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Gender differences and the role of estrogen in cognitive enhancements with nicotine in rats

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ABSTRACT

Research has reported that nicotine can increase accuracy, response times and rates of learning with evidence of different effects on males and females. The goal of our research was to study further sex differences by examining the role played by estrogen in the effects of nicotine on learning and memory in female rats. In experiment 1, 48 male and female rats were administered 0.3 mg or 0.7 mg/kg bwt of nicotine (nic) or vehicle only (veh) and tested in a visual spatial orientation (VSO) paradigm designed to maximize the benefits of nicotine on spatial working memory. Females exposed to 0.3 mg nic performed superior to all other groups of both genders. In experiment 2, ovariectomized females ($N=40$) were exposed to 30 μg estradiol/kg bwt (E2), 3 mg nicotine/kg bwt, a combination of both E2 and nic, or veh, and tested as in experiment 1. The rankings of scores in the VSO task by group were E2 + nic > nic alone > E2 alone > veh. The E2 + nic combination group also demonstrated the highest rate of acquisition. Collectively, the findings suggest that estrogen can synergize the ability of chronic nicotine to enhance acetylcholine–hippocampal interactions underlying performance in the VSO paradigm.

1. Introduction

There is a solid body of learning and memory research with both animal models and humans that have reported that nicotine can increase accuracy, response times and rates of learning (Levin et al., 1992; Pineda et al., 1998). Performance enhancements appear not to be simply from the stimulant properties of the drug. The mechanism is likely related to the activation of the nicotinic receptor (nAChR) of the cholinergic system that underlies many forms of learning and memory. Evidence includes that agonists of the nAChR can improve cognition while nAChR antagonists, such as mecamylamine, can impair cognitive behaviors (Levin et al., 1993; Poincheval-Fuhrman and Sara, 1993; Puma et al., 1999; Socci et al., 1995). Indeed, dysfunction of nicotinic cholinergic receptor is speculated to be involved in epilepsy, schizophrenia, autism, disease, and the dementia accompanying Parkinson's, Lewy bodies and Alzheimer's disease (Dani and Bertrand, 2007).

Subsequent research has limited the role of nicotinic enhancements to certain dosages and specific learning and memory paradigms (Rezvani and Levin, 2001; Scerri et al., 2006). Nonetheless, there appears to be a special relation between nicotine, spatial performance and working memory in rodents. Involvement of cholinergic pathways in spatial learning and memory is suggested by the high concentrations of nicotinic receptors in the hippocampal complex (Hasselmo, 2006; Teaktong et al., 2004). The hippocampus is a critical brain region for spatial performance, and cholinergic activity within the hippocampus plays an important role in spatial memory (Dunbar et al., 1993; Kim and Levin, 1996; Ren et al., 2007). Chronic administration of nicotine upregulates nAChRs in the hippocampus (Abreu-Villaca et al., 2003), suggesting a mechanism for the spatial memory benefits accrued with nicotine.

The bulk of the studies have used males. Still, there is evidence that cognition in both genders can benefit from chronic nicotine exposure, although there is evidence of sex differences in the effects of nicotine in both animal models and humans (Algan et al., 1997; Booze et al., 1999; Buccafusco et al., 1999; Levin et al., 1992, 1999). Women, for example, appear more sensitive than men to the physiological and behavioral effects of nicotine, and many other psychoactive drugs (Becker and Hu, 2008). Ovarian hormones have been speculated to be the source of these differences (Lynch, 2006).

The goal of our research was to study further sex differences by examining the role played by estrogen in the effects of nicotine on spatial working memory in female rats. A pair of experiments is presented, the first to confirm the existence of sex differences in the visual spatial orientation (VSO) paradigm, and the second to evaluate estrogenic interactions with nicotine in ovariectomized rats.

2. Methods

2.1. Experiment 1

The initial experiment was designed to investigate the influence of nicotine on cognitive behaviors in unoperated male and female rats.

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Both genders were chronically administered one of two dosages of nicotine and compared on rates of learning in a spatial task that required working memory to solve.

2.1.1. Subjects

Animals ($N=48$) were equal numbers of experimentally naïve male and female (4–7 months of age) Long–Evans rats from the animal colony maintained at the University of Missouri-St. Louis. All rats were individually housed for at least 30 days prior to testing in flat bottom plastic cages measuring $48.3\times25.4\times20.3$ cm. Standard lab diet and water were available as dictated by the food restriction protocol described below. The colony room lighting is a 12:12 h reversed light/dark cycle; room temperature (~20–22 °C) and relative humidity (50%) are controlled automatically. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Missouri-St. Louis.

2.1.2. Materials

Our VSO apparatus (Taylor et al., 2005) is based on a 5-choice structure described in the literature for testing of spatial learning and memory (Carli et al., 1983). Briefly, the Plexiglas structure measured $26\times31\times20$ cm. One end contained a curved wall divided into five equal sections separated by partitions. Each section contains a round food well in the floor with a green light-emitting diode (LED) positioned above each of the five wells. A start box measured $21\times10\times15$ cm with an opening into the apparatus proper that could be closed off with a manually operated clear Plexiglas sliding start door.

The open field apparatus (Taylor et al., 1996) was a platform, 122×91.5 cm, with 3 sides open and the fourth side flush against a wall, with the top marked into a grid of 48 equal squares, 15.25×15.25 cm, to allow for quantifying locomotor activity. During testing the room was dimly lit with a 60-watt light bulb positioned away from the apparatus.

Nicotine tartrate salt was purchased from Sigma Chemical Company (St. Louis, MO) and was solubilized in a 0.9% saline solution and adjusted with small quantities of 10 N NaOH to pH 7.0.

2.1.3. Experimental design

Rats from each gender were randomly assigned to 1 of 3 groups ($n=8$) to be injected daily with dosages of saline vehicle (veh), 0.3 mg nicotine/kg bwt (0.3 mg nic), or 0.7 mg nicotine/kg bwt (0.7 mg nic), both calculated as the weight of the base. The result was a $2\times3\times6$ factorial design, with main factors of gender, drug dosage, and repeated trials of behavioral testing. Daily injections were administered subcutaneously for 3 weeks in 0.2 ml of saline solution, with spatial testing conducted over the final week.

During the extensive habituation phase for the VSO task the subjects were not exposed to drug treatment. With successful completion of habituation, the animals began a daily regimen of drug injections for the following three weeks. No behavioral tests were conducted during week 1 of drug exposure. Rats were tested in the open field only during week 2 of drug exposure while testing in the open field and the VSO paradigm occurred during week 3 of drug exposure. Behavioral testing began 1 h after the daily injection to ensure absorption of the drug into the bloodstream and unimpeded mobility for testing. Each animal was tested at the same time each day. The subjects were food restricted by allowing access to food for only 1 h following daily testing, resulting in food restriction for 23 h before behavioral testing.

The order of testing subjects was counterbalanced within and between groups. General activity was assessed in the open field apparatus once during the week prior to spatial testing and then once again following the completion of spatial testing.

2.1.4. Procedures

2.1.4.1. Overview. Rats were tested in a VSO paradigm modified from a serial reaction time paradigm in which the animal chooses a distal hole signaled by a flashing light from among five holes that contain food (Muir et al., 1995; Stolerman et al., 2000). The task is uniquely suitable for the present research because it possesses many of the features to which nicotinic activation appears most sensitive. The paradigm is a spatial working memory task using selective attention to visual cues that can be learned with massed trial testing (Grilly et al., 2000; Hahn et al., 2002). Rather than a fixed location for the reward characteristic of many spatial learning paradigms, the VSO paradigm requires the animal to use working memory to locate in space the stimulus signaling the location of food (Nitz, 2009). Only three holes in the distal bank of five holes were signaled to contain food. Having unused holes on both sides of the signaled hole made a difficult task less difficult for rats (Panlilio et al., 2009).

Each animal was given 30 trials scheduled over 1 day or, more often, over 2 days or, occasionally, 3 days, with a 3 day maximum. The criterion to end a session was the animal failing to make a choice within 1 min on 3 consecutive trials, and, therefore, the session was ended and continued on the next day.

On each trial, the animal must attend to a brief, flickering LED before leaving a start area to approach the LED-signaled well containing food from among five possible food wells. We used discrete trials with an experimenter returning the animal to the start box for the next trial and the use of only three possible LEDs, at the one, three and five positions. The experiment had three phases: habituation, drug initiation, and testing.

2.1.4.2. Habituation. Animals were not injected during the habituation sessions. Only animals achieving criterion in the habituation phase were assigned to groups for the drug-exposure phases of the experiment. We observed notable individual differences in length of time to complete habituation, and, indeed, some animals (<15% from each gender) failed to achieve criterion.

Essentially, habituation was used to train the food restricted animal to orient toward the LEDs at the back wall of the apparatus and to find a piece of sweet breakfast cereal, Honey Nut Cheerios, in one of the food wells. All LEDs were illuminated and the animal was allowed to emerge from the start area and locate and eat a piece of cereal from one of the food wells. The animal was prevented from getting food from more than a single well before being taken from the apparatus for a 1 min inter-trial interval. The criteria for successful completion of habituation were to find and eat food from 1 well within 20 s for 4 of 5 trials.

2.1.4.3. Drug Initiation. After completion of habituation, drug injections were initiated and continued for three weeks. During the first two weeks of drug injections, the animals were not exposed to VSO testing, however, the animals were tested in the open field apparatus at the end of the second week of drug exposure. The VSO testing was conducted during the third week of drug injections.

2.1.4.4. Testing. In the test phase of the VSO paradigm, animals were tested until each had successfully completed 30 trials. On the occasion that a rat failed to make a choice and enter a stall within 1 min on three consecutive trials, the session was ended and continued on the next day. On each trial, only a single LED was illuminated indicating the stall and food well in which cereal was available. A ten hertz flickering LED stimulus of 1 s duration was presented in one of the stalls, and the start door was immediately opened. If the subject entered that stall without entering another stall, the cover was removed from the well 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 quickly removed, and the trial was recorded as incorrect. The next trial began immediately with the animal being returned to the start box and, when orientated toward the end of the apparatus containing the lights, the LED was activated and the start door was opened. Position of the LED activated to signal the correct choice was randomized.

On all habituation and test days the apparatus was cleaned with a weak soapy solution and wiped dry with paper towels before another animal was introduced into the apparatus.

2.1.5. Open field and body weights

Each animal was also tested in the open field apparatus twice during the experiment, at the end of week 2 and again at the end of week 3 of drug exposure. The open field was used 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 6 min session. The rat was allowed to roam the open field freely, and numbers of squares crossed were recorded to assess locomotor changes after chronic drug treatments. Body weights were recorded prior to the introduction of drug injections and food restriction and weekly thereafter.

2.1.6. Statistical analyses

Data analyzed from the VSO paradigm were percentages of correct choices over each of 6 blocks of 5 trials each. In addition, numbers of squares crossed in the open field apparatus were recorded and a total score over the two open field testing sessions was summed. Percentage of weight loss was calculated from body weights obtained before the initiation of drug injections and food restriction relative to body weights at the end of the experiment.

Means and standard errors were computed for each measure. Factorial analyses of variance were performed on data sets using the SPSS statistical program for PC computers. With a statistically significant interaction between main factors on the factorial ANOVA, simple main effects were calculated. With a statistically significant F value, the Tukey's HSD method was used as a post hoc test to compare means of each group with every other group. For a statistically significant F value for main and simple effects across within-subjects variables, pairwise comparisons were used to compare means over days. Multiple comparisons were Bonferroni adjusted. Probability value for all analyses was $p<0.05$.

2.2. Experiment 2

The second experiment was designed to clarify the role estrogen plays in the cognitive enhancements with nicotine administration in female rats. Estrogen receptors are concentrated in the hippocampus (Maggi et al., 1989), and estrogenic activity regulates dendritic density in the hippocampal complex of gonadally intact female rats (Gould et al., 1990; Woolley and McEwen, 1992).

Adult female rats were ovariectomized (OVX) and administered vehicle only, nicotine alone, estradiol alone, or a combination of estradiol and nicotine. Based on results of experiment 1, all nicotine dosages were 0.3 mg nicotine/kg bwt. The same VSO paradigm to assess spatial working memory performance used in the first experiment was again used.

2.2.1. Subjects

Subjects ($N=40$) were experimentally naïve female, ovariectomized Long–Evans rats (4–7 months of age) from the animal colony maintained at the University of Missouri-St. Louis. Housing conditions and other details were the same as in experiment 1.

2.2.2. Materials

The VSO apparatus and open field apparatus were the same as described in experiment 1. Nicotine was purchased from Sigma Chemical Company (St. Louis, MO) and solubilized in 0.9% saline solution and adjusted to pH 7.0. Estradiol was purchased from Sigma Chemical Company (St. Louis, MO) and suspended in olive oil.

2.2.3. Experimental design

Rats were randomly assigned to 1 of 4 treatment groups ($n=10$) to be injected with either estradiol only (E2 alone), nicotine only (nic alone), both E2 and nicotine (E2 + nic), or vehicle only (veh). Dosages were 30 μg estradiol benzoate/kg bwt (Taylor et al., 2004) and 0.3 mg nicotine/kg bwt. Nicotine dosages were calculated as the weight of the base. Daily injections were administered s.c. for 4 weeks either as a 0.2 ml solution of saline or oil.

2.2.4. Procedures

As in experiment 1, the rats were evaluated in the VSO paradigm. Similarly to the first experiment, the 40 naïve females of experiment 2 were assessed during the habituation phase in which no drug was administered and the females were gonadally intact. After achieving the criterion employed in experiment 1, the females were ovariectomized. Briefly, each animal was anesthetized with Halothane, and the ovaries were removed through an incision in the abdomen. Following the surgeries, the animals were allowed a week for recovery, followed by the spatial working memory test phase that began during the third week of the three weeks of drug exposure.

The same protocol used in the first experiment was used for behavioral tests in experiment 2. Testing began 1 h after the daily injection to ensure absorption of the drug into the bloodstream and unimpeded mobility for testing. The animals were food restricted by allowing each access to food for 1 h following daily testing, resulting in food restriction for 23 h before a behavioral test.

2.2.5. Statistical analyses

The data collection and analyses performed in experiment 1 were also used in the second experiment. Probability value for all analyses was $p<0.05$.

3. Results

3.1. Experiment 1

3.1.1. Visual spatial orientation task

Prior to analysis, SPSS and the R suite of software facilities from the GNU project were used to examine the spatial working memory scores in blocks 1–6 for missing values and fit between their distributions and the assumptions of univariate analysis. No missing values were found, preserving the original 48 subjects. Each treatment dosage group contained eight cases, exceeding the number of variables sufficient for the analysis.

Examination of univariate normality of sampling distributions revealed normal distributions for all 6 blocks of VSO testing (range of $g_1=-0.28$–0.40, $p>0.05$; range of $g_2=-1.14$–0.66, $p>0.05$). Transformation of all 6 blocks into z-scores revealed no univariate outliers with an extreme standard deviation limit of ± 3.5.

Levene's test of equality of error variances was non-significant for each of the 6 blocks (range of $p=0.893$–0.178). This indicates that the error variance is equal across all groups and there is no problem with the accuracy of the repeated measures ANOVA analysis.

A repeated measures $2\times3\times6$ ANOVA was performed on the spatial working memory scores with gender and nicotine dosage (veh, 0.3 mg nic, and 0.7 mg nic) as the between-subjects factors and blocks of trials (6 blocks of 5 trials each) as the repeated measure. The spatial memory scores were percentages of correct choices calculated for each animal for each block of trials.

Results revealed statistically significant values for each main effect of gender, nicotine dosage, and blocks of trials [$F\ (1,42)=37.48$, $p=0.000$, $F\ (2,42)=59.85$, $p=0.000$, and $F\ (5,210)=88.68$, $p=0.000$, respectively]. The 3-way interaction among factors failed to achieve statistical significance.

The only 2-way interaction that was statistically reliable was between gender and nicotine dosage [$F\ (2,42)=17.79$, $p=0.000$; Fig. 1]. Further analysis with simple main effects allowed for comparisons between males and females for each drug dosage and within each gender for the three drug dosages. Results of between

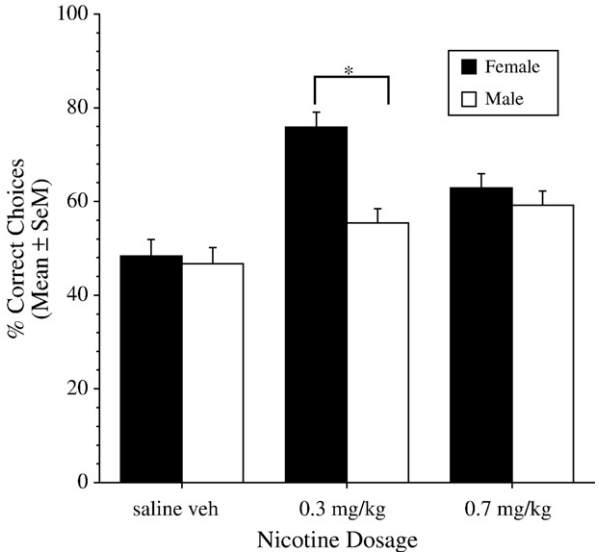

Fig. 1. Means ± SEM of percentage of correct choices in the VSO paradigm for all groups in experiment 1. Both males and females were treated with 1 of 2 dosage levels of nicotine (veh, 0.3 mg nic and 0.7 mg nic) during the 2 weeks prior and the week of testing. Comparisons of drug dosages independent of gender demonstrates both nicotine dosages yielded a greater number of correct choices over veh, but 0.3 mg nic was superior to 0.7 mg nic. Females chose correctly in a significantly higher percentage than males when collapsed across all groups. When taking into account treatment, females performed superior to males only when administered 0.3 mg nic. *Denotes a statistically significant difference between females treated with 0.3 mg nic and all other groups.

gender comparisons of each nicotine dosage revealed that females performed better than males only when exposed to 0.3 mg nic [F (1,42) = 70.22, p = 0.000; Fig 2]. Within gender comparisons revealed that females made more correct choices with 0.3 mg nic than with 0.7 mg nic or veh as well as more correct choices with 0.7 mg nic than with veh [F (2,42) = 63.78, p = 0.000]. Comparisons among the male groups at each nicotine dosage [F (2,42) = 13.86, p = 0.000] revealed that males performed better with both the 0.3 mg nic and the 0.7 mg nic dosages than the males receiving vehicle only.

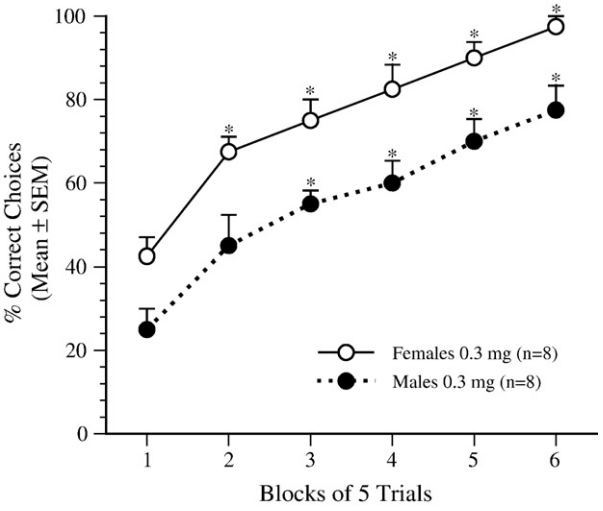

Fig. 2. Means ± SEM of percentage of correct choices across all 6 blocks of 5 trials each in the VSO paradigm for the groups of males and females in experiment 1 administered 0.3 mg nic across all 6 blocks of 5 trials. Females receiving 0.3 mg nic showed statistically significant improvement earlier relative to the first block of trials than males receiving the same dosage. *Denotes statistically significant difference from block 1.

The statistically significant main effect for nicotine dosage was analyzed further using Tukey's HSD. Results for both genders collapsed across nicotine dosage revealed a statistically significant increase in the percentage of correct choices with 0.3 mg nic relative to the correct choices made by either the 0.7 mg nic or veh, and a statistically significant increase in the percentage of correct choices with 0.7 mg nic relative to veh.

A statistically significant main effect for spatial memory performance across blocks of trials was analyzed further using pairwise comparisons. This revealed a statistically significant increase in the percentage of correct choices in the VSO paradigm between all blocks of trials except between those of blocks 4 and 5, and blocks 5 and 6 [F (5,38) = 90.68, p = 0.000].

Given our interest in changes over time in the male and female nicotine groups showing enhanced learning, pairwise comparisons were conducted between the blocks of trials for the 0.3 mg nic treated males and females. Results revealed that both males and females made more correct responses over blocks of trials [F (5,38) = 15.83, p = 0.000 and F (5,38) = 14.75, p = 0.000, respectively]. However, the 0.3 mg nic females showed improvement relative to block 1 trials earlier (block 2) than the 0.3 mg nic males (block 3). Also, the females had a higher percentage end-point than the males (Fig. 2).

3.1.2. Open field

A repeated measures 2 × 3 × 2 ANOVA was performed on open field activity scores with gender and nicotine dosage (veh, 0.3 mg nic, and 0.7 mg nic) as the between-subjects factors and testing session (1 and 2) as the repeated measure. The activity scores represented a total number of gridlines crossed over a 6 min period on the open field apparatus. Results revealed no statistically significant values for any of the interactions. Also, the main factor for gender failed to achieve statistical significance. The only statistically reliable main factor was for the nicotine dosage [F (2,42) = 3.61, p = 0.036; Table 1]. Post hoc (Tukey HSD) analyses revealed greater activity levels for animals administered 0.3 mg nic over animals that received veh (p<0.05). Animals treated with 0.7 mg nic did not differ in their activity levels compared to either the 0.3 mg nic or vehicle groups.

3.1.3. Body weights

After examining the percent body weight change from beginning of the experiment to the end of the experiment, mean substitutions for two data points that were found to be influential outliers at z<−2.5 corrected significant negative skew and kurtosis.

A univariate ANOVA was performed with gender and nicotine dosage (veh, 0.3 mg nic or 0.7 mg nic) as the fixed factors and the percent of change in body weight from the beginning of the experiment to the end of the experiment. This dependent variable was calculated with the formula [(beginning body weight minus

Table 1
Mean and SEM of gridline crossings during blocks one and two of open field testing and of percentage of body weight change for both genders across all treatment groups for both experiments 1 and 2.

	Treatment	Open field gridline crossings				% Body wt change	
		Block 1		Block 2			
		M	*SE*	*M*	*SE*	*M*	*SE*
Exp. 1	Female veh	102.00	10.83	88.88	6.62	1.12	2.76
	Female 0.3 mg nic	128.88	14.88	126.50	18.72	5.75	1.18
	Female 0.7 mg nic	94.63	13.38	126.32	17.06	5.39	1.21
	Male veh	106.62	2.63	124.88	11.70	6.65	1.65
	Male 0.3 mg nic	142.63	9.53	140.75	14.49	7.06	2.13
	Male 0.7 mg nic	124.88	9.81	129.00	11.03	6.26	0.82
Exp. 2	veh	106.3	10.93	111.7	11.23	1.64	2.34
	E2 alone	108.5	6.9	112.5	4.08	7.01	1.03
	nic alone	120.9	12.85	130.3	9.62	4.36	1.44
	E2 + nic	120.8	15.02	124.5	20.43	5.4	1.94

ending body weight)/beginning body weight]×100. No statistically significant differences were found between any groups in their percent difference of body weight (Table 1). This indicates that neither nicotine nor gender or the interaction significantly influenced body weight changes.

3.2. Experiment 2

3.2.1. Visual spatial orientation task

Examination of univariate normality of sampling distributions revealed normal distributions for all 6 blocks of VSO testing (range of $g_1 = -0.34$–0.38, $p > 0.05$; range of $g_2 = -0.84$–0.00, $p > 0.05$). Transformation of all 6 blocks into z-scores revealed no univariate outliers with an extreme standard deviation limit of ±3.5. Levene's test was conducted to ensure equality of error variances.

A repeated measures 4×6 ANOVA analysis was performed on the spatial working memory scores with treatment group (E2 + nic, nic alone, E2 alone, veh) as the between-subjects factor and blocks of 5 testing trials (1–6) as the repeated measure (Fig. 3). Statistically significant main effects for treatment group and for blocks of trials [F (3,36) = 61.97, $p = 0.000$ and F (5,180) = 66.91, $p = 0.000$, respectively] were revealed. A statistically significant main effect for treatment group was analyzed further using Tukey's HSD. Over all trials, the ranking of spatial working memory scores by group was E2 + nic > nic alone > E2 alone > veh. However, a closer analysis of the statistically significant interaction between treatment groups across blocks of trials [F (15,180) = 3.34, $p = 0.000$] revealed differences in the rate of learning.

Further analyses of simple main effects were conducted to detect between group differences at different blocks of trials. Results revealed statistically significant values on blocks 3–6 [F (3,36) = range 5.14–28.42, p = range 0.005–0.000]. The E2 + nic group was significantly different from the E2 alone ($p = 0.004$) and veh groups ($p = 0.004$) by block 3 of trials. On block 4, the E2 + nic group had higher spatial working memory scores than the E2 alone ($p = 0.007$) and veh groups ($p = 0.000$), but the nic alone group also had better scores than the vehicle group ($p = 0.000$). By block 5, the E2 + nic and nic alone groups had scores superior to the E2 alone ($p = 0.000$ and $p = 0.001$, respectively) and vehicle groups (both $p = 0.000$). By the last block or trials, unlike the E2 + nic group ($p = 0.001$), the nic alone group no longer demonstrated superior scores to the E2 alone group, which, along with both E2 + nic and nic alone groups, had superior scores to the veh group (all $p = 0.000$).

The F values obtained in the within group analysis indicated statistically significant values for all the drug exposed groups [all F (5,32) = range of 22.71–27.89, all $p = 0.000$]. Animals in the drug exposed groups had more correct responses over blocks of trials. However, the E2 + nic group showed improvement by block 2 ($p = 0.001$) while the other experimental animals, E2 + nic and nic alone treated, showed improvement only by block 3 ($p = 0.000$ and $p = 0.001$, respectively).

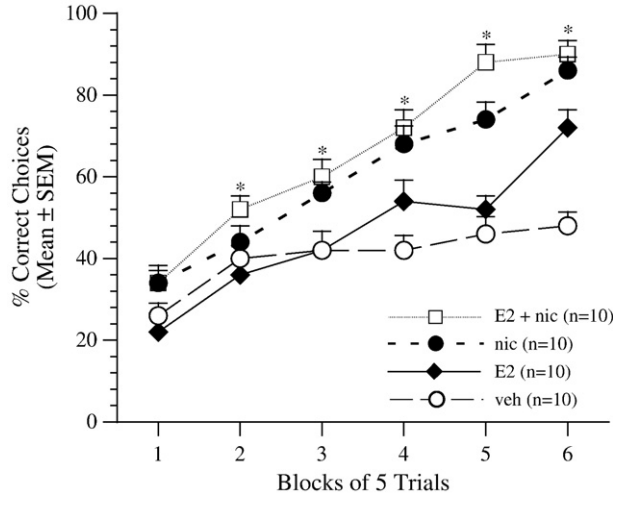

Fig. 3. Means ± SEM of percentage of correct choices in the VSO paradigm from experiment 2 for all groups across all 6 blocks of 5 trials. Ovariectomized females received 1 of 4 treatments (E2 + nic, nic alone, E2 alone, or veh) during the 2 weeks prior and the week of testing. Statistically significant differences were found between the groups during blocks 3 though 6. *Denotes a statistically significant difference in percentage of correct choices between treatment groups.

3.2.2. Open field

After examining the 2 blocks of open field activity data using the R suite of software facilities from the GNU project and SPSS, a square root transformation was found to be appropriate, along with mean substitutions for 4 outliers at $2.0 > z > -2.0$, for correcting skew and kurtosis, and for allowing the data to meet homogeneity assumptions appropriate for a repeated measures ANOVA.

A repeated measures 4×2 ANOVA was performed on open field activity with treatment group (E2 + nic, nic alone, E2 alone, veh) as the between-subjects factor and block of open field testing (1 and 2) as the repeated measure. Results revealed no significant interactions or main effects (Table 1). This indicates that an increase in overall activity level did not influence the animals' performance on the spatial working memory task.

3.2.3. Body weight

Changes in body weight from the beginning of the second experiment to the end of the experiment were analyzed similarly to those of experiment 1. Mean substitution for one data point that was found to be an influential outlier at $z < -2.5$ corrected significant negative skew. The subsequent univariate ANOVA revealed no statistically significant differences between the groups (Table 1). This indicates that neither nicotine nor E2, nor the interaction between the two variables, influenced body weight throughout the experiment.

4. Discussion

The two experiments were designed to investigate gender differences in the capacity of nicotine to enhance cognitive performance in a spatial working memory task. Previous research with humans and animal models has demonstrated that spatial improvements with nicotine exposure are contingent on a number of factors, including gender (Algan et al., 1997), dosage (Scerri et al., 2006), task requirements (Rezvani and Levin, 2001; Rusted et al., 1995) and sensory modality (Muir et al., 1995; Taylor et al., 2005). Experiment 1 was conducted to confirm that these factors influence acquisition and recall in the visual spatial orientation (VSO) paradigm. Experiment 2 examined the role of estrogen in the improvement of learning and memory in females exposed chronically to nicotine. These results on cognition were not a result of locomotor differences or differences in food motivation because both open field activity and weight loss were similar between treatment groups.

In experiment 1, nicotine improved performance on the VSO task of both males and females at both 0.3 mg nic and 0.7 mg nic dosages relative to controls. Although only two dosages were used, females performed better than males at the lower dosage. That the 0.3 mg nic females showed superior performance to that of the 0.3 mg nic males, but not at the higher dosage, demonstrated an interaction between gender and dosage.

The objective of experiment 1 was to compare genders. Because we wanted this to be a comparison in their natural endocrine state, the animals were left gonadally intact, and females allowed to cycle through estrus without interference. This allowed for the females to be tested during different phases of the estrous cycle, permitting for a more ecologically valid comparison of the two genders.

In the second experiment, only OVX females and only the 0.3 mg nic dosage were used, with or without concurrent estradiol (E2). Dosage was 30 μg E2/kg bwt. In a 2004 experiment (Taylor et al., 2004), we used a chronic administration of 50 μg E2/kg bwt because previous studies had suggested that dosage produced physiological titers of E2 (Ferretti et al., 1992). Yet, upon measuring the circulating estrogen in that experiment, we found values that were slightly above physiological levels. We reduced the dosage in the present study to produce, albeit high, physiological titers of estrogen. We were unable to measure the serum levels in the present study to confirm that goal was achieved.

Results of between groups comparisons in experiment 2 indicated that the E2 + nic animals performed better than all other groups over all trials in the VSO paradigm. The animals treated with nicotine alone performed better overall than the animals treated with E2 alone or vehicle alone, and the E2 alone group performed better than the controls. This demonstrates that E2 promoted spatial learning and memory, however, not to the extent at which nicotine did. Most important, however, the females treated with both E2 and nicotine experienced the greatest enhancement of cognition.

By evaluating differences in blocks of trials, it was possible to determine acquisition rates by evaluating the first block of trials in which the group demonstrated improved performance relative to the earlier trials. Those within group analyses revealed that the combination group administered both E2 and nicotine learned at a faster rate than any of the other groups. Importantly, this faster rate included the nicotine only and the E2 only groups.

The central roles of acetylcholine and the cholinergic system on cognition have been recognized for decades. Confirmation in humans has focused on memory dysfunction with antagonism of cholinergic pathways (Rosier et al., 1998) and memory enhancements with cholinergic agonists in aging women (Marin et al., 1995). Viewed independently, both estrogen and nicotine have been implicated in enhancing function of central cholinergic pathways. Estrogenic influences on cholinergic pathways include upregulation of the acetylcholine-synthesizing enzyme, choline acetyltransferase (ChAT) (McEwen and Alves, 1999). More specific to the hippocampus, estrogen increases the release and reuptake of local acetylcholine release (Spencer et al., 2008). Ovariectomized rats exhibit decreased ACh levels in septo-hippocampal cholinergic neurons while subsequent estradiol replacement fully restores the ACh levels (Mitsushima et al., 2008), suggesting the necessity for the steroid for normal cholinergic function. In a recent report that included a measure of cognition, E2 replacement to OVX females increased ChAT and improved performance in a spatial memory task (Ping et al., 2008).

Nicotine also can augment performance in various cognitive paradigms (Young et al., 2004), including spatial memory tasks (Poincheval-Fuhrman and Sara, 1993; Socci et al., 1995). The activation of nicotinic acetylcholine receptors (nAChRs) has been suggested to underlie various forms of cognitive enhancements (Curzon et al., 2006). Upregulation of nAChRs is observed with chronic exposure to nicotine (Collins et al., 2004; Besson et al., 2007) and perhaps with acute exposure. Abreu-Villaca et al. (2003) found increases in nAChRs in the hippocampus in as few as two days. These data are directly applicable to humans (Maki and Dumas, 2009). Upregulation of binding to nAChRs is observed in the brains of smokers of both genders (Vallejo et al., 2005).

The two experiments support the hypothesis that spatial performance of both genders of rats can benefit from nicotine. However, female rats accrued greater benefit than males, at least at the lower of the dosages used. Moreover, E2 played an important role in the benefits on cognition accorded by nicotine. A likely mechanism is the capacity of estrogen to act as a non-genomic modulator of neurotransmission and receptor binding in various brain regions.

Here, the findings point toward elevated cholinergic activity in the hippocampus via the nicotine-induced upregulation of the nAChR receptor (Dani and Bertrand, 2007; Hasselmo, 2006; Nott and Levin, 2006; Teaktong et al., 2004). The suggestion is that the combination of the capacity of chronic nicotine to upregulate the cholinergic receptor and estrogen's influence at the presynaptic region underlies the enhanced VSO performance observed by females in experiment 1 and the estradiol and nicotine combination group in experiment 2.

Closer scrutiny of the data from experiment 2 suggests the interesting notion that nicotine had a greater role in cognitive enhancement than did exogenous estrogen in OVX females. The nicotine only group performed almost as well as the combination group. The conclusion is that the addition of E2 to nicotine had a relatively modest, although statistically significant, effect on the performance of OVX females. These findings suggest that current levels of estrogen in adult females are less potent than we had speculated originally. Yet, it does not indicate that sex steroids play a minor role in the gender differences in learning and memory observed in the first experiment. It is likely that prenatal exposure to sex hormones, the so-called organizational stage of the organizational–activational model (Breedlove, 1992; Dwyer et al., 2009), established brain circuitry differences underlying cognitive behaviors that are influenced differently by nicotine.

Additional sources of gender differences may be peripheral (Taylor et al., 2009). Male and female rats experience different rates of hepatic clearance of nicotine, for example, male rats may metabolize nicotine faster than females due to androgenic stimulation of metabolizing hepatic enzymes (Kyerematen et al., 1988). Also, nicotine appears to have different effects on the peripheral pharmacodynamics in men and women (Dawkins and Potter, 1991; Girdler et al., 1997).

Other results of the two experiments deserving comment include the open field results from experiment 1, which were included to assess the stimulant properties of nicotine as influencing the learning and memory data. Results revealed that only the 0.3 mg animals were more active than the controls. An increase in activity suggests an increase in the rate at which the animal performs the VSO task. Such an increase would likely attenuate performance on such a task. The argument could be made that a rate increase would be detrimental to performance because the animal would decrease attention to the task. The results indicate that this was not the case since the animals treated with the 0.3 mg dosage actually performed the best on the VSO memory task.

In conclusion, findings with gonadally intact male and female rats demonstrated that females perform better on a spatial task when chronically exposed to a low dosage of nicotine. Combining exogenous estradiol with that same low dosage of chronic nicotine for OVX females enhanced their spatial working memory over nicotine alone, but only modestly. We suggest that these gender differences on cognition are a product of the capacity of nicotine and current circulating hormone levels to enhance central cholinergic pathways. Nonetheless, it is also likely that the differential influence of the sex steroids on male and female fetal brain development and peripheral pharmacodynamics played roles in our findings.

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