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Limits of learning enhancements with nicotine in old male rats

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Abstract: Findings with young adult humans and animal models suggest that nicotine may serve both neuroprotective and cognition enhancing roles in old animals. A pair of experiments was conducted to examine drug-induced modification of the cholinergic nicotinic receptor subtype on rates of learning by young and aged rats. In experiment I males (4–7 months or 20–25 months old) were administered nicotine (0.0, 0.3 or 0.7 mg/kg injected s.c. daily) and tested in both a T-maze non-spatial discrimination paradigm and a hole board spatial task. Nicotine failed to improve acquisition by young animals on either task. Nicotine also failed to improve non-spatial learning by old animals. However, both dosages of nicotine improved performance by the old males in the spatial paradigm. In experiment II, a 5-choice serial discrimination paradigm designed to better evaluate visual attention and spatial working memory in aging was used. Groups of old male rats were administered nicotine or mecamylamine (2 or 8 mg/kg), an antagonist of the nicotinic cholinergic receptor. Results were that the 0.3 mg nicotine group learned the task fastest and achieved the highest learning asymptote. Both learning rates and final levels of performance were worst in the 8 mg mecamylamine group. However, the 2 mg mecamylamine rats were the equals of the control group and both reached a higher asymptote than the 0.7 mg nicotine group. These data suggest that healthy old animals can accrue benefits from nicotinic activation but that the benefits are complex, being limited to certain dosages and to specific cognitive skills.

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GENERAL INTRODUCTION

Nicotine and the nicotinic receptors of the brain's cholinergic transmitter systems remain a research focus for cognitive impairments accompanying both normal aging and the pathological diseases of the aged (Albuquerque et al. 2001, Levin and Rezvani 2002). Despite the many good public health reasons to vilify nicotine, there is a considerable literature suggesting nicotine may protect the aging brain. These include epidemiological findings that smokers are less likely to develop age-related dementias (Fratiglioni and Wang 2000, Tyas et al. 2000) and identification of a possible mechanism.

Chronic nicotine exposure has the unusual capacity to up-regulate nicotinic receptor (nAChR) concentrations (Breese et al. 1997, Rowell and Li 1997). These findings have clinical relevance because *post mortem* examination of the brains of Parkinson's and Alzheimer's patients reveal unusually high losses of nAChR (Kelton et al. 2000, Patterson and Nordberg 2000) and in brain regions critical for learning and memory (McGehee and Role 1996, Perry et al. 1999). That the rodent brain shows similar nAChR losses with normal aging and similar up-regulation with nicotine treatments (Rogers et al. 1998, Schulz and Kuchel 1993) suggests rats as an appropriate animal model for the study of nicotine - cognitive relations during aging (Kalueff and Tuohimaa 2004).

The literature of young animal models administered, most often acutely, nicotine suggests enhancements of some aspects of cognitive performance with some dosages (Abdulla et al. 1996, Arthur and Levin 2002). Demonstrating a beneficial role for nicotine in young adults is most reliable in paradigms that are cognitively demanding, employ spatial working memory or require attention to visual cues (Granon et al. 1995, Hahn et al. 2002, Muir et al. 1995, Puma et al. 1999).

Behavioral studies with old animal models have yielded more fragile conclusions. For example, Levin and Torry (1996) found that the same dosages of nicotine that clearly improved working memory in young animals were ineffective in old animals. Moreover, infusion of mecamylamine, the prototypical nicotinic receptor antagonist, impaired performance of young but not of old rats. Another report suggested nicotine improved and mecamylamine impaired spatial memory performance in old male rats (Riekkinen and Riekkinen 1997). Other studies concluded that it is reference memory that receives the most benefits from nicotine in old rats (Arendash et al. 1995b) or that nicotine is most effective on visual attention in middle-aged and old rats (Grilly et al. 2000). Still other experiments with nicotine have reported no improvements in reference memory (Attaway et al. 1999, Kelton et al. 2000) or in visual discrimination in old animals (Turchi et al. 1996).

EXPERIMENT I

Introduction

Two experiments were designed to clarify the role of chronic nicotinic activation in the cognitive behaviors of healthy old rats. In experiment I, old and young animals (Koprowska et al. 2004) administered different dosages of nicotine were compared in paradigms requiring different cognitive skills. Only old rats were used in experiment II for a dose-response study of nicotine agonism and antagonism in a paradigm based on visual attention and working spatial memory.

Methods

ANIMALS

The Long-Evans rats (n=48) were equal numbers of young adult (4–7 months of age, range of body weights 445–584 g) and old males (20–25 months of age, range of body weights 506–658 g), respectively. The animals had been housed individually for at least 30 days before the experiment in hanging wire cages measuring 20.5 × 23.5 × 29.5 cm. Water and Richmond Standard Lab Diet 5001 were available as dictated by the food restriction protocol described below. Lighting in colony rooms are on a reversed cycle of 12 h light/dark, room temperature (20–22°C) and relative humidity (55 ± 5%) are controlled automatically.

APPARATUS

Our hole board apparatus (Taylor et al. 2004) is a 66.5 × 66.5 × 43 cm box constructed of clear Plexiglas. The floor has 4 holes, 4 cm in diameter and 4.5 cm in depth, located 12 cm from each corner. A small circular section of wire mesh screen was located halfway into each hole to render food inaccessible if placed below the screen. A portable cylinder positioned in the

center of the apparatus served as a start box. The apparatus sat on the floor in the center of the room to reveal various extra maze cues to the animal. Examples of extra maze stimuli were posters mounted on the walls, a set of shelves on another wall, and the experimenter standing behind the apparatus.

The T-maze apparatus used has been described in detail previously (Taylor and Weiss 1987). The 12 cm wide × 16 cm tall structure has a start box and runway totaling 129 cm, with 35 cm arms and 21 cm goal boxes. Manually operated guillotine doors were located immediately after the start box and at the choice point at the entrance to each of the arms. At the choice point located at the entrance to each arm were 0.5 cm diameter lights that could be activated to flicker at 30 Hz.

The open field apparatus (Taylor et al. 1996) was a platform 90 x 120 cm onto which 15 cm squares were drawn on the top to allow quantifying locomotor activity. Nicotine tartrate salt was purchased from Sigma Chemical Company (St. Louis, MO) and was solubilized in 0.9% saline solution and neutralized with small quantities of 10 N NaOH to pH 7.

EXPERIMENTAL DESIGN

For experiment I, rats from each age were assigned at random to 1 of 3 treatment conditions (n=8 per group) to be administered daily doses of vehicle only, 0.3 mg nicotine/kg or 0.7 mg nicotine/kg, both calculated as the weight of the base. The result was a 2 × 3 factorial design with main factors of age and drug dosage.

All substances were administered daily for 5 weeks as a s.c. injection of 0.2 ml of saline solution, with a behavioral session initiated 1 h later. Animals were food restricted by being allowed access to food in its home cages only for the hour following a session, after which time the food was removed. Consequently, the animals had not eaten for the 23 h prior to either a habituation or a test session. Testing of the animals in the T-maze and in the hole board were conducted during weeks 2 and 3.

Tests of general activity were conducted in the open field on each of the three weeks of drug exposure. Open field testing was conducted only on days in which no other behavioral testing was done. Body weights were obtained at the beginning prior to food restrictions and again were obtained at the end of the experiment.

PROCEDURES

Overview

Ordering of the procedures was as follows. Prior to any drug exposures, animals were placed on food restriction and habituated to the T-maze and the hole-board paradigms. Subsequently, drug administrations began (week 1) with food restrictions continuing for the duration of the experiment. During week 2 of drug exposure, testing in the T-maze began and continued into week 3. Testing in the hole board was conducted for half the animals in each group during week 2 of drug administrations and during week 3 for the other half of the animals. Injections were administered 1 h before a scheduled behavioral session.

T-maze paradigm

Each animal received 32 test trials in the T-maze, distributed as 4 trials per day, 4 days per week over 2 weeks. The paradigm used is a reference memory task (Jakubowska-Dogru et al. 2003) requiring the animal to attend to a continuously flickering stimulus light that signaled the arm containing a food reward. A piece of sweetened breakfast cereal (Honey Nut Cheerios) cut into quarters was placed at the back of the goal area of the correct arm. After 15 s the animal was removed from the goal area chosen and returned to a holding cage. The floor of the maze was wiped with a moistened paper towel and prepared for a second trial. Order of the arm containing the food reward was counterbalanced within each session.

Hole board paradigm

The hole board paradigm is a spatial task using massed trials. It also is a reference memory task in which a hungry rat must learn and remember extra-maze cues to find food (Brosnan-Watters and Wozniak 1997). A correct choice was defined by the animal selecting first the food-baited hole before searching in one of the other holes. Nicotine was administered prior to the test sessions.

We have described earlier details of our hole board procedures (Taylor et al. 2004). Briefly, each animal received a single test session in the hole board apparatus. The apparatus was prepared for discrete trials by cereal being placed on top of a small wire-mesh screen

positioned half way down into the hole designated as the correct choice. To equate food odor cues, cereal also was placed in the other three, incorrect holes under the screen, making the food inaccessible.

A trial began by placing the rat in the start box positioned in the center of the apparatus. After 5 s, the cylinder was lifted and the rat was allowed to move about the apparatus until it found the hole containing the accessible food. On trials in which the rat went directly to the food-baited hole without searching in the other holes, it was scored as a correct choice. On incorrect trials, the animal was allowed to visit the other holes until the accessible cereal was discovered. After eating, the rat was removed to a holding cage for a 1 min ITI during which time the apparatus was cleaned.

Testing continued until the rat met a criterion of choosing the correct hole first on eight of nine consecutive trials food (Brosnan-Watters et al. 1999) or until 60 trials had been given. Most often, testing was completed during a single test day. However, if the rat failed to search for the food for 3 minutes on 3 consecutive trials, the session was terminated and continued the next day.

Open field

Each animal also was tested in the open field to assess nonspecific drug effects. Testing was conducted in a dimly lit room, and the rat was placed at one end of the apparatus facing the open field to begin a 5 min session. The rat was allowed to roam the open field freely. Numbers of squares crossed were recorded to assess locomotor changes during each week of drug exposures.

Statistical Analyses

Data analyzed were numbers of trials required to achieve criterion (8 correct choices on 9 consecutive trials) in the hole board paradigm and percentages of correct choices in trials in the T-maze. Mean numbers of squares crossed in the open field apparatus and body weight changes also were analyzed. Percentage of weight loss was calculated from the differences in body weights taken at the beginning and at the end of the experiment.

Means and standard errors were calculated for each measure. Factorial analyses of variance and one-way ANOVAs were performed using the SPSS statistical program for Macintosh computers. With a statistically significant interaction between main factors on the factorial ANOVA, simple main effects were calculated (Kirk 1995) to more thoroughly assess the results of the same drug administered to different age groups.

With a statistically significant F value obtained on any of the ANOVA conducted, the Tukey's HSD method was used as a *post-hoc* test to compare means of each group with every other group. Multiple simultaneous pairwise comparisons of groups often yields results that cannot be adequately described simply, for example with an asterisk. In those situations, superscripts are used and explained further in the table captions. Probability value for all analyses was $P<0.05$.

Results

Group means and standard errors of the means on measures obtained in experiment I appear in Table I. Factorial analyses of variance with main effects of age and drug were conducted on each measure. The ANOVA on percentages of correct responses in the T-maze yielded non-significant differences for both age and drug main effects, $F_{1,42}=1.81$, ns, and $F_{2,42}=0.21$, ns, respectively, and for their interaction, $F_{2,42}=0.80$, ns.

Results of the trials required to reach criterion in the hole-board paradigm revealed statistically significant differences for both age and drug main factors, $F_{1,42}=5.82$ and $F_{2,42}=8.99$, respectively, both $P<0.05$. More important, a statistically reliable interaction between main factors was obtained, $F_{2,42}=4.83$, $P<0.05$. The initial analyses of simple main effects of the interaction were comparisons of the young group and the old group administered the same drug dosage. Results indicated the only statistically significant differences between drug groups were the vehicle controls, $F_{1,42}=7.44$, $P<0.05$, with the young controls achieving the learning criterion faster than the old controls. The young and old groups administered the 0.3 mg nicotine (Nic0.3) dosage did not differ nor did the young and old animals receiving the 0.7 mg (Nic0.7) dosage, $F_{1,42}=1.83$ and $F_{1,42}=1.11$, respectively, ns.

Simple main effects calculations on the different drug exposure conditions for each age provided a within age comparison. Results revealed statistical significance among the old groups, $F_{2,42}=6.12$, $P<0.05$, but not among for the young groups, $F_{2,42}=1.24$, ns. Subsequent comparisons of old group means with

Table I

Influence of nicotine injections on the results of behavioral tests in young and old rats

Group	*n*	T-maze % Correct	Hole Board Trials to Criterion	Open Field Squares Crossed	Body Weight % Loss
Young Controls	8	66 ± 4	40 ± 2 ^{a}	122 ± 6 ^{ab}	8 ± 2
Young Nic0.3	8	63 ± 2	38 ± 2 ^{a}	134 ± 11 ^{a}	11 ± 3
Young Nic0.7	8	62 ± 2	39 ± 2 ^{a}	105 ± 11 ^{bc}	12 ± 3
Old Controls	8	67 ± 4	52 ± 3 ^{b}	93 ± 12 ^{cd}	14 ± 4
Old Nic0.3	8	68 ± 3	36 ± 2 ^{a}	83 ± 12 ^{d}	12 ± 3
Old Nic0.7	8	65 ± 5	43 ± 2 ^{a}	67 ± 4 ^{e}	13 ± 4

Experiment I data with young (4–7 months) or old (20–25 months) male rats administered either vehicle only or nicotine at dosages of 0.3 mg or 0.7 mg/kg. All values are mean ± SEM. The overall ANOVA achieved statistical significance only on the hole board and open field data. Superscript letters are used to indicate statistically reliable group differences in the post-hoc comparisons with the Tukey's test ($P<0.05$). Reading down each column, any two groups with a different superscript letter differed significantly.

Tukey's HSD test demonstrated that all three old groups differed significantly from each other. More specifically, the Nic0.3 animals achieved criterion fastest of the old groups, and the old Nic0.7 met criterion faster than the old vehicle controls. The conclusion is that only among the old groups did both dosages of nicotine enhance learning of the hole board task, with the lower nicotine dosage producing superior performance to the higher dose.

The factorial analyses of exploratory activity in the open field indicated statistically significant values for the main effects of age, $F_{1,42}=38.89$, $P<0.05$, and of drug, $F_{2,42}=6.60$, $P<0.05$. The interaction between main effects was not significant, $F_{2,42}=1.78$, ns. Subsequent post hoc comparisons of age revealed complex results, as indicated by the superscript letters in Table I. Nonetheless, overall the young animals were more active than the old rats, and the higher 0.7 mg dosage of nicotine suppressed activity in the open field in both age groups.

With food restriction, all animals lost body weight during the experiment. There were, however, no statistically reliable differences among the groups. The factorial ANOVA on percentages of body weight lost revealed non-significant values for both main effects, $F_{1,42}=2.06$, ns, and $F_{2,42}=1.09$, ns, and for their interaction, $F_{2,42}=0.44$, ns.

EXPERIMENT II

Introduction

A second experiment was subsequently conducted using only old rats. Animals were administered either nicotine or mecamylamine, a nicotinic receptor antagonist. A 5-choice serial discrimination paradigm was chosen for optimal sensitivity to manipulation of aging nicotinic systems underlying cognitive behaviors.

Methods

ANIMALS

The Long-Evans subjects ($n=50$) were experimentally naive 20–25 months old males (range of body weights 552–681 g) in good health. Housing conditions and other details were the same as for the animals in experiment I.

APPARATUS

Construction of the 5-choice apparatus was based on descriptions in the literature (Muir et al. 1995, Stolerman et al. 2000). The main Plexiglas structure measured 26 × 31 × 20 cm. The curved rear wall was divided into 5 equal sections, or stalls, separated by partitions protruding 2 cm from the rear wall. Each stall contained a round food hole in the floor, 2.5 cm diameter, and a green light-emitting diode (LED) positioned in the middle of the rear wall. A single metal flap that could be retracted manually covered the food holes. A start box measured 21 cm in length with a 10 cm opening into the apparatus proper and included a manually operated sliding start door. The start door was clear Plexiglas to allow the animal to clearly see the rear wall and the LED that was activated to flicker at 10 Hz for 1 s duration. An experimenter activated all LED simultaneously (during habituation) or a single LED (for test trials) by pressing a button.

EXPERIMENTAL DESIGN

Subject rats were assigned at random to 1 of 5 treatment groups (n=10 per group) to receive daily s.c. injections either of vehicle only, 2 mg or 8 mg mecamylamine/kg, or 0.3 mg or 0.7 mg nicotine/kg calculated as the weight of the base. Animals were food restricted, but not exposed to drug, for habituation training in the learning paradigm. Subsequently, animals were administered drug for three weeks and tested in the 5-choice paradigm during the third week. As in experiment I, the animals were food restricted throughout the habituation and testing periods, open field tests were conducted once each week of drug exposure and body weights were recorded prior to drug exposure and at the end of the experiment.

During extensive habituation training for experiment II, the animals were food restricted but not exposed to drug treatments. After successful habituation, drug injections began and continued for three weeks. Testing in the 5-choice paradigm was conducted during week 3, with injections being administered 1 hour before a test session.

PROCEDURES

Old male rats were tested in a modified version of the 5-choice paradigm designed to assess visual attention and spatial working memory. Similar to the methods used by the earlier researchers (Muir et al. 1995, Stolerman et al. 2000), our animals also were given massed trials in which an animal must attend to a brief, flickering LED before leaving a start area to approach the LED-signaled hole containing food from among 5 possible food holes. Our methods were different in that we used discrete trials with the experimenter returning the animal to the start box for the next trial. Also, only 3 possible LEDs, at the 1, 3 and 5 positions, were illuminated and only those stalls were ever baited with food. LEDs in stalls 2 and 4 were never illuminated nor were food ever available in those two stalls. Nonetheless, we continue to refer to our paradigm as being a 5-choice task because the animal could choose from among all five stalls.

Extensive preliminary work with the 5-choice apparatus revealed that the paradigm requires considerable habituation in old male rats prior to testing for learning and memory. A series of habituation phases for pre-drug training of animals (Bushnell 2001) were used. Only animals achieving an adaptation criterion of finding and eating food from one well within 20 seconds for 4 of 5 trials were selected as subjects for the experiment. Typically, approximately 90% of our old, healthy rats reach this criterion.

After completion of habituation, each animal received 30 trials in the 5-choice paradigm. For a test session, a trial began with the animal in the start box orienting toward the wall containing the lights. A 10 Hz flickering LED stimulus of 1 s duration was illuminated indicating the stall and food well in which food was available. Position of the LED that was illuminated was randomized among stalls 1, 3 and 5.

After onset and offset of the single LED in a trial, the start door is opened. This is the feature that makes our 5-choice paradigm a working memory task. Working memory has been defined as "holding in mind, very briefly, information that is temporarily relevant, quickly updating that information, and implementing goal-directed behaviors" (Keenan et al. 2001).

Opening the start door allowed the subject access to the main section of the apparatus. If the animal entered the LED-signaled stall without entering another stall, the cover was retracted from the hole to reveal the food. The rat was allowed to eat, and a correct score was recorded. If the rat entered one of the other stalls, the cover remained shut, the animal was removed quickly, and the trial was recorded as incorrect. The animal was returned immediately to the start box and the next trial was initiated.

On the occasion that a rat failed to make a choice and enter a stall within 1 min on 3 consecutive trials, the ses-

sion was ended and continued on the next day. Finally, the apparatus was cleaned with a weak soapy solution and wiped dry with paper towels before another animal was introduced into the apparatus.

Statistical analyses

The primary data analyzed were the percentages of correct choices in the 5-choice paradigm over each of 3 blocks of 10 trials each. Similarly to experiment I, numbers of squares crossed in the open field apparatus and percentage of weight loss before and after treatments were also analyzed. Also as for the data of experiment I, one-way ANOVA, factorial ANOVA, simple main effects, and appropriate *post-hoc* tests were performed on the data. Probability value for all analyses was $P<0.05$.

In a factorial ANOVA when one of the main factors is repeated, the analyses of simple main effects of a statistically significant interaction is an important tool. Simple main effects provide a means to examine differences between groups at each time point of the repeated factor. Then the analyses allow examination of each group over the different time points. The latter is to assess statistically reliable performance changes by a group over the course of the experiment.

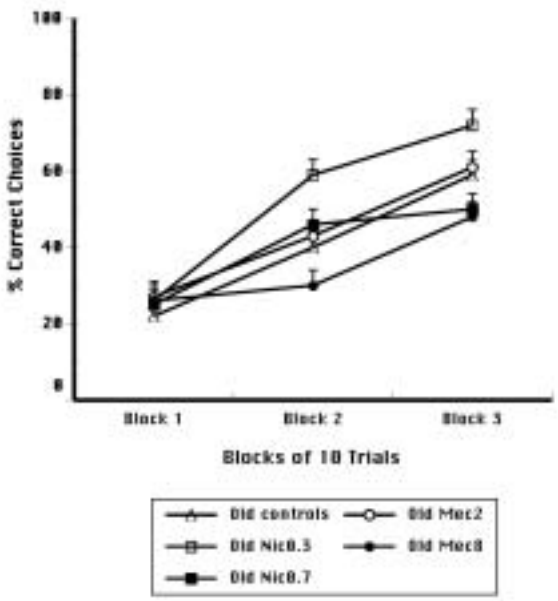

Fig. 1. Influence of nicotine and mecamylamine injections on spatial learning in the old rats. Percentage of correct choices in the 5-choice paradigm from experiment II. All values are mean ± SEM. The 5 groups are old (20–25 months) male rats administered vehicle only, nicotine (either 0.3 mg or 0.7 mg/kg) or mecamylamine (2 mg or 8 mg/kg). *Post-hoc* comparisons revealed complex differences between groups that are described in detail in the Results section.

Table II

Influence of nicotine and mecamylamine injections on the motor activity and weight of the old rats

Group	*n*	Open Field Squares Crossed	Body Weight % weight loss
Controls	10	93 ± 17	6 ± 2
Nic0.3	10	90 ± 12	10 ± 4
Nic0.7	10	79 ± 13	9 ± 5
Mec2	10	89 ± 15	10 ± 4
Mec8	10	85 ± 12	9 ± 3

Experiment 2 with 5 groups of old (20–25 months) male rats administered vehicle only, nicotine (either 0.3 mg or 0.7 mg/kg) or mecamylamine (2 mg or 8 mg/kg). All values are mean ± SEM. Neither of the ANOVA on the two measures achieved statistical significance ($P<0.05$).

Results

Group means and standard errors of the means on open field and body weights from experiment II appear in Table II. Open field data yielded a non-significant value, $F_{4,45}=0.23$, ns. Body weight loss was calculated as a percentage of weight loss from the beginning of the experiment. All groups lost weight, but there were no statistically reliable differences among the groups, $F_{4,45}=0.30$, ns.

The data of primary interest from experiment II were the percentages of correct trials in the 5-choice paradigm after exposure to the same dosages of nicotine used in experiment I (Nic0.3 or Nic0.7) or to either 2 mg (Mec2) or 8 mg (Mec8) of mecamylamine. To assess both rates of learning rates and asymptotic performance levels, correct choices were analyzed in 3 blocks of 10 trials each. Results appear in Fig. 1.

A 5 × 3 factorial ANOVA was performed on those data with main factors of groups and blocks of trials,

with the latter as a repeated measure. Results indicated statistically significant values for the group main factor, $F_{4,45}$=6.51, P<0.05, and for blocks of trials, $F_{2,90}$=31.56, P<0.05. The interaction between main factors also was significant, $F_{8,90}$=2.99, P<0.05.

Simple main effects were computed to further analyze the interaction. Between groups conclusions are based on group differences at block 1, at block 2, and at block 3. That is, these analyses allowed comparisons of the groups at beginning, middle and later stages of learning the task, with the latter being a comparison of final, asymptotic performance among the groups.

Results revealed statistically significant values only on blocks 2 and 3, $F_{4,90}$= 2.78 and $F_{4,90}$=3.85, respectively, both P<0.05. Tukey's test was used to determine which groups differed on these two blocks of trials. On block 2 trials, the old Nic0.3 rats had higher learning scores than all other groups. The vehicle-only controls, Nic0.7 and Mec2 groups were similar and superior to the Mec8 animals on block 2 trials. Comparisons on the final set of trials (block 3) revealed that Nic0.3 was the superior group with Nic0.7 and Mec8 as the worst performing groups. The other groups – Mec2 and controls – did not differ from each other on block 3 trials.

Within group comparisons were used to compare rate of learning the task. Results of the simple main effects analyses were statistically significant differences for each group, range of $F_{2,90}$ values = 3.24–6.31, all P<0.05. Tukey's comparisons within each group indicated that all groups showed learning, i.e., performance on the task improved for all groups from the initial set of trials to the last block of trials. However, only three groups – Nic0.3, Mec2 and controls – showed progressive learning over each of the 3 blocks of trials. The Nic0.7 animals improved from the first to the second block of trials but were no better at solving the problem on block 3 trials than they were on block 2 trials. The Mec8 group showed no improvement from block 1 to block 2 trials but improved by the third block of trials.

GENERAL DISCUSSION

The goal of this project was to employ an animal model to clarify the influences of chronic nicotine on cognitive declines accompanying aging. Aged brains are likely to be undergoing loses in cholinergic function (Luine et al. 1986, Rogers et al. 1998), and one indicator is that an older individual experiences decrements in learning (Spangler et al. 1989).

We compared old and young rats in experiment I in the acquisition of a hole board problem. As expected untreated, control old males experienced decrements relative to untreated young males. Treatment with nicotine at two dosages (0.3 mg or 0.7 mg) elevated the learning rates of the old animals to the levels of the young animals.

Experiment II compared old animals in which the nicotinic receptor (nAChR) was activated by nicotine or antagonized by mecamylamine. Groups of old animals were tested in a 5-choice paradigm. Findings were that the lower dosage of nicotine enhanced the acquisition rate and final performance levels of the old animals, and the higher dosage of mecamylamine did the opposite.

The presumed mechanism underlying these results is the capacity of chronic nicotine to upregulate nAChR (Breese et al. 1997, Rowell and Li 1997) and for mecamylamine to block nAChR. There were, however, findings from both experiments limiting a broad endorsement of nicotine as a cognitive enhancing agent. Results from experiment II also suggest a greater complexity than presumed for the role of the nicotinic receptor.

The picture that emerges is of nAChR involvement in cognitive benefits to the aged being limited to select dosages (Yilmaz et al. 1997) and to select cognitive tasks (Arendash et al. 1995b, Grilly et al. 2000). Our data help clarify the limitations imposed by both factors. A key factor is that spatial abilities are clearly sensitive to cognitive improvements with nicotine in old animals. Visual attention and learning under a regimen of massed trials appear to be other, albeit more complex, factors.

These conclusions are supported by the data from the two experiments. Experiment I compared young and old male rats on two learning and memory paradigms with contrasting skill requirements (Klimkiewicz 2001). The T-maze is a non-spatial discrimination problem, relying on reference memory and learned in multiple trials distributed over many days. The hole board paradigm is also a reference memory task but one that tests spatial abilities and is learned with massed trials.

As suggested by the literature with young adults (Granon et al. 1995, Ohno et al. 1993, Widzowski et al. 1994), nicotine had no reliable effects on the T-maze performance in young animals or in old animals. Indeed, the old rats showed no decrements in the

T-maze paradigm. It is not a unique finding that healthy old animals are the equals of young adults on the acquisition of a cognitive task (Goudsmit et al. 1990).

By contrast, untreated old animals showed a significant decrement in learning the hole board, a working memory test of visual attention, relative to their young counterparts. Nicotine at either 0.3 mg or 0.7 mg doses to old males enhanced hole board performance compared to the old controls. Notably, nicotine administration elevated performance on the hole board of the old animals to the levels of young controls. This stands in contrast to most studies comparing old and young groups where experimental treatments most commonly are unable to restore old animals performance to the levels of the young animals (Arendash et al. 1995a).

There was no similar nicotinic enhancement in the hole board paradigm for young adults. It may be that old males respond to nicotine differently than young animals (Levin and Torry 1996, White and Levin 1999). More likely, the young controls were performing at peak levels, i.e., they experienced a ceiling effect that masked any benefits from nicotine.

Experiment II employed the prototypical agonist and antagonist of the nAChR. Groups of old male rats were administered the same dosages of nicotine (Nic0.3 or Nic0.7) used in the first experiment or 2 mg or 8 mg mecamylamine (Mec2 or Mec8) for two weeks prior to testing. The cognitively demanding task was a 5-choice serial discrimination paradigm (Hahn et al. 2002, Muir et al. 1995, Stolerman et al. 2000) which is similar to the hole board in being a spatial task learned with massed training trials. However, different from the hole board is that the 5-choice paradigm was designed to be a more specific measure of visual attention and working memory.

Results revealed superior learning of the 5-choice by the Nic0.3 group of old males. The Nic0.3 group showed significant improvement over each of three blocks of 10 trials. Although the 2 mg mecamylamine group and the vehicle-only controls also improved over each block of trials, the Nic0.3 animals had the highest percentages of correct responses of all groups during the second and third blocks of trials. The latter block of trials indicates a higher asymptotic level of learning by the Nic0.3 group.

By contrast, the Nic0.7 group was no better than the control group in blocks 1 and 2, and worse than controls and the Mec2 animals in block 3. That is, the 0.7 mg nicotine dosage that had improved performance by old males in the hole board paradigm of experiment I was notably less effective in the 5-choice task. Finally, Mec8 old males displayed the poorest learning of all groups. The Mec8 group showed little evidence of learning the 5-choice problem until the final block of trials.

The open field data offered additional confirmation that the nicotine findings were unlikely due to the capacity of nicotine to stimulate general activity. The Nic0.7 old males were less active in experiment II than the Nic0.3 old males (Attaway et al. 1999, Levin and Torry 1996, Turchi et al. 1996). Moreover, the 5-choice paradigm is relatively independent of response speed and, indeed, increased general activity may be an impediment to careful attention to the stimulus light.

The 2 mg mecamylamine dosage is within ranges that have been commonly used to specifically antagonize nAChR and disrupt learning (Decker and Majchrzak 1992). Our Mec2 group, however, outperformed the Nic0.7 animals in the final block of trials in experiment II. These data cast doubts on the simple conclusion that antagonizing the nicotinic receptor in old rats has direct influence on cognitive outcome (Levin and Rezvani 2002, Moran 1993). Results with knockout mice and the various subtypes of nAChR confirm the complexity of nicotine effects on the central nervous system (Picciotto and Zoli 2002).

Another possibility is the evidence that mecamylamine dosages above 1 mg also influence the NMDA receptor (Newman et al. 2001). It is possible that modest NMDA modulation contributed to the absence of learning disruption by the Mec2 group, and that they outperformed the Nic0.7 group (Brioni et al. 1997). Regardless, our dose-related findings with nicotine and mecamylamine point to greater complexity in nicotinic influences on the aged brain than simply activation or inhibition of cholinergic pathways (Teo et al. 2004).

GENERAL CONCLUSION

The findings with an old male animal model suggest that some dosages of nicotine may prove useful in the treatment of cognitive declines with aging in settings that require specific skills (Rusted et al. 1998). Under those conditions, the benefits of nicotine to learning and memory are likely to be dependent upon influencing both cholinergic and non-cholinergic pathways (Brioni et al. 1997).

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REFERENCES

Abdulla FA, Bradbury E, Calaminici M, Lippiello P, Wonnacott S, Gray JA, Sinden J (1996) Relationship between up-regulation of nicotine binding sites in rat brain and delayed cognitive enhancement observed after chronic or acute nicotinic receptor stimulation. Psychopharmacology 124: 323–331.

Albuquerque EX, Santos MD, Alkondon M, Pereira EF, Maelicke A (2001) Modulation of nicotinic receptor activity in the central nervous system: A novel approach to the treatment of Alzheimer disease. Alzheimer Dis Assoc Disorders 15: S19–25.

Arendash GW, Sanberg PR, Sengstock GJ (1995a) Nicotine enhances the learning and memory of aged rats. Pharmacol Biochem Behav 52: 517–523.

Arendash GW, Sengstock GJ, Sanberg PR, Kem WR (1995b) Improved learning and memory in aged rats with chronic administration of the nicotinic receptor agonist GTS-21. Brain Res 674: 252–259.

Arthur D, Levin ED (2002) Chronic inhibition of alpha4beta2 nicotinic receptors in the ventral hippocampus of rats: impacts on memory and nicotine response. Psychopharmacology 160: 140–145.

Attaway CM, Compton DM, Turner MD (1999) The effects of nicotine on learning and memory: A neuropsychological assessment in young and senescent Fischer 344 rats. Physiol Behav 67: 421–431.

Breese CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC, Leonard S (1997) Effect of smoking history on [3H]nicotine binding in human postmortem brain. J Pharmacol Exp Therapeut 282: 7–13.

Brioni J, Decker M, Sullivan J, Arneric S (1997) The pharmacology of (-)- nicotine and novel cholinergic channel modulators. In: Advances in Pharmacology, Vol. 37 (August J, Anders A, Murad F, Coyle J, eds.). Academic Press, San Diego, p. 153–214.

Brosnan-Watters G, Wozniak DF (1997) A rotating holeboard procedure for testing drug effects on spatial learning and memory in mice. Brain Res Protocols 1: 331–338.

Brosnan-Watters G, Wozniak DF, Nardi A, Olney JW (1999) Parallel recovery of MK-801-induced spatial learning impairment and neuronal injury in male mice. Pharmacol Biochem Behav 62: 111–122.

Bushnell PJ (2001) Assessing attention in rats. In: Methods of Behavior Analysis in Neuroscience (Buccafusco JJ, ed.). CRC Press, Boca Raton FL, p. 111–122.

Decker M, Majchrzak M (1992) Effects of systemic and intracerebroventricular administration of mecamylamine, a nicotine cholinergic antagonist, on spatial memory in rats. Psychopharmacology 107: 530–534.

Fratiglioni L, Wang HX (2000) Smoking and Parkinson's and Alzheimer's disease: Review of the epidemiological studies. Behav Brain Res 113: 117–120.

Goudsmit E, van de Poll N, Swaab D (1990) Testosterone fails to reverse spatial memory decline in aged rats and impairs retention in young and middle-aged animals. Behav Neural Biol 53: 6–20.

Granon S, Poucet B, Thinus-Blanc C, Changeux JP, Vidal C (1995) Nicotinic and muscarinic receptors in the rat prefrontal cortex: Differential roles in working memory, response selection and effortful processing. Psychopharmacology 119: 139–144.

Grilly DM, Simon BB, Levin ED (2000) Nicotine enhances stimulus detection performance of middle- and old-aged rats: a longitudinal study. Pharmacol Biochem Behav 65: 665–670.

Hahn B, Shoaib M, Stolerman IP (2002) Nicotine-induced enhancement of attention in the five-choice serial reaction time task: The influence of task demands. Psychopharmacology 162: 129–137.

Jakubowska-Dogru E, Gumusbas U, Kara F (2003) Individual variation in the spatial reference and working memory assessed under allothetic and idiothetic orientation cues in rat. Acta Neurobiol Exp (Wars) 63: 17–23.

Kalueff AV, Tuohimaa P (2004) Experimental modeling of anxiety and depression. Acta Neurobiol Exp (Wars) 64: 439–448.

Keenan PA, Ezzat WH, Ginsburg K, Moore GJ (2001) Prefrontal cortex as the site of estrogen's effect on cognition. Psychoneuroendocrinology 26: 577–590.

Kelton MC, Kahn HJ, Conrath CL, Newhouse PA (2000) The effects of nicotine on Parkinson's disease. Brain Cogn 43: 274–282.

Kirk RE (1995) Experimental Design: Procedures for the Behavioral Sciences. Brooks/Cole, Pacific Grove, CA.

Koprowska M, Krotewicz M, Romaniuk A, Strzelczuk M (2004) Age-related changes in fear behavior and

regional brain monoamines distribution in rats. Acta Neurobiol Exp (Wars) 64: 131–142.

Klimkiewicz T (2001) Memory effects of arginine vasopressin (AVP) and [7-9] fragment of its peptide chain in rats. Acta Neurobiol Exp (Wars) 61: 267–276.

Levin ED, Rezvani AH (2002) Nicotinic involvement in cognitive function of rats. In: Nicotinic Receptors in the Nervous System (Levin ED, ed.). CRC Press, Boca Raton, FL, p. 167–178.

Levin ED, Torry D (1996) Acute and chronic nicotine effects on working memory in aged rats. Psychopharmacology 123: 88–97.

Luine VN, Renner KJ, Heady S, Jones KJ (1986) Age and sex-dependent decreases in ChAT in basal forebrain nuclei. Neurobiol Aging 7: 193–198.

McGehee DS, Role LW (1996) Neurobiology: memories of nicotine. Nature 383: 670–671.

Moran PM (1993) Differential effects of scopolamine and mecamylamine on working and reference memory in the rat. Pharmacol Biochem Behav 45: 533–538.

Muir J, Everitt B, Robbins T (1995) Reversal of visual attentional dysfunction following lesions of the cholinergic basal forebrain by physostigmine and nicotine but not by the 5-HT3 receptor antagonist, ondansetron. Psychopharmacology 118: 82–92.

Newman MB, Manresa JJ, Sanberg PR, Shytle RD (2001) Nicotine induced seizures blocked by mecamylamine and its stereoisomers. Life Sci 69: 2583–2591.

Ohno M, Yamamoto T, Watanabe S (1993) Blockade of hippocampal nicotinic receptors impairs working memory but not reference memory in rats. Pharmacol Biochem Behav 45: 89–93.

Patterson D, Nordberg A (2000) Neuronal nicotinic receptors in the human brain. Prog Neurobiol 6: 75–111.

Perry DC, Davila-Garcia MI, Stockmeier CA, Kellar KJ (1999) Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. J Pharmacol Exp Therapeut 289: 1545–1552.

Picciotto MR, Zoli M (2002) Nicotinic receptors in aging and dementia. J Neurobiol 53: 641–55.

Puma C, Deschaux O, Molimard R, Bizot JC (1999) Nicotine improves memory in an object recognition task in rats. Eur Neuropsychopharmacol 9: 323–327.

Riekkinen M, Riekkinen P (1997) Nicotine and D-cycloserine enhance acquisition of water maze spatial navigation in aged rats. Neuroreport 8: 699–703.

Rogers SW, Gahring LC, Collins AC, Marks M (1998) Age-related changes in neuronal nicotinic acetylcholine receptor subunit alpha4 expression are modified by long-term nicotine administration. J Neurosci 18: 4825–4832.

Rowell PP, Li M (1997) Dose-response relationship for nicotine-induced up-regulation of rat brain nicotinic receptors. J Neurochem 68: 1982–1989.

Rusted JM, Graupner L, Tennant A, Warburton DM (1998) Effortful processing is a requirement for nicotine-induced improvements in memory. Psychopharmacology 138: 362–368.

Schulz D, Kuchel G (1993) Decline in response to nicotine in aged rat striatum: Correlation with a decrease in a subpopulation of nicotinic receptors. J Neurochem 61: 2225–2232.

Spangler EL, Chachich ME, Curtis NJ, Ingram DK (1989) Age-related impairment in complex maze learning in rats: Relationship to neophobia and cholinergic antagonism. Neurobiol Aging 10: 133–141.

Stolerman IP, Mirza NR, Hahn B, Shoaib M (2000) Nicotine in an animal model of attention. Eur J Pharmacol 393: 147–154.

Taylor GT, Weiss J (1987) Behaviour and fecundity of female rats mated with preferred and non-preferred males. Anim Behav 35: 115–121.

Taylor G, Bardgett M, Csernansky J, Early T, Haller J, Scherrer J, Womack S (1996) Male rat reproductive systems under chronic fluoxetine or trimipramine treatment. Physiol Behav 59: 479–485.

Taylor GT, Farr S, Klinga K, Weiss J (2004) Chronic fluoxetine suppresses circulating estrogen and the enhanced spatial learning of estrogen-restored ovariectomized female rats. Psychoneuroendocrinology 29: 1241–1249.

Teo M-Y, van Wyk M, Lin J, Lipski J (2004) Differential effects of nicotine on the activity of substantia nigra and ventral tegmental area dopaminergic neurons in vitro. Acta Neurobiol Exp (Wars) 64: 119–130.

Turchi J, Holley LA, Sarter M (1996) Effects of benzodiazepine receptor inverse agonists and nicotine on behavioral vigilance in senescent rats. J Gerontol: Biol Sci 51: B225–B231.

Tyas SL, Koval JJ, Pederson LL (2000) Does an interaction between smoking and drinking influence the risk of Alzheimer's disease? Results from three Canadian data sets. Stat Med 19: 1685–1696.

White HK, Levin ED (1999) Four-week nicotine skin patch treatment effects on cognitive performance in

Alzheimer's disease. Psychopharmacology 143: 158–165.

Widzowski D, Cregan E, Bialobok P (1994) Effects of nicotinic agonists and antagonists on spatial working memory in normal adult and aged rats. Drug Develop Res 31: 24–31.

Yilmaz O, Kanit L, Okur BE, Pogun S (1997) Effects of nicotine on active avoidance learning in rats: sex differences. Behav Pharmacol 8: 253–260.

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