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Paternal Nicotine Exposure in Rats Produces Long-lasting Neurobehavioral Effects in the Offspring

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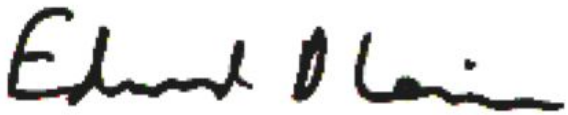
Anthony R. Isles, PhD
Section Editor, *European Journal of Neuroscience*
Behavioral Neuroscience Section

Dear Dr. Isles:

Enclosed is our article "Paternal Nicotine Exposure in Rats Produces Long-lasting Neurobehavioral Effects in the Offspring." It describes studies that we conducted testing the efficacy of drugs affecting nicotinic receptors for reducing stimulant self-administration in rats. We think it would be of interest to the readers of the *European Journal of Neuroscience*. I think the Behavioral Neuroscience section would be the best one for our article. My ORCID is: https://orcid.org/0000-0002-5060-9602. This study was conducted under protocols approved by the Institutional Animal Care and Use Committee of Duke University and meets the requirements of state, federal and international regulatory bodies. It has not been published previously and is not under consideration at any other journal. We look forward to hearing from you soon.

Sincerely,



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Paternal Nicotine Exposure in Rats Produces Long-lasting Neurobehavioral Effects in the Offspring

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Running Head: Paternal Nicotine and Offspring Behavioral Impairment

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Abstract

Studies of intergenerational effects of parental chemical exposure have principally focused on maternal exposure, particularly for studies of adverse neurobehavioral consequences on the offspring. Maternal nicotine exposure has long been known to impair neurobehavioral function. in the offspring. However, paternal toxicant exposure can also cause neurobehavioral toxicity in their offspring. Recent work suggests that paternal nicotine exposure can have epigenetic effects, although it remains unclear whether such changes lead to neurobehavioral effects. In this study, we investigated the effects of paternal nicotine exposure on neurobehavioral development of their offspring. Male Sprague-Dawley rats were exposed to 0 or 2 mg/kg/day nicotine (sc) for 56 consecutive days with two consecutive 2ML4 osmotic minipumps. Following treatment, these males were mated with drug-naïve female rats. Offspring of both sexes were tested in a behavioral battery to assess locomotion, emotional function and cognition. Paternal nicotine exposure did not impact offspring viability, health or growth. However, behavioral function of the offspring was significantly altered. Male offspring with paternal nicotine exposure exhibited locomotor hyperactivity in the Figure-8 apparatus when tested during adolescence. When retested in adulthood and regardless of sex, offspring of the nicotine exposed father had reduced habituation of locomotor activity. Compared to controls, female offspring of nicotine-exposed fathers showed significantly reduced response latency in the radial arm maze test. The offspring of nicotine-exposed fathers also showed significantly diminished habituation in the novel object recognition test. These results indicate that chronic paternal nicotine exposure can impact the behavior of offspring, producing locomotor hyperactivity and impaired habituation.

Introduction

Parental tobacco smoking is a major risk factor for offspring health and is associated with a variety of neurobehavioral effects, including increased risk for psychiatric disorders and alterations in cognitive and affective functions (He, 2017; Meier, 2017; Sciberras, 2017). Studies investigating how this risk is conferred to offspring have largely focused on how tobacco smoke constituents impact the developing brain, linking maternal smoking during pregnancy to adverse outcomes later in life. One of the primary neurotoxins of concern in tobacco smoke is nicotine, which is found in both combustible cigarettes and alternative products such as smokeless tobacco and e-cigarettes. Prenatal exposure to nicotine produces neurobehavioral effects in animal models which mirror psychiatric effects seen in children of tobacco smokers (Hall, 2016; Schneider, 2017). Maternal nicotine exposure during pregnancy may then account for some of the risk presented by parental smoking, although these are not the only mechanisms likely to be involved. Advances in epigenetic analyses of toxicity have identified additional mechanisms that may be capable of altering neural development and impairing behavioral health.

Of particular interest, epigenetic toxicity studies have observed that exposure to tobacco products can alter the methylation patterns of DNA. Active adult smokers exhibit changes in methylation at hundreds of CpG sites (Zeilinger *et al.*, 2013) and certain effects may persist for decades (Ambatipudi, 2016). Sperm are also vulnerable to these effects (Jenkins, 2017) raising concern that smoking-induced methylation changes in males may be passed on to their offspring (Soubry, 2014). Although the majority of genomic DNA methylation in sperm is removed after fertilization, some methylation is retained (Tang *et al.*, 2015). Any remaining methylation could

be passed on to the offspring and alter gene regulation and expression. Recently, Jenkins et al. (2017) reported widespread effects of smoking on methylation in the sperm of male participants. More specifically, smoking was shown to alter methylation at diverse CpGs or within specific regions of the genome, as well as generally increasing the variability in genome-wide methylation patterns (Jenkins, 2017). Although it has been observed that sperm are vulnerable to toxic insults, we do not yet understand the importance of these effects. Additional studies are needed to establish which modifications are actually passed on to offspring and whether these modifications result in neurobehavioral effects that are relevant to those observed in the children of smokers. Additionally, it needs to be determined whether nicotine, which is heavily implicated in prenatal tobacco exposure effects, is also a main toxin of interest with respect to epigenetic effects of tobacco smoking on behavioral health in offspring.

The present study evaluated the multigenerational effects of paternal nicotine exposure in rats. Male rats were passively exposed to nicotine (2mg/kg/day) or vehicle through osmotic minipumps over a 56-day period prior to mating. This exposure period ensured that exposure would cover the length of a full spermatogenic cycle in rats. Male and female offspring were reared and tested in a behavioral battery which spanned from juvenile to young adult development. Behavioral assays were selected to measure a range of locomotor, affective and cognitive functions.

Methods

Design

Young adult male Sprague-Dawley rats (Charles Rivers Labs, Raleigh, NC, USA) were administered nicotine detartrate via osmotic minipump (Alzet model 2ML4, Durect Inc, Cupertino, CA, USA) delivering 2 mg/kg/day of nicotine detartrate (dose calculated as of the nicotine base weight). The exposure duration was 56 days with two consecutively implanted (SC) minipumps placed on opposite flanks of the body. Controls received the same model minipumps and the same surgery, but the pumps contained only the saline vehicle. For the surgery, animals were anesthetized with a mixture of ketamine (60 mg/kg) and dormitor (15 mg/kg). After the second pump was removed the male rats were mated with drug naïve young adult female Sprague-Dawley rats from the same source. A total of eighteen breeding pairs, split among the two treatment groups, were used. Two dams matched with control studs failed to become pregnant. These studies were conducted under a protocol approved by the Institutional Animal Care and Use Committee of Duke University and meet the requirements of state, federal and international regulatory bodies.

Clinical Effects

The females were weighed weekly during gestation and the offspring were weighed weekly after birth. One male and one female from each litter were kept after weaning for behavioral assessment.

Behavioral Test Battery

The behavioral test battery assessed locomotor activity, emotional function and cognition. The tests were given in sequence starting at four weeks of age through adulthood.

Week 4: Elevated Plus Maze

The rats were tested on the elevated plus maze (Med Associates, St Albans, VT, USA) to assess their anxiety-like behavior vs. risk-taking behavior. The maze measured 142-cm x 104-cm x 76-cm high and consisted of two arms with 15-cm high enclosed walls and two open arms with 2-cm railings. Each rat was assessed individually on the elevated plus maze for a single five-min session. The percentage of time the rat spent in the open vs. enclosed arms of the maze was calculated as an index of anxiety vs. risking taking. Also, the number of crossings across the center was counted as a measure of activity. The dependent measures were percent of time in the open arms to index anxiety-like behavior and the number of center crossings to measure locomotion in this five-min test.

Week 5: Figure-8 Apparatus Test of Locomotor Activity

Locomotor activity and its habituation were assessed in an enclosed maze in the shape of a figure-8 with two side alleys. The Figure-8 apparatus had a continuous alley measuring 10-cm x 10-cm, with the entire maze measuring 70-cm x 42-cm. Animals were permitted to freely explore the apparatus. Locomotor activity was indexed by the crossing of eight photo-beams located at approximately equal points throughout the alley. Photo-beam breaks were tallied in 5-min blocks across the one-hour test session. The mean number of photobeam breaks per five-min block within the session indexed locomotor activity. The linear trend of decreasing beam breaks over the twelve sequential time blocks within the session indexed the habituation of activity with experience in the apparatus over the one-hour session. In order to track locomotor activity effects across development, subjects were also tested in the Figure-8 maze as young adults (week 11) and full adults (following completion of attention task).

Week 6: Novelty Suppressed Feeding

To assess fear responsivity, the offspring rats were tested for the suppression of feeding behavior in a novel environment. Each rat had food restricted for 24-h prior to the test session. The novel environment consisted of a plastic rectangular cage (different from the home cage) placed in the middle of a brightly lit testing room, with no cage top and no bedding in the cage. Twelve standard rat-chow pellets were weighed before testing and were spread across the cage floor in 4 rows of 3 pellets each. The sessions lasted 10 min. Eating was defined as the act of chewing the food and not merely sniffing, holding, or carrying the food around in the mouth. The food pellets which remained after the test session were weighed to determine the amount of food eaten. The dependent measures were: amount of food eaten, latency to begin eating, the number of eating bouts and the duration of eating.

Week 7: Novel Object Recognition

Recognition of a novel vs. familiar object was used to test attention and memory in a low-motivational state. Tests were conducted in opaque plastic enclosures measuring 70-cm x 41-cm x 33-cm. Objects consisted of plastic, glass, or ceramic material and were randomized for each animal. Animals were first habituated to the apparatus in two 10-min sessions over the course of two days. Testing began on day 3 with a 10-min familiarization session in which two identical objects (A/A) were placed in the cage for the animal to explore. The A/A session was then followed by a 1-h period spent in the animal's home cage. The animal was then placed back in the enclosure with one object from the A/A session and with another, dissimilar, "novel" object (A/B session). Between sessions, the objects were wiped clean in order to avoid odor recognition cues by the rats. The test session lasted for ten min. Analysis considered the preference in the first and second halves of the sessions. In the first five-min of the session there

was more clearly differential novelty between the two objects compared with the second five min of the test session when there had been more experience with the newer object. The time spent actively exploring each object was recorded (in seconds) during each five-min block during the ten-min session and used for analysis.

Week 8-11: Radial-Arm Maze

Spatial learning and memory were tested in the 16-arm radial maze. The maze was made of wood painted black with a central platform (50-cm diameter) and 16 radiating arms, each 10-cm wide x 60-cm in length. A food cup was positioned 2 cm from the end of each arm. Visual cues (cardboard shapes) were on the walls of the testing room to facilitate spatial orientation. The rats were habituated in the maze for two different 10-min sessions in which they were placed on the central platform inside a large, black, round, opaque cylinder, with half-pieces of sugar-coated cereal (Froot Loops®; Kellogg's Inc, Battle Creek, MI, USA). For the test sessions, twelve of the arms were baited at the beginning of each session to test working memory performance and the other four arms were always left un-baited to test reference memory (Hall, 2016). The baited arms of the maze for each rat remained constant throughout the entire series of testing sessions, but which arms were baited differed randomly between rats. Each trial began by placing the rat on the central platform inside the opaque cylinder for 10 seconds. Then the cylinder was lifted and the rat was allowed to roam the maze freely. Each session lasted 10 min or until the rat had entered all twelve baited arms, whichever occurred first. Each rat was assessed for working and reference memory errors over 18 sessions. Working memory errors were counted as repeat entries into baited arms, and reference memory errors were counted as entries into one of the arms that was never baited. Latency was calculated as the total session time divided by the number of arm entries. There was one session run per day. The dependent

measures were the number of working and reference memory errors as well as response latency (seconds per arm entry).

Weeks 12-40: Operant Visual Signal Detection Task for Attention

The attention test was conducted as described in detail previously (Hall, 2016). Each rat was placed in an operant chamber and was trained to press one of two retractable levers in response to a visual cue-light that was illuminated for a duration of 500 ms. If the cue-light became illuminated ("signal" trial), the animal needed to press the lever designated as the "signal" lever to receive a 20 mg food pellet reward. If the cue-light was not illuminated ("blank" trial), the animal needed to press the opposite lever in the chamber to receive the reward. The choice of "signal" and "blank" levers was randomized among the rats. If the rat made no response within 5 s of insertion of the response levers into the chamber, both levers retracted and a response "failure" was recorded. There were equal numbers of "signal" and "blank" trials in each test session with a total of 240 trials. "Hit" responses were correct choices on the signal trials while "correct rejection" responses were correct choices on blank trials. Percent correct hit and percent correct rejection per session were the dependent measures for response accuracy on this attention task. Analysis was conducted of the choice accuracy data including hits, correct rejections, and failures.

Data Analysis

For each behavioral test, the data were evaluated by analysis of variance with litter as the unit of variance. The between-litters factor was paternal treatment. The within-litter factor was sex. Within-subjects or repeated measures factors were sessions and time block within session. Because each litter contributed one male and one female, sex was treated as a repeated measure within litter. Significance was assumed at the level of $p < 0.05$ (two-tailed). For interactions at p

< 0.1, we also examined whether lower-order main effects were detectable after subdivision of the interactive variables (Snedecor & Cochran, 1967). The $p < 0.1$ criterion for interaction terms was not used to assign significance to the effects but rather to identify interactive variables requiring subdivision for lower-order tests of the main effects of nicotine, the variable of chief interest. A cut-off of $p<0.05$ (two-tailed) was used as the threshold for final statistical significance.

Results

Clinical Signs

No effects of paternal nicotine exposure were seen in measures of clinical health. Maternal and offspring body weight and growth were not significantly affected by paternal nicotine exposure. Neither were litter size and sex distribution.

Elevated Plus Maze

No significant effects of paternal nicotine exposure on offspring were seen in percent open arm time or center crossings in the elevated plus maze test.

Locomotor Activity in the Figure-8 Apparatus

Locomotor activity in adolescent offspring showed a significant nicotine x sex interaction ($F(1,13) = 6.26$, $p< 0.05$). This prompted follow-up tests of the simple main effects of paternal nicotine in each sex (Fig. 1). Paternal nicotine exposure caused a significant ($p < 0.025$) degree of locomotor hyperactivity in juvenile male offspring (41.1 ± 1.7) vs. male controls (34.5 ± 1.7). In contrast, no significant effects were seen in females (Control = 38.1 ± 1.8, Nicotine = 37.1 ±

2.4). The rats were re-tested for locomotor activity in the Figure-8 apparatus two more times: during young adulthood and as full adults. In the young adult test there was a significant paternal nicotine treatment x time block within session interaction ($F(11,143) = 1.92$, $p < 0.05$). Tests of the simple main effects showed a pattern of slower habituation of locomotor behavior by the offspring of the nicotine-treated males (Fig. 2). Paternal nicotine exposure caused significantly increased activity during time blocks 4 ($p < 0.005$), 6 ($p < 0.05$) and 7 ($p < 0.01$), with a nearly significant increase during time block 5 ($p < 0.06$). In the third locomotor activity test during full adulthood, there was a significant three-way interaction ($F(11,132) = 1.92$, $p < 0.05$), but the simple main effects tests of paternal nicotine exposure did not detect any significant paternal nicotine effects for males or females at any of the time blocks within the session (Fig. 3).

Novelty Suppressed Feeding

There was no significant paternal nicotine effect on latency to begin feeding in the novelty suppressed feeding test. The other measures for the novelty suppressed feeding test (amount of food eaten, number of feeding bouts and the total duration of feeding) also did not show significant paternal nicotine effects.

Novel Object Recognition

There was a significant paternal nicotine effect in novel object recognition. This was detected as a significant three-way interaction of paternal nicotine x familiar/novel object x time block ($F(1,13) = 6.89$, $p < 0.025$). There was the expected greater investigation of the novel object than the familiar object ($F(1,13) = 8.71$, $p < 0.025$). Analyses of the simple main effects showed that the controls showed a typical pattern for this task, as they significantly preferred the novel object during the first five minutes of the test ($p < 0.01$) and showed a significant ($p <$

0.025) drop-off in investigation of the novel object during minutes 6-10 of the test as the previously novel object became familiar. In contrast, the offspring of the nicotine-treated fathers did not show a robust preference for the novel object during the first half of the test ($p < 0.08$) but did have a significant ($p < 0.01$) preference for the novel object during the second half of the test.

Radial-Arm Maze

No significant effects of paternal nicotine treatment were seen with performance on the radial-arm maze. The other results showed the validity of the test. There was a significant main effect of session block ($F(3,39) = 3.52$, $p < 0.025$) with improvement as training progressed. There was also a significant effect of error type ($F(1,12) = 321.78$, $p < 0.0005$) with fewer reference than working memory error types committed.

An additional factor of interest was latency, or the amount of time spent per arm entry. There was a significant interaction of paternal nicotine x sex ($F(1,13) = 7.02$, $p < 0.025$). Follow-up tests of the simple main effects of paternal nicotine in each sex showed that female offspring of nicotine-treated males were significantly ($p < 0.001$) faster than control females while there was no significant paternal nicotine effect in male offspring (Fig. 5). There was also a significant ($F(3,39) = 75.88$, $p < 0.0005$) main effect of session block, reflecting a steady decrease in latency over the course of training. This is typical with radial-arm maze training.

Signal Detection Attention Task

No significant effects of paternal nicotine treatment were seen with performance on the signal detection attention task either as a main effect or interaction with the other factors of sex, trial type or session. The other results showed the validity of the test. There was a significant

effect of session ($F(5,60) = 56.60$, $p < 0.0005$) with choice accuracy continually improving over the six sessions of training. There was also a significant effect of trial type ($F(1,12) = 35.33$, $p < 0.0005$) with the typical better performance on correct rejection trials than hit trials.

Discussion

The current study showed that chronic paternal nicotine exposure prior to mating, which modeled nicotine exposure in moderate tobacco use, caused significant, long-lasting behavioral effects in the offspring. In particular, locomotor hyperactivity was apparent following paternal nicotine exposures. Additionally, paternal nicotine exposure led to alterations in the investigation of novel objects over time and response speed in the radial arm maze. Paternal treatment effects appeared to be relatively specific inasmuch as behavioral measures of activity and habituation were impacted, while tests of emotional function and cognition were spared. These patterns of hyperactivity and impaired adaptability are concerning, as similar problems are often seen in children with attention deficit hyperactivity disorder, a disorder which is associated with parental smoking (Biederman *et al.*, 2017; Huang *et al.*, 2017)

Of the measures assessed in this study, locomotor activity appeared to be particularly sensitive to paternal nicotine exposure effects. Paternal nicotine exposure generally produced hyperactivity in offspring, although this effect varied considerably across development. Among adolescent offspring, there was a sexually dimorphic effect, whereby males showed hyperactivity while females were unaffected. This dimorphism did not persist into adulthood. Young adult offspring of nicotine-exposed males showed a more selective increase in activity during the middle of the session, with no change in maximum or minimum activity levels early and late in

the session. By full adulthood, no alterations were apparent. These data show that paternal nicotine exposure may lead to hyperactivity in offspring but that these effects may not appear equally in male and female subjects, and may attenuate over the course of development. It is notable that adolescent rats showed a sex difference in vulnerability to paternal nicotine-induced hyperactivity, although there is limited evidence to suggest why males may be more susceptible to paternal exposure effects than females. Future research should evaluate the impacts of paternal nicotine on sexually-dimorphic factors which could preferentially affect males, including genetic or neuroendocrine factors. Additionally, these sex differences should be investigated in the context of adolescent development. The attenuation and eventual elimination of hyperactivity in the young and at full adulthood suggests that developmentally typical processes may alleviate these alterations. So, the male-specific hyperactivity in adolescence could indicate a developmental delay in those processes among males, rather than a uniquely male risk factor. Such a developmental delay could represent either an additional epigenetic mechanism or a typical sex difference. A more detailed temporal analysis of these transitions may better demonstrate when and under what circumstances these sex differences are expressed and what range of development will be most impacted by hyperactivity effects.

In addition to hyperactivity, paternal nicotine exposure led to impairments in habituation, a basic learning process which reduces responding across repeated or extended exposure to a stimulus. In the Figure-8 maze, activity levels are reduced across the session as rats acclimate to the new environment and explore less. Similarly, rats in the novel object recognition task show a strong preference for a novel object over a familiar one, with that preference reducing over time as the novel object is repeatedly investigated. Offspring of nicotine-exposed males showed altered patterns of habituation in both of these tasks. Young adult offspring of nicotine-exposed

males showed slower locomotor habituation, indicating that these animals required greater time or exploration to achieve the same reduction in activity shown by controls. Similarly, adolescent offspring of nicotine-exposed males failed to show the characteristic reduction in preference for a novel object across two 5-min time blocks. Rather, these subjects showed a preference for the novel object which failed to reach significance in the first time block, but was significant in the second time block. This indicates that these subjects recognized the familiar object and preferred a novel one, but that a 10-min session was not sufficient to observe habituation to the novel object in these animals.

The final behavioral alteration observed in this study was in the radial arm maze. Specifically, female offspring of nicotine-exposed males moved from arm to arm more quickly than control females did. This effect was not detrimental to task performance overall, as the error rate was unaffected by paternal treatment, although it may indicate a subtle behavioral effect not related to learning and memory. For instance, faster food retrieval may simply reflect a hyperactivity effect, as indicated by other tasks, or perhaps greater motivation for the food reinforcers. Male offspring of nicotine-treated fathers did not differ from controls on this outcome.

Taken together, the present data suggest that paternal nicotine exposure can have multigenerational effects on neurobehavioral function. Although the literature on paternal exposure effects still has substantial gaps, our data appear to fall in line with existing behavioral studies following paternal drug exposure. Current evidence generally supports the conclusion that commonly abused substances such as ethanol, opiates and psychostimulants can have behavioral effects on the next generation (Yohn *et al.*, 2015; Goldberg & Gould, 2018), although these effects can vary based on species and strain of animal used, age tested, sex of the offspring,

and dose of exposure. More work is needed to evaluate the potential effects of nicotine. To date, there are no known published studies examining the offspring of nicotine-exposed male rats, although two papers have been recently published with mice. Dai et al. (Dai *et al.*, 2017) reported that paternal nicotine exposure (0.05 mg/100 g nicotine, i.p., 4x daily for 5 weeks) produced highly selective effects on behavior, leading to locomotor hyperactivity and decreased immobile time in the forced swim test, with all other social, affective and cognitive tests unaffected. These effects were similar to those produced by paternal exposure to inhaled tobacco smoke, though tobacco smoke had an additional effect on social motivation. Vallaster et al (Vallaster *et al.*, 2017) provided voluntary nicotine exposure (200 µg/mL in water for 5 weeks) to males prior to mating. This exposure had some limited effects on nicotine sensitivity in the offspring, but no significant effects on behavior. The present data are generally consistent with these studies, in that certain specific functions are impacted by paternal nicotine exposure, while the bulk of behavioral functions are spared.

Additional analyses will be needed to determine the mechanisms by which paternal nicotine exposure causes behavioral alterations in their offspring. Sperm samples were collected from the paternal males (F0) in this study for epigenetic analysis, as were sperm and/or tissue samples from the F1 and F2 generations. Nicotine-induced alterations in sperm DNA methylation are likely candidate mechanisms (Dai *et al.*, 2017) although other transferrable germline modifications may also need to be investigated in the future (Soubry, 2014; Vassoler *et al.*, 2014). Further studies should also be undertaken to clarify the importance of nicotine in the context of tobacco smoke, a complex mixture containing thousands of chemicals, many of which are known to be toxic. Comparisons of paternal nicotine with paternal exposure to tobacco smoke (Dai *et al.*, 2017), tobacco smoke extract (Hall, 2016), or other individual tobacco smoke

constituents may improve our understanding of how shifting trends towards smokeless or electronic tobacco products among men (Anic *et al.*, 2018) may impact behavioral health in future generations.

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Competing Interests

The authors have no competing interests with regard to this article.

Data Accessibility

Data for this study are available to interested parties. Contact Dr. Edward D. Levin (edlevin@duke.edu) for access.

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Figure Legends

Figure 1: Adolescent locomotor activity in the Figure-8 maze (mean ± sem). There was a significant paternal nicotine x offspring sex interaction. Paternal nicotine exposure caused significant locomotor hyperactivity in the male offspring; female locomotor activity was not found to be affected by paternal nicotine exposure.

Figure 2: Young adult locomotor activity in the Figure-8 maze (mean ± sem). There was a significant interaction of paternal nicotine treatment x 5-min time block within the 1-h session. A slower habituation of locomotor activity was noted in offspring of nicotine exposed males.

Figure 3: Full adult locomotor activity in the Figure-8 maze (mean ± sem). There was a significant three-way interaction of paternal nicotine x sex of the offspring x 5-min time block within the 1-h session. However, none of the simple main effects of paternal nicotine exposure were significant.

Figure 4: Novel Object Recognition, time in seconds spent investigating the familiar and novel objects during the first and second five-min time blocks of the session (mean ± sem). There was a significant interaction of paternal nicotine x familiar vs. unfamiliar object x five min time block ($p < 0.025$).

Figure 5: Radial-arm maze response latency (seconds per arm entry) showed a significant ($p < 0.025$) paternal nicotine x sex interaction with female offspring of nicotine-treated fathers having significantly ($p < 0.001$) longer latencies than control females. No significant paternal nicotine effects were seen in male offspring with this measure (mean ± sem).

Figure 1

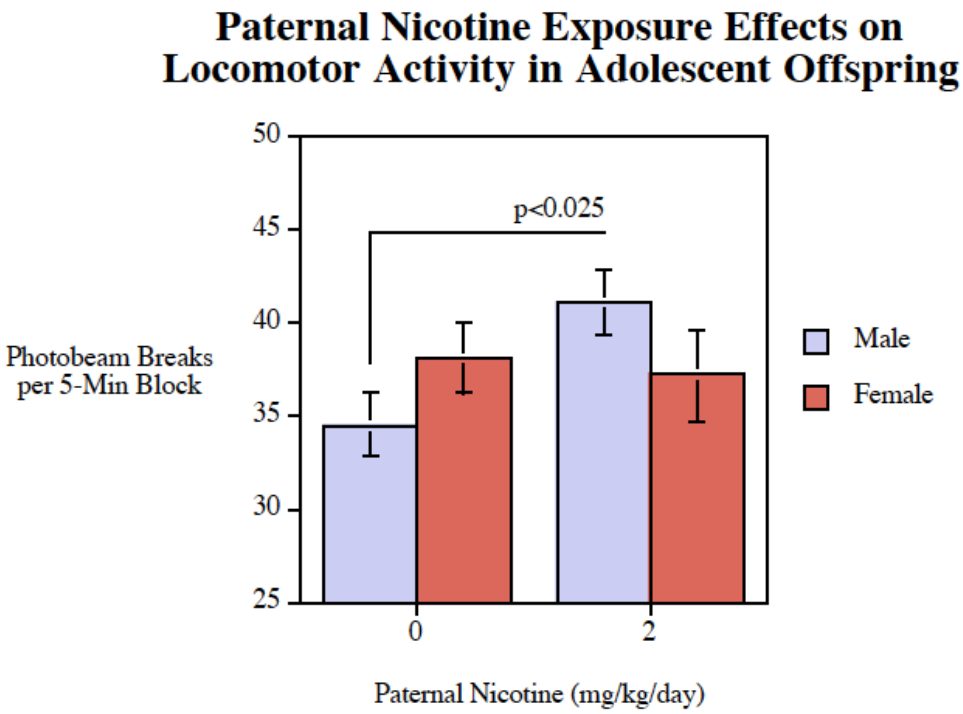

Paternal Nicotine Exposure Effects on
Locomotor Activity in Adolescent Offspring
Photobeam Breaks
per 5-Min Block
50
45
40
35
30
25
p<0.025
0
2
Paternal Nicotine (mg/kg/day)
Male
Female

Figure 2

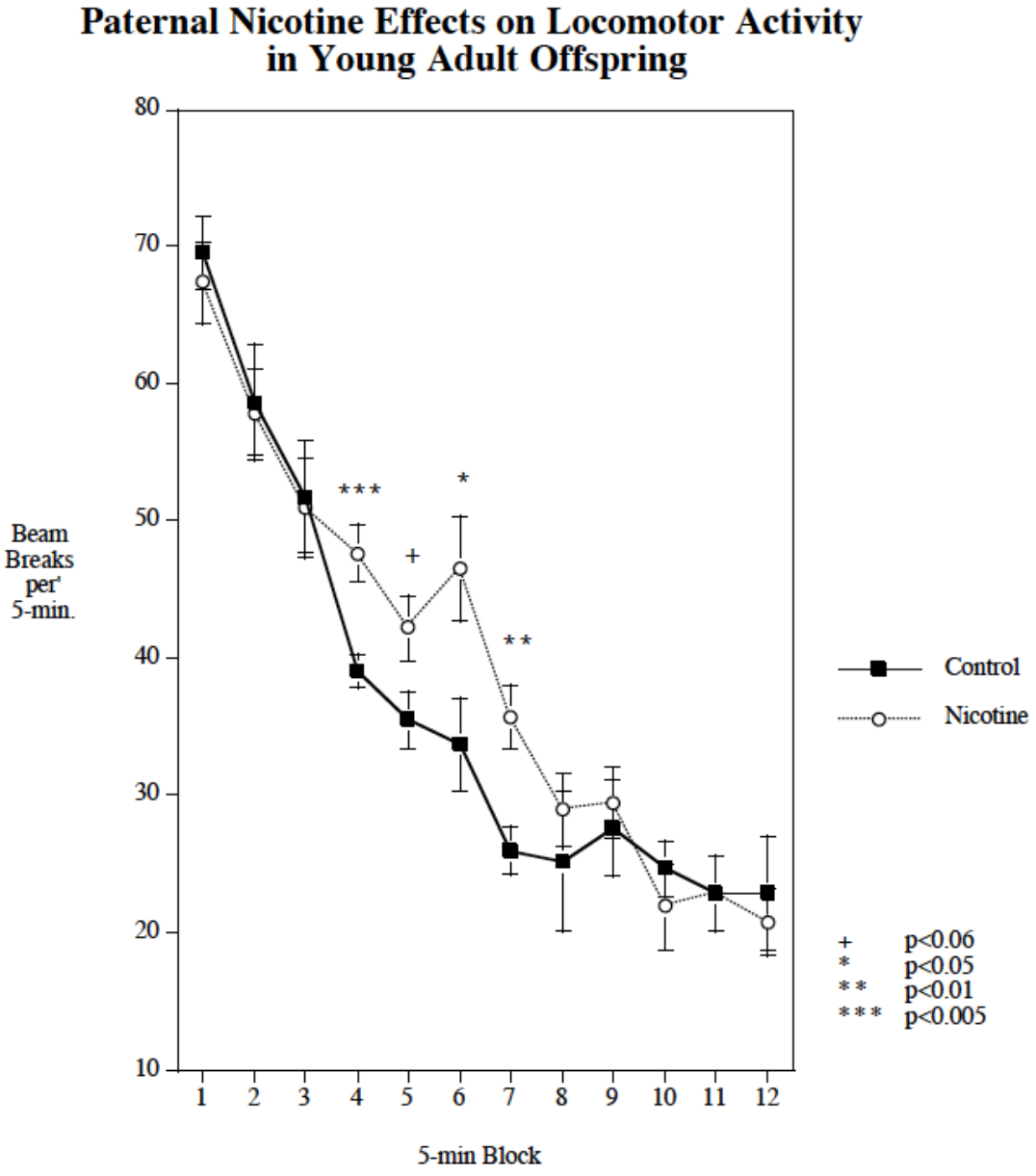

Paternal Nicotine Effects on Locomotor Activity
in Young Adult Offspring
Beam Breaks per 5-min.
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
5-min Block
***
+
*
**
Control
Nicotine
+ p<0.06
* p<0.05
** p<0.01
*** p<0.005

Figure 3

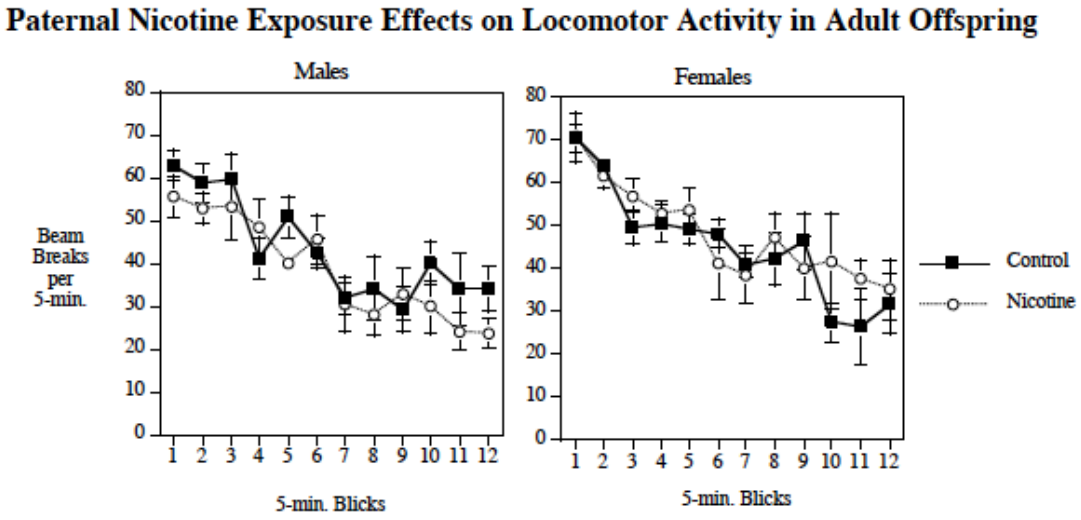


Figure 4

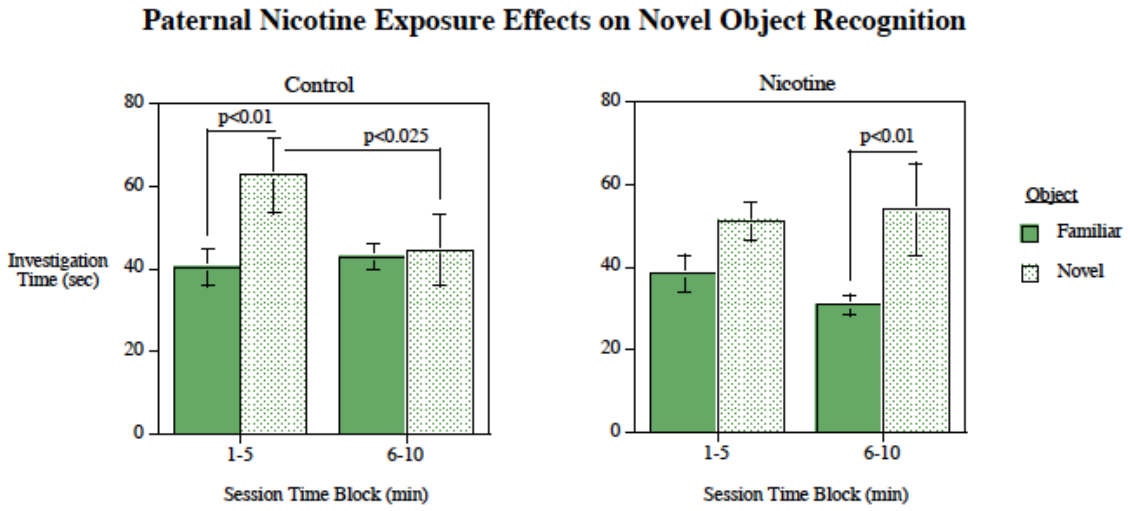
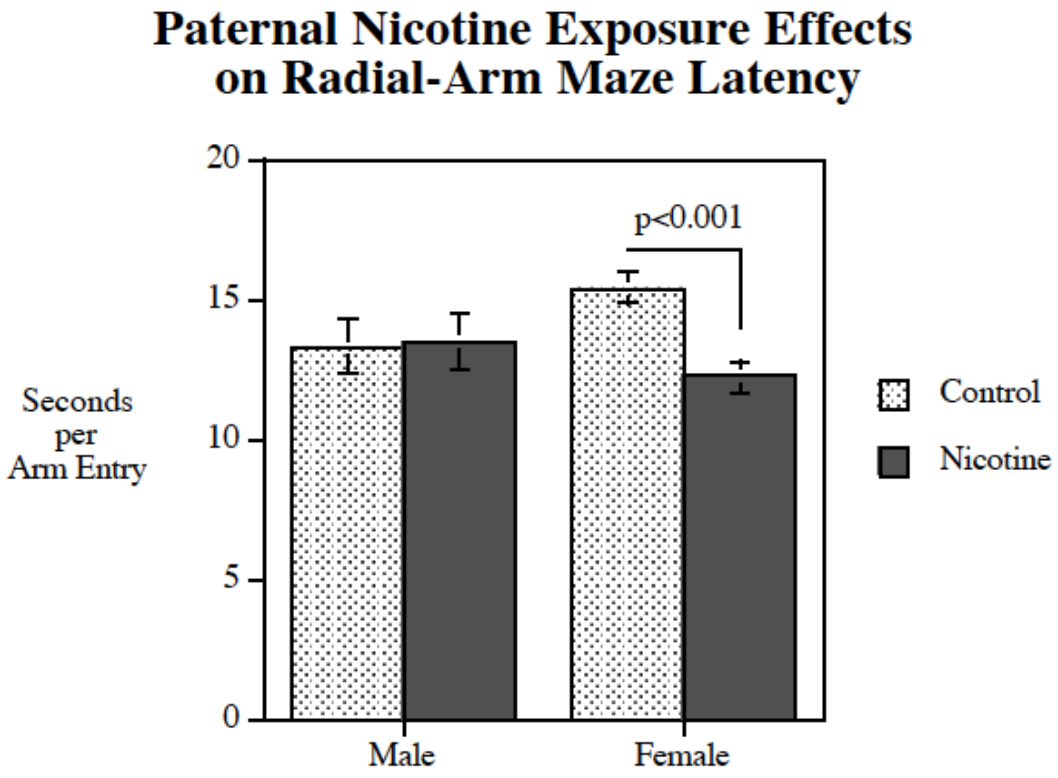
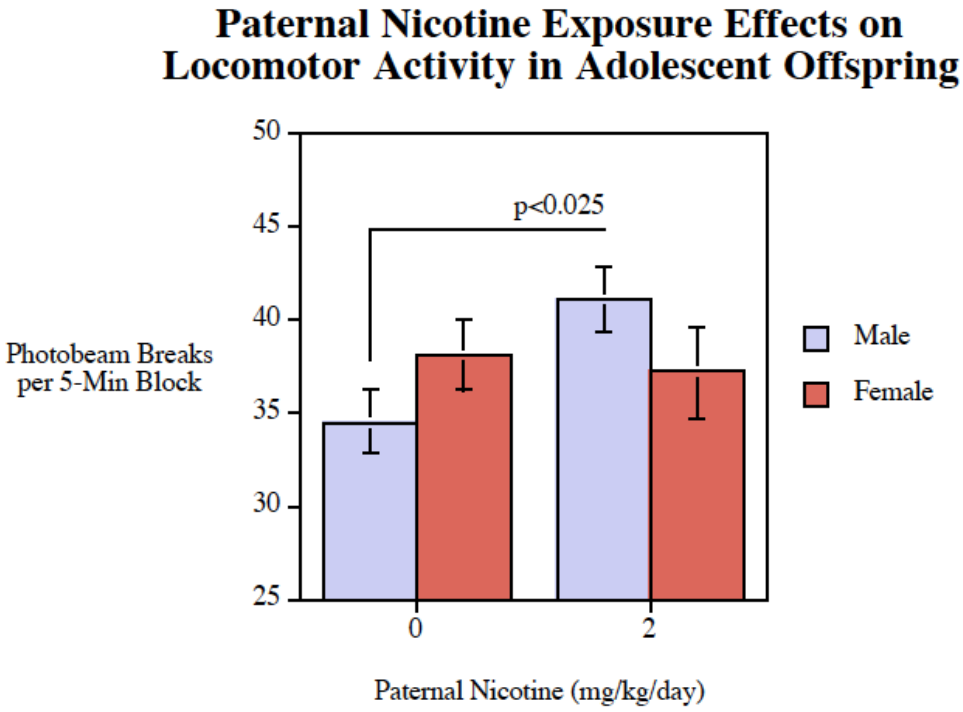
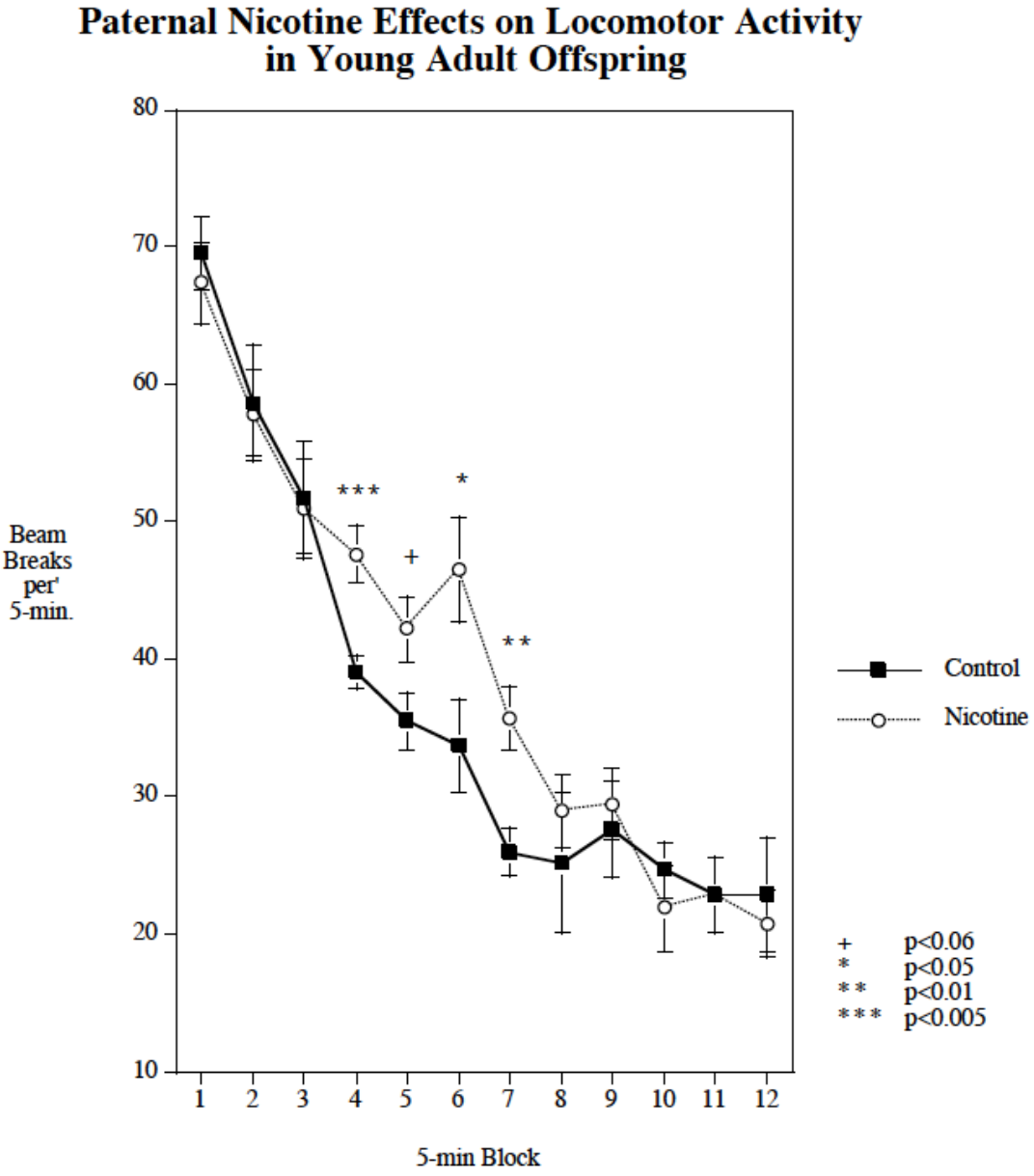


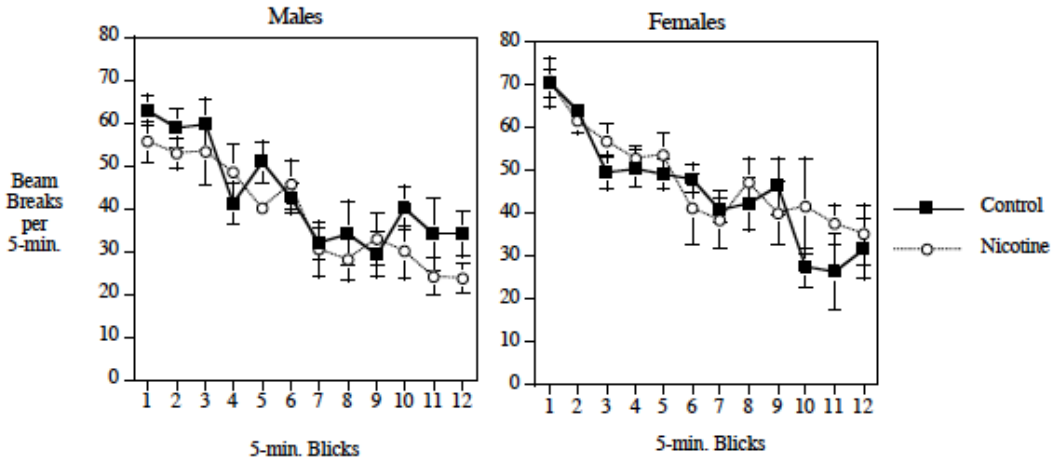
Figure 5

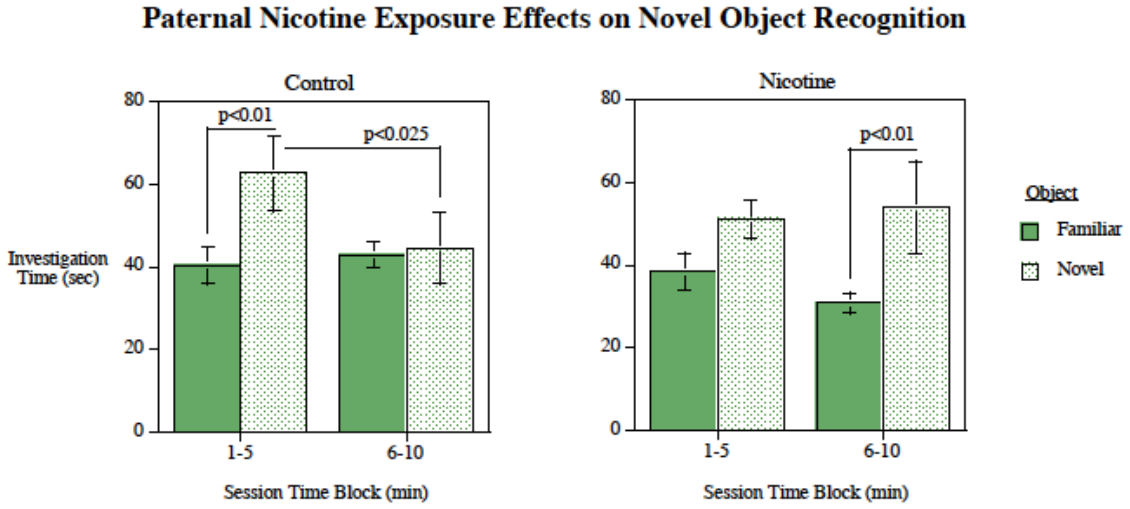

Paternal Nicotine Exposure Effects
on Radial-Arm Maze Latency
Seconds
per
Arm Entry
20
15
10
5
0
p<0.001
Male
Female
Control
Nicotine

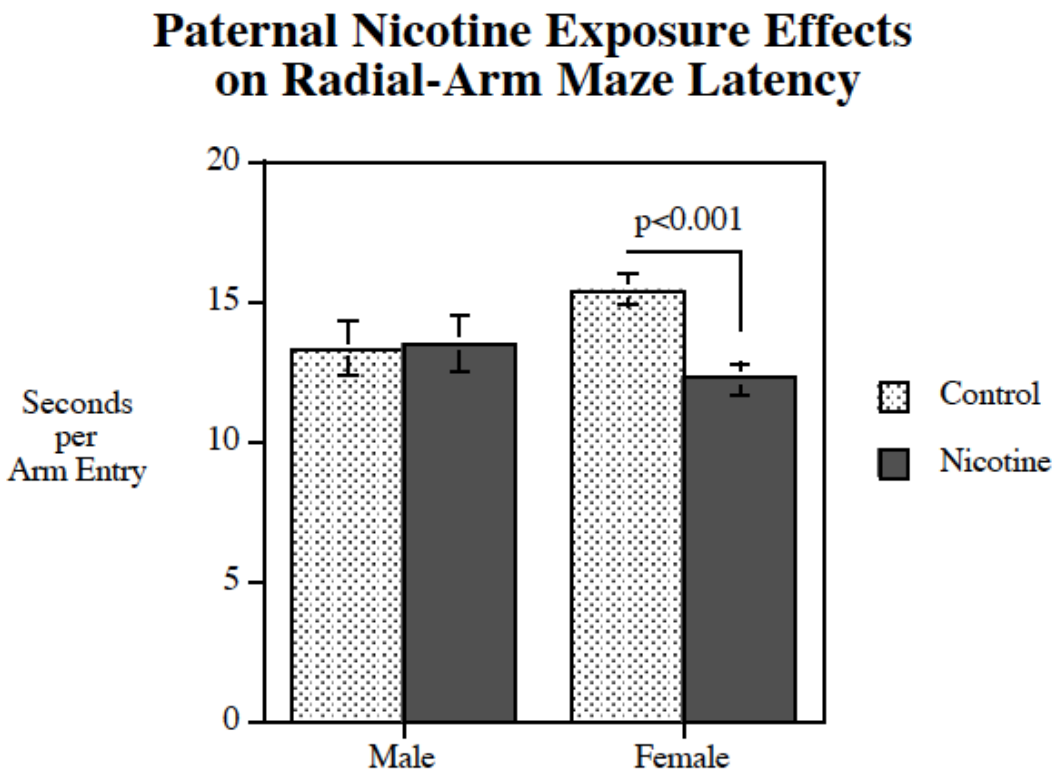

Paternal Nicotine Exposure Effects on Locomotor Activity in Adolescent Offspring
Photobeam Breaks per 5-Min Block
50
45
40
35
30
25
p<0.025
0
2
Paternal Nicotine (mg/kg/day)
Male
Female


Paternal Nicotine Effects on Locomotor Activity
in Young Adult Offspring
Beam Breaks per 5-min.
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
5-min Block
***
*
+
**
Control
Nicotine
+ p<0.06
* p<0.05
** p<0.01
*** p<0.005

Paternal Nicotine Exposure Effects on Locomotor Activity in Adult Offspring






Paternal Nicotine Exposure Effects
on Radial-Arm Maze Latency
Seconds
per
Arm Entry
20
15
10
5
0
p<0.001
Male
Female
Control
Nicotine