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Chromaproline and Chromaperidine, nicotine agonists, and Donepezil, cholinesterase inhibitor, enhance performance of memory tasks in ovariectomized rats

V.N. Luine[a,*], G. Mohan[a], Z. Tu[b], S.M.N. Efange[b,c,d,e]

[a]*Department of Psychology, Hunter College of CUNY, 695 Park Avenue, New York, NY 10021, USA*
[b]*Department of Radiology, University of Minnesota, Minneapolis, MN 55455, USA*
[c]*Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55455, USA*
[d]*Department of Neurosurgery, University of Minnesota, Minneapolis, MN 55455, USA*
[e]*Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455, USA*

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Abstract

Chromaproline and Chromaperidine, two recently synthesized and pharmacologically characterized nicotinic agonists, and Donepezil (Aricept), an acetylcholinesterase inhibitor approved for the treatment of memory loss, were evaluated for effects on performance of a visual recognition memory task (object recognition) and a spatial memory task (object placement). Ovariectomized female rats received the drugs chronically via subcutaneous Alzet minipumps. None of the drugs altered activity in the open field or the time spent exploring objects in the field. One week following initiation of treatment, all three drugs enhanced performance of the visual recognition task, but only Donepezil enhanced performance of the spatial memory task. With a longer period of treatment (3 weeks), the nicotinic agonist Chromaproline also enhanced object placement performance. Current results show the memory-enhancing efficacy of Donepezil in two additional memory tasks in rats and suggest that the novel nicotinic agonists, Chromaproline and Chromaperidine, may also be useful new drugs for the treatment of memory impairments/loss.

Keywords: Object recognition memory; Spatial memory; Nicotinic agonists; Chromaproline; Chromaperidine; Aricept

1. Introduction

Nicotine displays a diverse array of cellular and pharmacological properties, including the ability to potentiate neurotransmitter release, modulate cardiovascular function, protect neurons from excitoxin-mediated or β-amyloid-induced injury and enhance memory (reviewed by Benowitz, 1986, 1996; Kihara et al., 1997; Rezvani and Levin, 2001). Recent advances in molecular biology suggest that the diverse actions of nicotine can be attributed to the existence of multiple nicotinic acetylcholine receptor (nAChR) subtypes (for review, see Decker et al., 1995; Gotti et al., 1997; Zoli et al., 1998). Mammalian CNS nicotinic receptors belong to a class of pentameric ligand-gated ion channels where the channels are composed of two kinds of subunits, designated α and β, and at least eight α ($\alpha_2-\alpha_9$) and three β ($\beta 2-\beta_4$) subunits have been cloned. Functional channels can be obtained from homopentamers or from pairwise combinations of α and β subunits. At least eight nAChR subtypes have been identified in heterologous expression systems, and many of these display pharmacological and physiological properties that are similar to the native receptors found in the CNS (Decker et al., 1995; Gotti et al., 1997; Willens et al., 1999).

The pharmacology of naturally occurring and synthetic nicotine agonists/antagonists suggests that selective nAChR subtype activation may have considerable potential in the treatment of human neurological disorders such as Parkinson's disease (Quik and Jeyarasasingam, 2000; Schneider et al., 1998), Alzheimer's disease (Potter et al., 1999; Sahakian et al., 1989), Tourette's syndrome (Sanberg et al., 1997), attention-deficit hyperactivity (Conners et al., 1996; Willens

* Corresponding author. Tel.: +1-212-772-4223; fax: +1-212-772-5620.
E-mail address: vluine@hunter.cuny.edu (V.N. Luine).

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et al., 1999), anxiety (Pomerleau, 1986) pain (Decker and Meyer, 1999; Marubio et al., 1999), addiction liability (Benowitz, 1996) and memory loss (Brown et al., 2000; Rezvani and Levin, 2001). However, use of nicotine has been limited because of its adverse effects on heart rate, body temperature, respiration and incidence of seizures. Recently, novel nicotinic agonists such as RJR-2403, ABT-089 or ABT-418, and AR-R17779, which have reduced side effects compared to nicotine, have been synthesized. They also improve passive avoidance acquisition and/or spatial memory in rats and mice with impaired memory due to lesions of the basal forebrain cholinergic system (Decker et al., 1994; Levin et al., 1999; Lippiello et al., 1996; Potter et al., 1999; Willens et al., 1999; Van der Staay et al., 1996). Consequently, the development of subtype-selective nicotine agonists without untoward side effects and with memory-enhancing properties has become a fertile area of investigation.

This study describes a preliminary investigation of two members of a new class of subtype-selective, nonpyridine, nicotinic agonists, Chromaperidine and Chromaproline, on memory (Efange et al., 2001). These compounds are semi-rigid analogues of the previously characterized nicotinic agonist ABT-089 (Decker et al., 1997), in which the 3-pyridyl group has been replaced by a hydroxyphenyl group (Fig. 1). Based on numerous precedents, the increased rigidity was expected to be accompanied by enhanced nAChR subtype selectivity. In fact, relative to ABT-089, Chromaproline and Chromaperidine display significantly altered neurochemical and pharmacological properties (Efange et al., 2001) and were therefore chosen for further characterization on memory. Both compounds activate $\alpha7$ and $\alpha3\beta2$ nAChRs in a dose-dependent manner, thus confirming nicotinic activity. Chromaproline was significantly more potent than Chromaperidine on both receptor subtypes. On the other hand, both compounds displayed poor affinity for [^{125}I]-α-bungarotoxin binding sites, suggesting selectivity for non-$\alpha7$ nAChRs. In functional assays, both

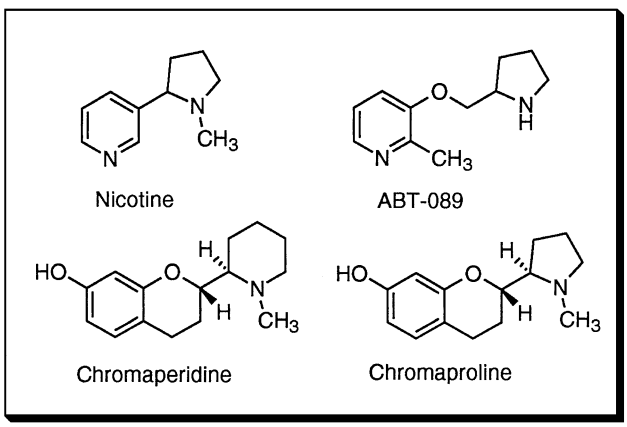

Fig. 1. Chemical structure of nicotine and nicotinic drugs. Structures for the parent compound, nicotine, and three nicotinic agonists, ABT-089, Chromaperidine and Chromaproline, are shown.

compounds were found to stimulate the release of [^{3}H]dopamine from rat striatal synaptosomes; however, only Chromaperidine stimulated the release of [^{3}H]ACh from cortical synaptosomes.

In this study, these nonpyridine, subtype-selective nicotinic agonist compounds were tested in rats for potential memory-enhancing effects. Subjects were evaluated for overall activity in the open field and then for memory function using object recognition (visual memory) and object location (spatial memory) tasks (Ennaceur and Aggleton, 1994; Ennaceur et al., 1997). In addition, Donepezil (Aricept), a drug that enhances memory in animal and human models (Poorheidari et al., 1998; Rogers et al., 1998) and is approved for the treatment of memory loss in mild to moderately impaired Alzheimer's disease patients (Sugimoto, 2001), was also tested for its effectiveness in these memory tasks and as a standard of comparison for the nicotinic agonists. Donepezil acts by inhibiting acetylcholinesterase—degradatory enzyme for acetylcholine (ACh)—resulting in increased ACh that can act at both nicotinic and muscarinic AChRs.

2. Methods

2.1. Subjects, treatments and general procedures

Female Sprague–Dawley (Harlan-Sprague Dawley, Indianapolis, IN) rats, 2 months old upon arrival, were used. All subjects were double-housed in plastic tubs in accordance with the Hunter College IACUC and the NIH Guide for Care and Use of Animals. Rats were on a 14:10 light–dark cycle (lights on at 05:00 h, lights off at 19:00 h). In the first experiment, there were three cohorts, which consisted of three to five subjects in each group. The number of rats for groups varied because some animals were determined to carry the SDHV virus and, though asymptomatic, were euthanized before testing was completed. In the second and third experiment, one cohort consisting of 12 and 15 rats, respectively, was tested.

Ovariectomies and implantation of miniosmotic pumps for drug delivery were performed on subjects 1 week after arrival, utilizing Metofane (Mallinckrodt Veterinary, Mundelein, IL). Subjects were ovariectomized, because lack of ovarian hormones is associated with poor performance of some memory tasks, including object recognition, object placement and the Morris water maze in rats (Frankfurt et al., 2001; Sandstrom and Williams, 2001; Singh et al., 1994) and a variety of memory tests in postmenopausal women who have little circulating estradiol (Sherwin, 2000). Alzet pumps (Alzet Model 2002) were 3 cm in length and were subcutaneously implanted through the same incision as the ovariectomies, but they were placed further below at the level of the posterior rib-spine. Drugs, synthesized in the laboratory of S.M.N. Efange as described previously (Efange et al., 2001), were provided as a dry powder and

were dissolved in sterile, physiological saline and placed in Alzet pumps with a capacity of 236 μl and continuously dispensed 0.5 μl/h for 2 weeks. Control subjects were implanted with Alzet pumps filled with saline or empty gelatin capsules (Lilly, Indianapolis, IN), which were the same size and shape as the Alzet pumps. The dosage for Donepezil (a kind gift from Pfizer-Eisai) was 2.4 μmol/kg/day (1 mg/kg/day). Chromaproline and Chromaperidine were given as a 1 μmol/kg/day dose (270 and 279 μg/kg/day, respectively). Rats gained approximately 15 g/week, which resulted in a 5% diminution in the dosage level each week. In all of the experiments, the procedures and behavioral paradigms were the same, but, in the third experiment, the drug was given for a longer period before behavioral testing (see below).

Behavioral testing was conducted in an open field (102.5 × 61.5 cm) with a Formica base and four sides. Two sides were the walls of the room and the other two sides were painted wood (16 in. high) secured together. For the initial trial, the floor was marked off into 15 equal (20.5 cm) squares (3 × 5). For the object recognition and object placement trials, the area was shortened to nine squares (3 × 3).

2.2. Open field

In Experiments 1 and 2, subjects rested and recovered for 2 days after surgery/implantation of the Alzet minipumps containing drugs. On Day 4 (3 days following surgery), behavioral testing began with the open field. In Experiment 3, ovariectomy surgery and minipump implantation were completed as in the previous experiment, but behavioral testing was delayed. Two weeks after pump implantation, new drug-containing pumps were implanted, and on Day 4 after implantation, behavioral testing in the open field began (17 days of drug treatment). Subjects were placed at the center of the field, and grid crossings (moving from one square to another), which include outside sector visits and inside sector visits, rearing (raising up on haunches), wall climbs (raising up on haunches and touching the walls) and defecations, were recorded. Subjects were observed for 6 min. Behaviors were tabulated for each subject for the first 3 min and the second 3 min of the 6-min trial. The open field was cleaned with disinfectant spray after each subject trial. Data were analyzed by between-subjects two-way ANOVAs, Group (control, drugs) × Time (first 3 min, second 3 min) for each behavior.

2.3. Object recognition

During the afternoon of Day 4, object recognition testing began according to the general methods of Ennaceur and Aggleton (1994), Ennaceur et al. (1997), and Beck and Luine (1999). Trials were conducted as previously described (1, 2) and consisted of a sample trial (T1) and a recognition trial (T2). The two trials were separated by an intertrial interval of 4 h. In T1, two identical objects were placed at one end of the open field, and the amount of time spent exploring the two objects was recorded for 3 min. For T2, or the recognition trial, one object was replaced by a new object. In T2, the time spent exploring the old (familiar object) and the new (novel) object was recorded for 3 min. Exploration was defined as when the subject sniffed at, whisked at or looked at the object from no more than 2 cm away. On Days 4–7, subjects received trials with intertrial delays of 1 and 10 min, and 1 and 2 h in order to acclimate the subjects to the field and to the task. On Day 8 of Experiments 1 and 2 (1 week following pump implantation), subjects were tested in trials with a 4-h intertrial delay. A 4-h intertrial delay interval was chosen to test for performance enhancements by drugs because previous studies show that ovariectomized rats do not significantly discriminate between old and new objects at this delay (Beck and Luine, 2002; Frankfurt et al., 2001). In Experiment 3, object recognition was tested 3 weeks following initiation of drug treatment. The objects used for trials were various bottles, cans and containers. The position of the objects, and which object was novel, was fully counterbalanced across groups.

2.4. Object placement

Object placement is a variation of the object recognition task and requires use of spatial memory (Ennaceur and Aggleton, 1994). The sample trial, T1, was conducted in the same manner as in the object recognition task. However, during the delay period, instead of a new object being placed in the field, one of the objects was moved to a new location. Therefore, in T2, the time spent exploring objects at the old and new location was recorded. In order to acclimate the subjects to this task, trials with a 1-h intertrial delay were conducted. Then, 12 days following initiation of drug treatment and 1 day following the 1-h delay trial, testing with a 2-h intertrial delay was conducted for subjects in Experiments 1 and 2. In Experiment 3, object placement was tested following 26 days of treatment. A 2-h intertrial delay was chosen to test for performance enhancements, because previous results show that ovariectomized rats do not significantly discriminate between the old and new objects at this delay interval (Beck and Luine, 2002; Frankfurt et al., 2001). The objects used for object placement testing, candleholders and funnels, were more intricate and complex than objects used in object recognition trials. The position of the objects across groups was fully counterbalanced.

2.5. Statistical analyses

One-way ANOVAs were utilized to test for differences among groups in exploration time during T1. For the recognition trial (object recognition or object placement), two-way ANOVAs were completed, Group (control, drugs) × Object (old, new). If significant F values were found ($P < .05$), then post hoc tests were applied. Paired t tests on each group tested

whether the time spent with the old object (location) was less than the time spent with the new object (location). All experiments had sufficient power with coefficients for α=.05 ranging from .82 to .98 (NCSS Statistical Software, 2000; Kaysville, UT). If subjects spent significantly more time exploring the new object (or location), they were considered to have discriminated/remembered.

3. Results

3.1. Experiment 1

3.1.1. Open field

In the first experiment, subjects received saline, Donepezil or Chromaproline. Neither drug significantly altered behavior in the open field, but all subjects, regardless of treatment, showed less activity in the second 3 min compared to the first 3 min in some parameters (Fig. 2). In the second 3 min compared to the first 3 min, subjects made significantly less outside sector visits [$F(1,34)=49.90$, $P<.001$], inside sector visits [$F(1,34)=5.21$, $P<.029$] and wall climbs [$F(1,34)=33.29$, $P<.001$]. There were no significant differences between the first and the second 3 min for rears, grooms and defecations, or Group × Time interactions (data not shown).

3.1.2. Object recognition and placement

All groups—control, Donepezil and Chromaproline—spent more than 6 s exploring the objects in T1, and there were no group differences in exploration time (data not shown). Fig. 3 shows the time spent exploring objects in the recognition trial. Control subjects did not appear to recognize the new object, because they spent the same amount of time exploring the old and the new objects, which is approximately 4–5 s. In contrast, both Donepezil- and Chromaproline-treated subjects spent significantly more time exploring the new than the old object, $P<.01$ and $P<.05$, respectively.

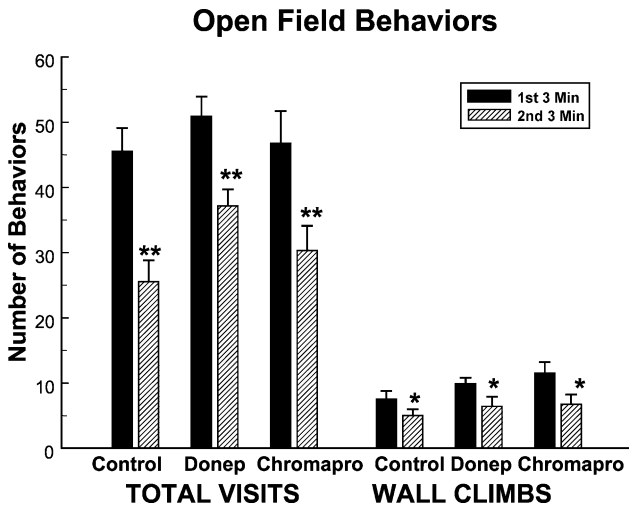

Fig. 2. Effect of Donepezil and Chromaproline on open-field behavior. The number of sector visits and wall climbs are shown for control ($n=13$), Donepezil (Donep)-treated ($n=14$) and Chromaproline (Chromapro)-treated ($n=9$) rats. Entries are averages ± S.E.M. during the first 3 min (closed bars) and second 3 min (striped bars) on the field. Data were analyzed by a two-way ANOVAs (Group × Time). No group effects, but time effects, were significant, $*P<.05$ and $**P<.01$.

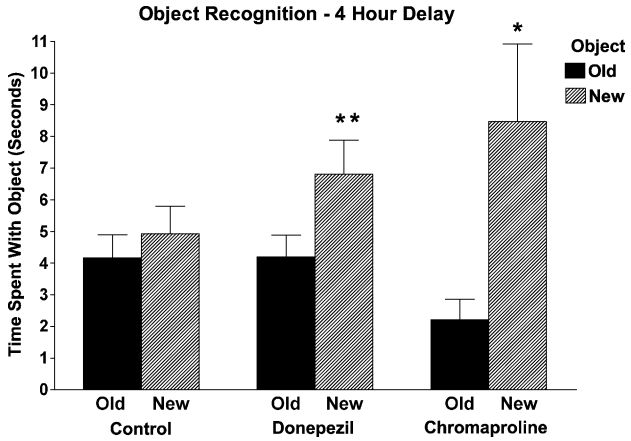

Fig. 3. Effect of Donepezil and Chromaproline on object recognition. The time spent exploring the old (solid bars) and the new (striped bars) objects in the recognition trial are shown for control ($n=13$), Donepezil-treated ($n=14$) and Chromaproline-treated ($n=9$) rats. The intertrial delay between the sample trial and the recognition trial was 4 h. Entries are averages ± S.E.M. Data were analyzed by two-way ANOVAs (Group × Object), where $F(1,66)=12.4$, $P<.0008$ for Object and $F(2,66)=3.11$, $P<.05$ for the Group × Object interaction. Differences between the time spent (s) with the old and new objects for each group were tested by paired t tests, $*P<.05$ and $**P<.01$.

In object placement testing, all groups (control, Donepezil and Chromaproline treated) explored the object during T1, and there were no differences between groups in exploration times (data not shown). In the recognition trial, control subjects spent the same amount of time exploring the old and new locations in the recognition trial, suggesting that they did not remember the old location (Fig. 4). Likewise, Chromaproline-treated subjects did not spend more time exploring at the new location; however, Donepezil-treated subjects spent significantly more time exploring at the new than at the old location, approximately 8 s versus 6 s, respectively, $P<.05$.

3.2. Experiment 2

3.2.1. Open field

Because the cholinesterase inhibitor Donepezil enhanced both object recognition and placement while the nicotinic agonist, Chromaproline, did not, an additional nonpyridine, subtype-selective agonist, Chromaperidine, was tested. Similar to results with Chromaproline and Donepezil, there were no effects of treatment in the open field except for reduced activity in the second 3 min compared to the first 3 min in both the control and Chromaperidine-treated groups for some parameters (data not shown).

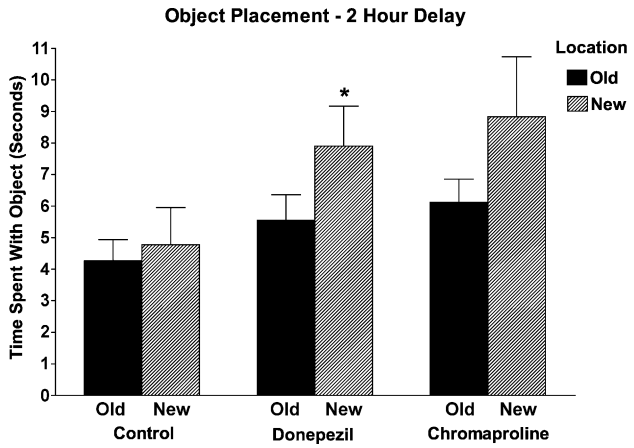

Fig. 4. Effect of Donepezil and Chromaproline on object placement. The time spent exploring objects at the old (solid bars) and the new (striped bars) location in the recognition trial are shown for control ($n=7$), Donepezil-treated ($n=8$) and Chromaproline-treated ($n=8$) rats. The intertrial delay between the sample trial and the recognition trial was 2 h. Entries are averages ± S.E.M. Data were analyzed by two-way ANOVAs (Group × Location), where $F(1,45)=5.36$, $P<.05$ for Location. Differences between the time spent (s) at the old and new location for each group were tested by paired t tests, $*P<.05$.

3.2.2. Object recognition and placement

Similar to the results in Experiment 1, agonist-treated subjects spent the same amount of time as control subjects exploring objects in T1 for object recognition and placement trials (data not shown). Also similar to the pattern of results with Chromaproline- and Donepezil-treated subjects, Chromaperidine-treated subjects spent significantly more time with the new object than the old object during a recognition trial with a 4-h intertrial delay, $P<.03$ (Fig. 5). Control subjects did not spend more time with the new object. In object placement testing, neither control nor Chromaperidine-treated subjects spent more time exploring at the new location than the old location, suggesting that they did not remember the old location (Fig. 6).

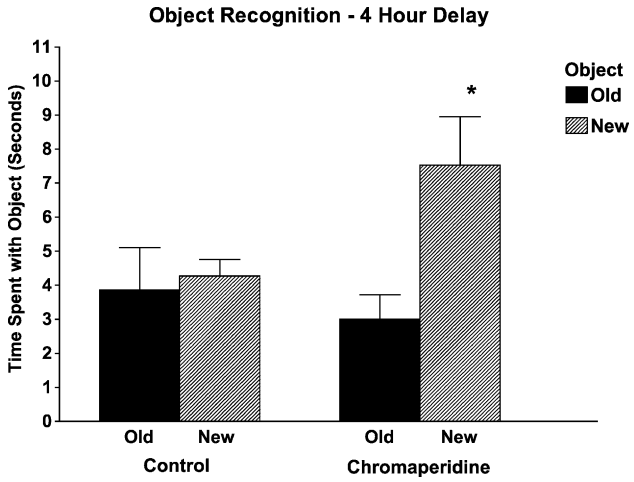

Fig. 5. Effect of Chromaperidine on object recognition. The time spent exploring the old (solid bars) and the new (striped bars) objects in the recognition trial are shown for control ($n=7$) and Chromaperidine-treated ($n=5$) rats. The intertrial delay between the sample trial and the recognition trial was 4 h. Entries are averages ± S.E.M. Data were analyzed by two-way ANOVAs (Group × Object), where $F(1,20)=5.7$, $P<.03$ for Object and $F(1,20)=4.0$, $P<.058$ for the Group × Object interaction. Differences between the time spent (s) with the old and new objects for each group were tested by paired t tests, $*P<.027$.

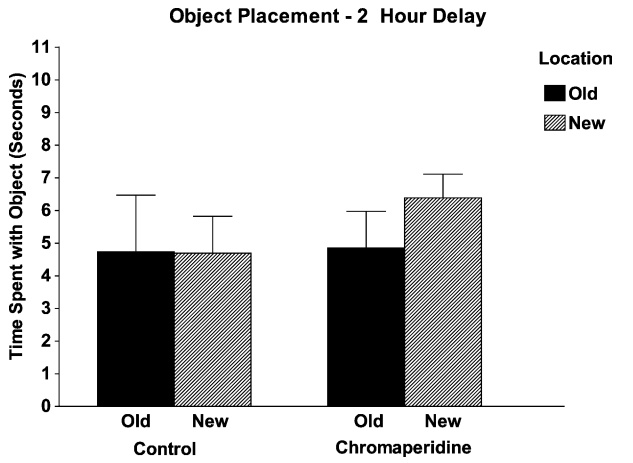

Fig. 6. Effect of Chromaperidine on object placement. The time spent exploring objects at the old (solid bars) and the new (striped bars) location in the recognition trial are shown for control ($n=7$) and Chromaperidine-treated ($n=5$) rats. The intertrial delay between the sample trial and the recognition trial was 2 h. Entries are averages ± S.E.M. No differences were found by two-way ANOVAs (Group × Location).

3.3. Experiment 3

Results in Experiments 1 and 2 showed that Donepezil enhanced both object recognition and placement perform-

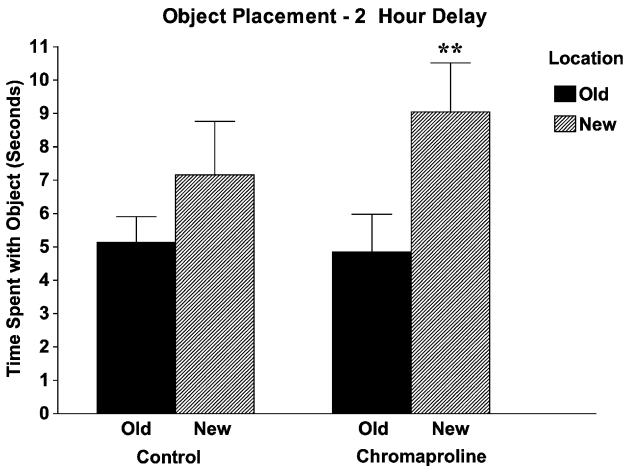

Fig. 7. Effect of 25 days of Chromaproline treatment on object placement. The time spent exploring objects at the old (solid bars) and the new (striped bars) location in the recognition trial are shown for control ($n=8$) and Chromaperidine-treated ($n=7$) rats. The intertrial delay between the sample trial and the recognition trial was 2 h. Entries are averages ± S.E.M. Data were analyzed by two-way ANOVAs (Group × Location), where $F(1,26)=5.8$, $P<.02$ for Location. Differences between the time spent (s) at the old and new location for each group were tested by paired t tests, $**P<.01$.

ance following treatment for 7 and 11 days, respectively, while the nicotinic agonists enhanced only object recognition performance. In this experiment, Chromaproline was administered for a longer period, 21 days before object recognition testing and 25 days before object placement testing. Similar to results in experiment 1, Chromaproline treatment did not alter the time spent exploring objects in T1 for either object recognition or placement trials (data not shown). Also similar to the results in Experiment 1, Chromaproline-treated subjects, but not saline-treated subjects, spent significantly more time exploring the new object than the old object during a recognition trial with a 4-h intertrial delay, $P < .003$ (data not shown). In contrast to results with a shorter drug treatment time (Experiments 1 and 2), Chromaproline-treated, but not control, subjects spent significantly more time exploring the new location than the old location during a recognition trial with a 2-h intertrial trial ($P < .01$, Fig. 7).

4. Discussion

Experiments tested the effects of drugs acting at cholinergic terminals on the performance of a visual recognition memory task and a spatial memory task. Chromaproline and Chromaperidine, two recently synthesized and characterized nicotinic agonists (Efange et al., 2001), were evaluated along with Donepezil (Aricept). Donepezil, which inhibits acetylcholinesterase, increases availability of ACh at both nicotinic and muscarinic cholinergic receptor sites. Donepezil has been previously shown to enhance memory in animal and human models (Poorheidari et al., 1998; Rogers et al., 1998; Rupniak et al., 1997). None of the drugs, at the doses and durations given, altered activity in the open field nor the time spent exploring objects in the field. However, all three drugs enhanced performance of a visual recognition task and a spatial memory task. Longer treatment with the nicotinic drug was necessary to enhance performance of the spatial memory task.

The current results are consistent with a large body of evidence showing that ACh containing neural systems contribute to normal memory function and loss of these neurons contributes to disease- and age-related losses in memory (Bartus et al., 1985; Baxter et al., 1999; Sanberg et al., 1997). These results also provide additional support for a growing body of information that nicotinic, in addition to muscarinic, receptors contribute to memory (Rezvani and Levin, 2001). In addition, most previous studies investigating cholinergic effects on memory in rats have utilized tasks that measure spatial memory and/or require either rewarding or aversive stimuli, for example, the eight-arm radial maze, Morris water maze task and active and passive avoidance, in order to show enhancements of memory by cholinergic agents (Bancroft and Levin, 2000; Brown et al., 2000; Decker et al., 1994, 1997; Levin et al., 1999; Lippiello et al., 1996; Van der Staay et al., 1996). In this study, we have avoided the use of noxious stimuli or appetitive-reinforcement contingencies. Ennaceur et al. showed that rodent memory can be assessed through the use of novelty exploration (Ennaceur and Aggleton, 1994; Ennaceur et al., 1997). Testing behavioral responses to novel stimuli removes the necessity for aversive stimuli and food deprivation to encourage performance. In the current tasks, the rationale is that the rat will explore a new object, or an object in a new location, more than one that it has previously explored a few hours earlier. The tasks appear to give a relatively sensitive measure of working memory, because drug-dependent enhancements were found in subjects that were not lesioned or treated with cholinergic antagonists before testing as is the case in many previous studies in rats (Bancroft and Levin, 2000; Brown et al., 2000; Decker et al., 1997; Lippiello et al., 1996, Poorheidari et al., 1998). This object recognition task in rats is similar to object recognition tasks employed in nonhuman primate studies and, thus, may provide a useful task for screening possible cognitive enhancing drugs. For example, daily administration of Donepezil or ABT-089 to monkeys enhances choice accuracy in a visual recognition task (Rupniak et al., 1997; Decker et al., 1997), a result similar to the current object recognition testing in rats.

Both Donepezil and the nicotinic agonists enhanced object recognition 1 week after initiation of treatment, but only Donepezil enhanced object placement performance 11 days following treatment. Chromaproline received additional testing for effects on spatial memory following 25 days of treatment, and with this longer duration of treatment, performance was enhanced, suggesting that the nicotinic agents may require a longer period for efficacy. Since only one dose of the nicotinic drugs was tested (approximately 250 μg/kg, 1 μmol/kg), it is also possible that higher doses may be effective at shorter intervals. It should be noted that approximately 1–5 mg/kg are required for nicotine to enhance performance of memory tasks (Bancroft and Levin, 2000; Brown et al., 2000); thus, the nicotinic drugs are clearly more potent than the parent compound. The current nicotinic agents were synthesized based on the structure of the agonist ABT-089. ABT-089 has been previously tested via Alzet minipumps at a dose of 1.3 μmol/kg in septal lesioned young rats and in young and aged rats (Decker et al., 1997). ABT-089 treatment improved water maze performance in the lesioned rats and in aged rats. It did not affect performance of either aged or young rats on passive avoidance and induced a small impairment in young rats on the water maze. While similar tasks were not employed for Chromaproline and Chromaperidine testing, it appears that these agents are at least comparable with, and may be more potent than, ABT-089 in enhancing performance of memory tasks. Donepezil enhanced both recognition and spatial memory at 1 mg/kg (2.4 μmol/kg), a higher dose than the nicotinic agents; we have not tested lower doses of this drug. Clearly, a complete dose–response curve for the agonists, as well as direct comparisons with other agonists, is necessary to determine

their ultimate therapeutic usefulness. Overall, however, these newly synthesized nonpyridine, subtype-selective, nicotinic agonists appear promising for treating memory loss.

The mechanism and areas of the brain responsible for Chromaproline and Chromaperidine's effects on memory tasks are unknown. Zoli et al. (1998) have identified four classes of brain nicotinic receptors using β2 knockout mice, and a variety of these receptors are expressed in the hippocampus. Recently though, results of Levin et al. suggest that α7 and α4β2 nicotinic receptors in the hippocampus maybe critical for mediating nicotinic effects on cognition (Bancroft and Levin, 2000; Levin et al., 1999, 2002; Rupniak et al., 1997). Chromaproline and Chromaperidine bind with moderate to high affinity to α4β2 but poorly to α7 receptors; however, in cloned human nicotinic receptors expressed in *Xenopus* oocytes, both drugs activated, in a dose-dependent manner, α7 and α3β2 nicotinic receptors, but did not activate α4β2 receptors. Since the agents bind to α4β2 receptors but do not activate them, they may be antagonists at this receptor (Efange et al., 2001). Thus, with this spectrum of binding and activity, these drugs may not be as effective in enhancing hippocampal dependent spatial memory as recognition memory. Evaluation in combination with α4β2 and α7 antagonist drugs, dihydro-β-erythrodine and methylcaconitine, respectively, would be useful in understanding the basis for their effects on memory. Nonetheless, the activation of α3β2 nicotinic receptors by Chromaproline and Chromaperidine may be important for the enhancements of recognition memory because these receptors have been implicated in memory (Decker and Meyer, 1999; Rezvani and Levin, 2001). The currently tested nicotinic agonists also release dopamine (Efange et al., 2001), which may contribute to their cognitive efficacy because dopamine is also implicated in promoting memory function, especially in the frontal cortex (Goldman-Rakic, 1998).

Relevant to the drug-dependent enhanced performance of the memory tasks is that the behavioral evaluations were completed in ovariectomized female rats. Estradiol is known to activate cholinergic neurons in the basal forebrain system, which contributes to cognitive function (Gibbs and Aggarwal, 1998), and, thus, lack of estrogen may have influenced the outcome. The temporal differences in nicotinic drug effects on visual versus object spatial memory may also be related to estrogen lack because the hippocampus receives major cholinergic input (Gibbs and Aggarwal, 1998). Major sex differences in response to the drugs appear unlikely because previous studies of nicotinic drugs have also been completed in female rats with results comparable to those in males (Levin et al., 1999, 2002).

In conclusion, a preliminary assessment of Chromaproline and Chromaperidine, α7 and α3β2 subtype-selective nicotinic agonists, shows that at 1 μmol/kg doses, delivered chronically, performance of the memory tasks objection recognition and placement is enhanced. The drugs did not alter activity in the open field or the time spent exploring objects in sample trials. Donepezil, a well-known and approved drug for memory loss, also enhanced performance of both tasks. Further evaluation of these novel nicotinic receptor agonists are necessary to determine whether they may be of benefit for disease- and age-dependent memory loss.

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