to distinguish between these possibilities.

The mnemonic improvement observed in the present study after oxotremorine infusion in middle-aged rats may be related to decreased ChAT activity in the dorsal hippocampus, rather than to an expected increase. A decrease in hippocampal ChAT activity is consistent with observations that intraseptal infusions of oxotremorine decreased hippocampal acetylcholine release in young rats, as measured by *in vivo* microdialysis [50]. However, decreased ChAT activity in the basal forebrain and hippocampus of aged rats has been reported to correlate with impaired spatial reference memory in the water maze and impaired spatial working memory in the radial arm maze [27,41,61]. In contrast to these studies, the present study found increased hippocampal ChAT activity in middle-aged rats with spatial reference memory impairments. It is likely that age and dissection procedures contribute to this discrepancy. In our own laboratory using more discrete dissections of the hippocampus than used in previous studies, we have observed increased hippocampal ChAT activity in 11- and 17-month rats relative to 4-month rats [49]. The reason for the observed increase is unclear, but possibly reflects a compensatory mechanism to counteract preliminary age-related septohippocampal degeneration [29].

Both middle-aged and aged brains appear to be capable of compensatory change. Increased dendritic extent has been reported in the middle-aged human brain [16], and expansion of the distribution of kainic acid and acetylcholinesterase staining has been observed in the dentate gyrus of Alzheimer's brains [42]. Hypertrophy of cholinergic medial septal neurons has been observed in aged rhesus monkeys, and interestingly, this hypertrophy was observed in mnemonically impaired aged monkeys but not in mnemonically unimpaired aged or young monkeys [77]. In young rats, nerve growth factor-producing fibroblasts implanted in the nucleus basalis induced cholinergic neuron hypertrophy and mnemonic impairments [14], suggesting that hypertrophy of cholinergic basal forebrain neurons may contribute to impaired memory. If the increased ChAT activity observed in middle-aged brains is designed to compensate for cholinergic degeneration, then it appears not to be sufficient to maintain accurate spatial reference memory function. The increased ChAT activity may

possibly contribute to the observed spatial reference memory deficit, either by interfering with normal synaptic function or by inducing excessive release of acetylcholine.

The following three conclusions may be drawn from the present study: (1) Intraseptal infusions of oxotremorine in middle-aged rats modestly improved the rate of acquisition of place discrimination, but did not increase the level of asymptotic performance achieved; (2) Oxotremorine infusions significantly decreased ChAT activity in the CA2/3 region of the dorsal hippocampus relative to middle-aged controls; and (3) The improved acquisition observed in middle-aged rats may be related to the decreased ChAT activity observed in the dorsal hippocampus. The results presented here illustrate that oxotremorine can improve spatial reference memory as well as spatial working memory, although this improvement is not as robust as that previously described for spatial working memory [65]. The finding that mnemonic improvements may be observed in younger impaired rats suggests that early treatment of age-related mnemonic decline in middle-aged humans and patients with Alzheimer's disease may be beneficial.

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