Published in final edited form as:

Int J Dev Neurosci. 2011 April ; 29(2): 153–161. doi:10.1016/j.ijdevneu.2010.12.001.

Prenatal IV Nicotine Exposure Produces a Sex Difference in Sensorimotor Gating of the Auditory Startle Reflex in Adult Rats

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Abstract

Maternal smoking during pregnancy is associated with auditory processing deficits in children; these effects have been confirmed with animal models of continuous high-dose prenatal nicotine exposure. The present experiments utilized a novel, low-dose, intermittent, intravenous (IV) gestational nicotine exposure model to investigate potential deficits on the preattentive process of sensorimotor gating, as indexed by prepulse inhibition (PPI), in preweanling and adult rat offspring. Pregnant dams received bolus IV injections of nicotine (0.05 mg/kg/injection) 3x/day on gestational days 8–21. Auditory and tactile stimulus modalities were probed with tone and air puff prepulse stimuli, respectively. These prepulse stimuli preceded a 100 dB(A) startle tone by six different interstimulus intervals (ISIs; 0, 8, 40, 80, 120, 4000 ms) to define a curve of response inhibition. The magnitude of PPI increased with age, from 59 to 81% inhibition. Preweanlings (PND 14 and 18) and adults (PND 75) gestationally exposed to nicotine exhibited altered startle responding relative to controls, but the nature of the deficit became more localized at later ages. The entire curve of response inhibition in preweanlings exposed to prenatal nicotine (PND 14) was shifted up relative to controls, and notably, did not interact with prepulse stimulus modality, suggesting a generalized increased sensorimotor responsiveness as a function of prenatal nicotine. At PND 18, a shift in the response curve across all ISIs was again noted, but varied as a function of prepulse stimulus modality; the increased sensorimotor responsiveness was specific to the auditory, but not tactile, sensory modality. In adulthood, male and female animals prenatally exposed to nicotine were differentially sensitive to modulation by the ISIs, relative to control male and female animals. Specifically, despite robust PPI, adult females exposed to gestational nicotine were relatively insensitive to changes in ISI from 8–120 ms; in contrast, the robust PPI of nicotine-exposed males demonstrated a clear focal point of inhibition at 40 ms. These findings indicate that a low, daily dosing of IV prenatal nicotine produces long-lasting alterations in auditory PPI. An important implication of this research is that “chipping” with smoked-tobacco products during pregnancy may produce enduring changes in sensorimotor processing.

Keywords

gestational nicotine; intravenous; prepulse inhibition; sensorimotor gating; preweanling; adult; rat

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1. Introduction

Maternal tobacco smoking during pregnancy is a major societal health concern for developing offspring. Gestational tobacco smoke exposure produces restricted intrauterine growth (Kramer, 1987; Kramer et al., 1990) and low birth weight (Meyer and Comstock, 1972; Simpson, 1957; Wang et al., 2002; Ward et al., 2007), and is associated with sudden infant death syndrome (Fleming and Blair, 2007). The negative health outcomes of maternal smoking are not limited to early development, however, as an assortment of neurobehavioral and cognitive problems are documented in children and adults prenatally exposed to tobacco smoke (see Heath and Picciotto, 2009; Pauly and Slotkin, 2008). Smoking during gestation increases the incidence of attention-deficit hyperactivity disorder (Button et al., 2007; Button et al., 2005; Linnet et al., 2003; Thapar et al., 2003), conduct disorder (Cornelius et al., 2007; Fergusson et al., 1998; Stene-Larsen et al., 2009; Wakschlag et al., 2006), and substance use disorder in offspring (Brennan et al., 2002; Buka et al., 2003; Weissman et al., 1999). Problems in auditory processing and cognitive function are also seen in offspring of maternal smokers (Fried et al., 1998, 2003; Jacobsen et al., 2007; Jacobsen et al., 2006; McCartney et al., 1994; Picone et al., 1982; Saxton, 1978; Sexton et al., 1990). For example, Jacobsen et al. (2007) assessed auditory and visual attention in adolescents who were prenatally exposed to maternal tobacco smoke and reported that relative to non-exposed controls, females exhibited impairments in auditory and visual attention, whereas males showed impairments in auditory processing. Thus, prenatal tobacco smoke exposure not only yields direct health consequences for the infant, it also produces persistent neurodevelopmental consequences that can result in long-lasting psychopathology and compromises in cognitive processing. Given that the prevalence of maternal smoking during pregnancy is as high as 13% in the United States (Martin et al., 2008; Tong et al., 2009), it is important to investigate how maternal tobacco smoking produces neurodevelopmental alterations in offspring.

Nicotine is the key component of tobacco that maintains long-term smoking behavior (Corrigall and Coen, 1989). It is rapidly absorbed from tobacco smoke, readily passes the blood brain barrier, and acts as an agonist at nicotinic acetylcholinergic receptors (nAChRs) located throughout the central and peripheral nervous systems (Livingstone and Wonnacott, 2009). In the mesocorticolimbic pathway, nicotine activates nAChRs in the ventral tegmental area and nucleus accumbens, and chronic nicotine exposure can alter motivation and shape behaviors that favor increased smoking, and hence substance use disorder (SUD). Thus, maternal smoking represents the maintenance of brain nicotine levels by a female exhibiting SUD, and this behavior introduces nicotine, and other tobacco by-products, to the developing fetus.

Preclinical research has utilized animal models of maternal nicotine exposure to determine if nicotine alone produces teratogenic effects on neurodevelopment (LeSage et al., 2006; Pauly et al., 2004; Slotkin, 1998). The most common method of administering gestational nicotine has been through the use of the osmotic minipump, which delivers continuous nicotine levels throughout the gestational period. This exposure method has been claimed to reduce the fetal hypoxia (Slotkin, 1998) that may occur in rat pups exposed to nicotine via high-dose subcutaneous injections of nicotine, but nicotine delivered by osmotic minipump (2 – 9 mg/kg/day) has been reported to cause significant reductions (~40%) in uterine and placental blood flow (Birnbaum et al., 1994). Continuous gestational nicotine disrupts the processes of cell replication, cell survival, and synaptogenesis *in utero* (Navarro et al., 1989; Slikker et al., 2005; Slotkin, 2004), and produces up-regulation of neuronal nicotinic receptors in the offspring's brain (Slotkin et al., 1987; Tizabi and Perry, 2000). It has been hypothesized that changes in motivated behaviors (Franke et al., 2008; LeSage et al., 2006; Levin et al., 2006; Pauly et al., 2004), including the increased incidence of SUD (Azam et al., 2007; Franke et

al., 2008; Paz et al., 2007), and compromises in auditory processing (Popke et al., 1997) observed in the offspring of maternal smokers are, in part, due to gestational nicotine s activity at nAChRs receptors during neurodevelopment (Navarro et al., 1989; Slotkin et al., 1987).

Investigation of the neurodevelopmental consequences of prenatal nicotine by administering intravenous (IV) nicotine to pregnant rats, through an internalized jugular catheter, is of interest because it closely models the pharmacokinetics of nicotine absorption and distribution via the route of inhalation (Benowitz et al., 2009; Booze et al., 1999; Mactutus, 1989; Russell and Feyerabend, 1978). Previous research shows that an IV nicotine administration model produced behavioral alterations in offspring (LeSage et al., 2006). In that study, female rats were implanted with externalized IV catheters, bred, and administered IV nicotine boli multiple times per day throughout gestation to approximate the nicotine levels achieved by tobacco smokers (Hukkanen et al., 2005; LeSage et al., 2003). The findings demonstrated that gestational IV nicotine produced decreased locomotor behavior in preweanling rats, and suggested that further research using the IV model will yield important information about the neurodevelopmental effects of nicotine.

The IV injection method (Mactutus et al., 1994) may be used to deliver less overall daily amounts of nicotine relative to continuous exposure models, such as osmotic minipump and nicotine via drinking water and the aforementioned IV procedure while still providing a translationally relevant exposure (see Dwyer et al., 2008). With the current IV model, pregnant females are injected 3x/day, at 1000, 1300, and 1600. The elimination half-life for IV 0.05 mg/kg/infusion nicotine is ~ 50 minutes (Booze et al., 1999) and thus represents a unique model in that the dam and fetuses experience the bolus delivery of nicotine to the brain followed by a rather precipitous clearance (Mactutus, 1989; Russell and Feyerabend, 1978).

Research investigating the effects of IV gestational nicotine is of interest because maternal tobacco smoking compromises auditory processing in children (Fried et al., 1998, 2003; McCartney et al., 1994; Picone et al., 1982) and prenatal exposure of rats to continuous nicotine produces sex-dependent reductions in auditory PPI (Popke et al., 1997). The present research determined if prenatal IV nicotine altered the process of sensorimotor gating in preweanling and adult rat offspring using the prepulse inhibition (PPI) procedure. The PPI task is commonly used to study preattentive processes in humans and animals because it measures the ability of a low intensity prepulse stimulus to inhibit or gate the startle response to a subsequent suprathreshold startle stimulus (Hoffman and Fleshler, 1963; Hoffman and Searle, 1965; Swerdlow et al., 1994). The modality of the prepulse stimulus can vary from auditory, visual, to tactile and it need not match the modality of the startle stimulus to observe PPI (Hord et al., submitted; Varty et al., 1998; Young et al., 2010). The brain regions responsible for inhibition of the startle response are localized primarily in the midbrain (see Fendt et al., 2001). The areas primarily responsible for PPI, which are the superior and inferior colliculi, and the thalamus, are innervated by acetylcholine projections (Beninato and Spencer, 1986; Broide et al., 1995; Broide et al., 1996; Happe and Morley, 2004). Thus, the development of these regions may be disrupted by gestational nicotine exposure (see Heath and Picciotto, 2009; Popke et al., 1997).

For the present study, two experiments were conducted to test if IV gestational nicotine altered PPI in preweanling and adult offspring. Preweanlings were tested on postnatal days (PND) 14 and 18 and adults were tested on PND 75. The strategy in the present experiment was to define a curve of response inhibition by varying the interstimulus interval (ISI; 0–4000 ms), consistent with other studies examining alterations in the development of the PPI response (Fitting, Booze, Hasselrot, et al., 2006; Fitting, Booze, et al., 2006a, 2006b; Ison,

1984). It was predicted that animals treated with gestational nicotine would demonstrate alterations in the development of PPI.

2. Methods

2.1. Animals

A total of 42 adult male and female Sprague-Dawley rats were acquired from Harlan Laboratories, Inc., (Indianapolis, IN) for breeding. Thirty-two virgin females were implanted with IV access ports at Harlan Laboratories, according to the methods of Mactutus et al. (1994). The females, together with 10 males for breeding, were transported to the animal care facilities at the University of South Carolina. Rodent food (8604 Teklad Rodent Diet, Harlan Laboratories, Inc., Indianapolis, IN) and water were provided *ad-libitum* throughout the course of the experiment. The animal colony was maintained at $21 \pm 2^\circ \text{C}$, $50\% \pm 10\%$ relative humidity and a 12L: 12D cycle with lights on at 0700 h. The protocol for this research methodology was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of South Carolina.

2.2. Breeding

Following a 7-day habituation period, female rats were housed three per cage. In the evening one male rat was placed in the cage with females for breeding. Males remained in the female cages from approximately 1700 to 0900. Dams were checked for pregnancy daily via vaginal lavage. Lavage samples were then analyzed under a microscope to assess the results of the previous night's breeding. If a sample was sperm-positive the corresponding female was single-caged and that day was considered gestational day (GD) 0. If the result was sperm-negative the animal remained group-housed and potential mating occurred the following evening(s). The breeding cycle continued until all females were determined to be sperm-positive. The weights of the pregnant dams were recorded every day during pregnancy.

2.3. Surgery

Catherization was performed according to the methods of Mactutus et al. (1994). Animals were anesthetized with a mixture of Ketamine hydrochloride (100 mg/kg/ml) and xylazine (3.3 mg/kg/ml). Following anesthesia a sterile Intracath IV catheter (Becton, Dickinson and Co., Franklin Lakes, NJ) with a Luer-lock injection cap (Medex, Inc., Carlsbad, CA) was implanted dorsally in a subcutaneous pouch. The distal end of the catheter was inserted into the left jugular vein and advanced toward the heart. Animals were kept under periodic post-operative observation and returned to the colony upon recovery. On the day following surgery, catheters were flushed with 0.2 ml of heparinized saline.

2.4. Drugs

Nicotine dihydrogen ditartrate (Sigma, St. Louis, MO) was weighed as base and was dissolved in physiological saline (0.9%). Control dams were administered physiological saline at the volumetric equivalent of the nicotine dose. Heparinized saline (2.5%) was used to flush the IV catheters.

2.5. Prenatal Nicotine Treatment

Nicotine (0.05 mg/kg/injection) or saline (SAL) was administered 3x/day to pregnant dams via internalized IV catheters from gestational day 8 – 21. Injection volume was 1 ml/kg. Intravenous injections were delivered through the Luer-lock injection cap of the subcutaneously implanted injection port. Following the first and second injection catheters were flushed with 0.2 ml of 0.9% physiological saline while 0.2 ml of heparinized saline

was used to flush the catheter after the final daily injection to maintain catheter patency. All injections were performed during the light portion of the photoperiod; injections were administered at approximately 1000, 1300, and 1600 daily.

2.6. Surrogate fostering, litter composition, and postnatal testing

The day of birth for pups was considered PND 0. On PND 1 litters were culled to 10 with 5 males and 5 females whenever possible. All pups were surrogate-fostered to timed-pregnant, drug naïve dams to preclude possible maternal care deficits from nicotine-exposed females. The righting reflex, negative geotaxis, and eye opening of the pups were assessed on PND 3–5, 7–9, and 13–17, respectively. PPI was assessed with one male and one female randomly selected from each litter (Holson and Pearce, 1992).

The righting reflex was assessed by placing animals on to their backs. The time from release to the completion of the righting response (rolling over to stomach) was recorded. This procedure occurred in blocks of 3 consecutive trials during PND 3–5, and the maximum latency for each animal to right itself was 25 sec per trial. Negative geotaxis was measured by placing animals on a wire mesh grid, positioned in a 25° downward angle. Rats were placed with their heads facing towards the downward slope of the apparatus. The latency for animals to turn 180° to face up the slope was recorded for every trial. A maximum latency of 30 sec was allowed for these trials. Animals were tested in three trial blocks across three consecutive days (PND 7–9) for negative geotaxis. Every animal's eyes (left and right) were checked for degree of openness. The degree of openness was rated on a scale of 0–3: 0 = completely closed; 1 = any part of the eye visible; 2 = partially open; 3 = fully open. All animals weights were recorded on PND 1, 7, 14, and 21. Rats were weaned on PND 21.

2.7. Apparatus

A sound attenuating chamber with 10 cm thick double-wall construction (Industrial Acoustics Company; Bronx, NY) was used to eliminate ambient sound from the testing environment. The internal measurements of the chamber were 60 × 60 × 81 cm. A SR- Lab Startle Reflex System (San Diego Instruments, Inc.) was used to initiate prepulse/startle stimuli and to record startle responses. A high-frequency loudspeaker of the SR-Lab system (Radio Shack model #40-1278B, frequency range of 5k-16k Hz) positioned 30 cm above the testing platform was used to present auditory prepulse and startle tones. A semi-rigid plastic tube (0.64 mm diameter) connected to a compressed air tank via a dual-stage regulator (SR2500, Victor Equipment Company, Denton, TX) was used to provide 16 psi airpuff tactile stimuli. The plastic tubing was routed through a port in the wall of the chamber and terminated 2 cm above the dorsal surface of the rat. During testing, animals were confined within a perspex test cylinder which varied in size with the age of the animal: 3.8 cm internal diameter (ID) for the preweanling rats and 8.9 cm ID for the adult rats. All perspex test cylinders were positioned on a 12.5 × 20-cm perspex platform, centrally located within the test chamber. A piezoelectric accelerometer, permanently affixed to the base of each perspex cylinder, converted the deflection of the perspex cylinder produced by the animal's startle response to an analog signal. All accelerometer signals were then digitized (12 bit A to D) and saved to a hard disk on a Pentium class computer. Acoustic stimulus intensities were measured and calibrated with a sound level meter (Extech Instruments, Waltham, MA) with the microphone placed inside the perspex test cylinder. Response sensitivities were calibrated using the SR-LAB startle calibration system. The startle chamber provides well over 30dB(A) of sound attenuation relative to the external environment with an ambient sound level in the chamber, in the absence of any stimuli presentation, of 22dB(A).

2.8. Procedure

2.8.1. Experiment 1: The effect of gestational IV NIC on PPI in male and female preweanling offspring—

A total of 36 animals were used in experiment 1. There were 20 animals in the gestational nicotine group (GN; 10 males and 10 females) and 16 animals in gestational saline group (GS; 8 males and 8 females). Experiments were conducted on PND 14 and 18, according to a within-subjects design. Each session began with a 5-min adaptation period during which a 70 dB(A) white noise was initiated. Six habituation trials were administered after the adaptation phase by presenting the startle tone alone (100 dB(A) inside the test cylinder, 120 dB(A) at the location of the audio speaker). The prepulse (test) trials followed the habituation trials. The prepulse stimuli (tone or airpuff) were presented in blocks of 6 trials at 6 different interstimulus intervals (ISIs) according to a Latin-square design. The ISIs were 0, 8, 40, 80, 120, and 4000 ms, and the intertrial interval averaged 20 sec (15–25 sec range). For each PPI session there was a total of 78 trials; 6 blank, 36 tone and 36 airpuff prepulses. All animals were tested in the same sound attenuating chamber.

2.8.2. Experiment 2: The effect of gestational IV NIC on PPI in male and female adult offspring—

A separate cohort of adult animals was used in experiment 2. A total of 30 male and female offspring were tested on PND 75. The GN groups included 6 males and 8 females, while the GS groups were comprised of 8 males and 8 females. The male GN and GS groups contained 1 and 3 pairs of rats from the same litter, respectively. All female animals were from different litters. The procedures were identical to experiment 1 with two exceptions: only the auditory prepulse stimulus was tested, and the size of the restraining tube was larger (8.9 cm ID) to accommodate the adult rats. The tactile prepulse was not used in the adult assessment as it became clear from the latter preweanling tests that nicotine had no differential effect with that prepulse modality.

2.9. Data Analysis

The data were analyzed using mixed factorial analysis of variance (ANOVA) techniques (SPSS 2009, Version 17.0 for Windows; SYSTAT 2006, Version 12.0 for Windows).

2.9.1. Litter parameters—The between-subjects factors for the litter parameter analyses were gestational treatment (Trt) and Sex. The within-subjects factor was age (either PND or GD). A one-way ANOVA was conducted for the total number of pups born to GN and GS dams. A Trt X Sex factorial ANOVA was used to analyze the ratio of males to females born to GN and GS dams. A Trt X Sex X PND mixed-factorial ANOVA was conducted for the pup weight gain, righting reflex, negative geotaxis and eye opening data. A Trt X GD mixed-factorial ANOVA determined if there were differences between GS and GN dams on the measure of maternal weight gain.

2.9.2. PPI Experiments—The dependent measure for each experiment was peak acoustic startle responses (ASR). For all analyses the ASR was calculated by averaging across all 6 PPI trials at each ISI. A 2 (Trt) X 2 (Sex) X 2 (PND: 14 vs. 18) X 2 (Modality; auditory vs. tactile) X 4 (ISI; 8, 40, 80, and 120 ms) mixed-factorial ANOVA was conducted on the data from experiment 1. Trt and Sex were between-subjects measures, whereas Age, prepulse Modality, and ISI were within subject s measures. A 2 (Trt) X 2 (Sex) X 4 (ISI) mixed-factorial ANOVA was conducted for the ASR data from experiment 2. This analysis, however, did not include the factors of Age and Modality. Age was not included because animals were only tested on PND 75, and the tactile modality was not tested. Further, more than one male animal from the same litter was used in experiment 2. In order to determine if a litter effect confounded the data, a preliminary ANOVA was conducted with a conservative alpha level set at 0.25. The main effect of litter was non-significant [$F(1, 26) =$

0.742, $P = 0.397$], demonstrating that the results of the ANOVA were not influenced by litter, and was not included in any subsequent analyses.

In general, the data from 0 and 4000 ms ISI were considered control trials to provide a baseline of PPI; data from the 0 and 4000 trials were analyzed separately using one-way ANOVA with Trt and Sex as between-subjects factors. Percent PPI was calculated by expressing the mean of the 8 – 120 ms ASR as a percentage of the average of 0 and 4000 ms control trials. Analysis of percent PPI data utilized ANOVA techniques with Trt and Sex as between-subjects factors and Age, Modality, and ISI as within-subjects measures. Statistical significance was determined with an alpha level of 0.05 for all analyses. Polynomial contrasts were conducted to characterize the linear, quadratic, or cubic components of the ISI function.

2.9.3 Graphical Presentation—For Figures 1, 2, and 4, data are presented on log-log plots. The data presented in these graphs were not transformed. Rather, all statistical analyses were performed on the raw data. The use of the $\log_{10}$ scale is preferred for graphical presentation because a large range of values, as many as 3 log units, are derived from the PPI procedure used in the present experiments. If data of such a range are presented on a linear plot there is an artificial compression of the data, particularly in the lower range of scores. Additionally, the $\log_{10}$ scale has the added advantage of conveying relative PPI effects, such that lines of comparable slope depict equivalent levels of percent inhibition.

3. Results

3.1. Pup and dam weight gain, righting reflex, negative geotaxis, and eye opening

No significant effects were observed for the total number of pups born to dams, the number of male vs. female pups, the righting reflex, negative geotaxis, or eye opening. There were also no differences in pup weight gain across PND 1, 7, 14, 21 or in maternal weight gain across GD 1, 7, 14, 21.

3.2. The ontogeny of the PPI response during the preweanling period and adulthood

There was a marked increase in inhibitory responding across the developmental period examined in the present experiments. At PND 14 there was a relative immaturity of the PPI response with an overall percent inhibition of $59.23(\pm 1.82)$ when averaging across the 8 – 120 ISIs. There was a significant increase in percent inhibition [$F(1, 32) = 47.677$, $P < .001$] following 4 days of maturation with PND 18 animals inhibiting at $72.69\% (\pm 1.02)$ on average. This significant increase in inhibitory responding, present at PND 18 is indicative of a more mature and thus more accurate index of sensorimotor gating during the preweanling period. At PND 75, animals were inhibiting at $80.78\% (\pm 1.28)$ overall and this was expected as the animals reached adulthood. These findings suggest that the present PPI model reliably measured inhibitory responding across the entire developmental period.

3.3. The effect of gestational IV nicotine on PPI in male and female preweanlings

The ANOVA for peak ASR revealed significant main effects of Trt [$F(1, 32) = 8.311$, $P < .01$], Age [$F(1, 32) = 8.848$, $P < .01$], Modality [$F(1, 32) = 21.349$, $P < .001$], and ISI [$F(1, 32) = 24.428$, $P < .001$, quadratic], but not Sex. The analysis identified a significant interaction of Trt x Age x Modality x ISI [$F(1, 32) = 4.457$, $P < .05$, quadratic]. Further interactions of Age x Modality x ISI [$F(1, 32) = 43.471$, $P < .001$, predominantly quadratic], and Trt x Modality x ISI [$F(1, 32) = 6.909$, $P < .05$, quadratic] were found to be significant. These findings suggest that GN animals exhibited increased startle responding relative to GS rats. Independent of gestational treatment, the magnitude of the ASR, on inhibitory trials, decreased from PND 14 to PND 18, suggesting a maturation of the inhibitory response,

similar to results from the percent inhibition analysis (Section 3.2.). Additionally, tactile prepulses elicited more inhibition of startle responding compared to auditory prepulses. The main effect of ISI defined an overall curve of inhibition best described by a quadratic function in which lower ASRs were exhibited at 40 and 80 ms, relative to the 8 and 120 ms ISI.

The significant Age x Modality x ISI interaction suggested that the tactile prepulses produced maximal inhibition at the 80 ms ISI and this occurred across both testing ages with PND 18 animals exhibiting a greater magnitude of inhibition relative to the PND 14 animals. In contrast, the inhibition response profile for tone prepulses shifted across ISI as a function of age, with the greatest inhibition at 8 ms on PND 14, and 40 ms on PND 18, indicating maturation of the PPI response (data not shown). Similar to responding on tactile trials, the overall magnitude of inhibition for tone prepulse trials was greater on PND 18.

3.4. Gestational IV nicotine-induced differences in PPI responding at PND 14

The Trt x Modality x ISI interaction, from the overall ANOVA, suggested that GN exposure decreased inhibitory responding relative to saline treated controls, regardless of age at testing. Therefore, further analyses were conducted to elucidate the effect of GN treatment on PPI at both PND 14 and 18. First, a Trt x Sex x Modality x ISI ANOVA was conducted on the PND 14 data. The analysis revealed main effects of Trt [$F(1,32) = 7.568, P < .05$] and ISI [$F(1,32) = 12.237, P < .01$, quadratic], indicating that prenatal IV nicotine treatment increased the overall magnitude of the ASR relative to GS controls. The Trt x ISI interaction was not significant, which is characteristic of a parallel, upward shift in the response inhibition curve. Furthermore, the interaction of Trt x Modality was not significant and the response inhibition curves for GN and GS animals are presented collapsed across modality in Figure 1. These results suggest that gestational nicotine treatment produced a generalized facilitation of startle responding which speaks to the relative immaturity of inhibitory responding at PND 14.

3.5. Gestational IV nicotine-induced differences in PPI responding are modality specific at PND 18

Further analyses examined tactile and tone PPI trials separately for the PND 18 ASR data in order to examine the impact of gestational nicotine on PPI when the preweanling PPI response was more mature. These data are shown in Figure 2. The Trt x Sex x ISI ANOVA conducted on the tactile data revealed a main effect of ISI [$F(1,32) = 45.7, P < .001$, cubic]. No other main effects or interactions were significant, indicating that gestational nicotine treatment did not alter PPI in response to tactile prepulse stimuli (see Figure 2A). The Trt x Sex x ISI ANOVA conducted on the tone data identified main effects of ISI [$F(1,32) = 36.603, P < .001$, primarily quadratic], and Trt [$F(1, 32) = 4.270, P < .05$]. The lack of a Trt x ISI interaction supported the parallel, upward shift in the response inhibition curve suggesting that PND 18 animals pretreated with gestational nicotine exhibited hyperresponsive startle responding that was specific to auditory prepulse stimuli (see Figure 2B).

3.6. The effect of gestational IV nicotine on 0 and 4000 ms control trials across PND 14 and 18

Analyses were performed on the mean peak ASR data collected from control trials (0 and 4000 ms ISI) averaged across both PND 14 and 18. This analysis revealed a significant effect of Trt, [$F(1,34) = 5.622, P < .05$] with GN animals displaying higher peak startle responses (359.366 ± 18.860) than GS animals (292.289 ± 21.086 ; Figure 3). These results supported the above mentioned findings at both testing ages with GN animals exhibiting increased startle responding relative to GS controls.

3.7. The effect of gestational IV nicotine on PPI in male and female adult offspring

The mixed-design ANOVA conducted on the tone ASR data revealed no significant main effects of gestational treatment or sex. However, a significant main effect of ISI [$F(1,26) = 9.889$, $P < .01$, quadratic] was observed and ISI was found to significantly interact with Sex, [$F(1,27) = 5.236$, $P < .05$, quadratic]. The significant Trt x Sex x ISI interaction, [$F(1,26) = 4.271$, $P < .05$, cubic] suggested that male and female GS animals exhibited similar ASR scores (Figure 4A), whereas the response was sex-dependent for GN animals (Figure 4B). Specifically, the GN female inhibition curve was flattened across 8–120 ms ISIs, whereas the male curve showed a clear maximal inhibition point at 40 ms. Thus, the sensorimotor gating response of the GN females was insensitive to modulation by ISI, relative to males. Percent PPI analysis for the adult data were also performed, and the ANOVA revealed a significant effect of ISI [$F(1,26) = 11.293$, $P < .01$, quadratic] and an interaction of ISI x Sex [$F(1, 26) = 5.626$, $P < .05$, quadratic]. These results suggest that male rats inhibit to a greater degree across ISI compared to females when assessing percent PPI (data not shown). Lastly, analysis of the mean peak ASR for the 0 and 4000 ms control trials revealed no significant differences.

4. Discussion

The present study investigated the neurodevelopmental effects of prenatal IV nicotine on the sensorimotor gating processes measured by PPI in male and female offspring. Experiment 1 focused on PPI during the preweanling period. Maximal inhibitory responding increased as a function of postnatal development, as animals exhibited greater inhibition for the auditory and tactile prepulse modalities at PND 18 compared to PND 14. Animals of both ages exhibited greater inhibition for tactile, relative to tone, prepulse stimuli. Animals exposed to gestational nicotine displayed a generalized increase in startle responding at PND 14, and furthermore, at PND 18, gestational nicotine animals showed increased startle responding specifically with auditory prepulse stimuli relative to controls. Experiment 2 investigated the long-term effects of gestational nicotine exposure on PPI by testing offspring during adulthood (PND 75, tone prepulse only). The important finding from experiment 2 is that gestational IV produced a sex-difference in sensorimotor gating in adult rats. Females in the GN group displayed an insensitivity of inhibition to modulation by ISI (8–120 ms) during adulthood, whereas males in the GN group displayed clear, maximal inhibition at the 40 ms ISI. Together, the present findings show that 3x/day IV nicotine produced long-term alterations in sensorimotor gating that appeared modality and sex specific despite the early alterations reflecting a more generalized reflex hyperresponsiveness.

The current results are in accord with previous research, which reported that prenatal nicotine exposure, delivered via osmotic minipump (i.e. 6 mg/kg/day), produced a reduction in PPI in adolescent offspring (PND 35; Popke et al., 1997). That study showed that gestational nicotine reduced PPI in offspring with a startle stimulus intensity of 98, but not a 112 or 122, dB tone, when measured by a strain gauge sensitive to body weight change. The present findings indicate that gestational IV nicotine exposure produced altered sensorimotor processing in adult female offspring using a startle tone of 100 dB (inside the test cylinder) as measured with an accelerometer. Thus, our findings extend those of Popke et al. (1997) by showing that gestational IV nicotine induced alterations in sensorimotor gating in adult animals using a startle stimulus of similar intensity (100 vs. 98 dB) with a response measure independent of differences in body weight. Further, Popke et al. (1997), reported that gestational nicotine exposure impaired adolescent females sensorimotor gating to a greater extent than males; the present data indicated that the sensorimotor gating of gestational nicotine exposed females was insensitive to modulation by ISI, relative to that of the males. These results are in accord with findings in humans exposed to prenatal nicotine,

which reported that males and females exhibited differential impairments in auditory vs. visual sensory processing (Jacobsen et al., 2007).

Previous data suggest that testing at different time points during the estrous and menstrual cycles can result in alterations of PPI in rats (Koch, 1998) and women (Jovanovic et al., 2004; Swerdlow et al., 1997), respectively. Several studies, however, have failed to detect differences in PPI amongst female rodents when compared at different stages of the estrous cycle (Bubenikova et al., 2005; Gulinello et al., 2003; Plappert et al., 2005). These null findings may be due to the difficulty in discerning the influence of cycling hormones in female rats during their relatively short estrous cycle (approximately 4 days). It is well established that the variation of circulating hormones across the rat estrous cycle is characterized by peak levels of many hormones, e.g., luteinizing hormone, prolactin, progesterone and estradiol, all occurring during proestrus (Gorski et al., 1975). Thus, suggesting that a specific hormone, such as estrogen, may explain a specific effect on PPI is confounded by the presence of several other hormones. Estrous cyclicity was therefore not monitored in the present study, and the specific stage of the estrous cycle for each rat during testing is not known. The sex difference observed in experiment 2, however, was observed in the GN animals, and not in the GS controls. Thus, the sex-dependent alteration of PPI in GN animals is likely related to a complex interaction of gonadal hormones and gestational nicotine exposure. Future studies will need to utilize hormone replacement procedures in gonadectomized animals to investigate specific interactions of hormones and nicotine on sex differences in the early organizational effects of hormones on the brain.

The current findings indicate that the IV route of administration, which delivers comparatively less daily nicotine relative to the osmotic minipump (i.e., 0.15 mg/kg/day vs. 6 mg/kg/day), produced altered sensorimotor gating in offspring. This indicates that significantly smaller amounts of gestational nicotine are sufficient to alter auditory processing in offspring when the IV route of administration is used. Taken together, the findings from experiments using different routes of prenatal nicotine administration provide converging evidence that gestational nicotine exposure is detrimental to auditory processing under controlled experimental conditions. These findings further strengthen the suggestion that animal models of gestational nicotine exposure adequately mimic the detrimental effects of maternal smoking on auditory processing in human offspring (Heath and Picciotto, 2009; Jacobsen et al., 2007; Slotkin, 2008).

The PPI procedure used in the present experiments varied the ISI (i.e., 0–4000 ms) and maintained the startle stimulus intensity at 100 dB to establish a curve of inhibition (Parisi and Ison, 1979). This procedure revealed that maximal inhibition occurs at approximately 40 and 80 ms ISI for auditory and tactile prepulse stimuli, respectively. Analyzing the curve of inhibition exhibited by treated and control animals showed that in the first experiment gestational nicotine treatment produced an upward shift in the curve on PND 18 relative to controls during auditory prepulse stimulus trials. The upward shift in the curve of inhibition indicates hyperresponsive reflexive behavior at all ISI during tone prepulse trials in animals prenatally treated with IV nicotine. The gestational effects of nicotine observed in experiment 2, however, showed that adults exhibited a different pattern of inhibition that was sex dependent. Although the point of inhibition remained at 40 ms for control males and females, the female rats treated with prenatal nicotine exhibited a curve of inhibition that was flat across the 8–120 ms ISI trials. In other words, the prenatal nicotine-treated adult females exhibited the same magnitude of inhibition when tone prepulse stimuli preceded the startle stimulus by 8, 40, 80, and 120 ms, which indicates that the females were insensitive to variations in ISI. Males that were prenatally exposed to nicotine did not exhibit a flattening of the inhibition curve, but rather, showed a clear point of inhibition at 40 ms ISI like that of controls. These findings indicate that holding the magnitude of the startle

stimulus constant and establishing a curve of inhibition by varying the ISI is a sensitive procedure to measure the detrimental effects of IV prenatal nicotine on sensorimotor gating in a rat model.

The statistical strategy used in the present experiments was to compare response inhibition curves generated from male and female animals from each gestational exposure condition. The analytical approach of comparing the curves of inhibition was based on previous research utilizing multiple ISI to investigate PPI (Parisi and Ison, 1979). Many studies utilize a single ISI (i.e. 100ms) and calculate percent of inhibition as the dependent measure; and this protocol has been widely used to assess PPI in animals and humans (Geyer and Swerdlow, 2001). The presentation and statistical analysis of the ASR data as a function of ISI affords the opportunity of detecting 1) sensitivity to modulation of ISI, 2) shifts in the point of maximal inhibition, and 3) the relative degree of PPI. For example, in experiment 1 of the present study, there was a parallel shift in the mean curves of inhibition when comparing GN to GS animals. In this case, the calculation of percent inhibition for the 8–120 ms ISI from control trials is disadvantageous because a parallel shift in the curve of inhibition will produce a null result, or “equivalent” inhibition, according to this measure. The data from experiment 1 do not represent a null finding, and thus using percent inhibition as the dependent measure would misrepresent this data set. This example demonstrates that calculating percent PPI for a single ISI, rather than manipulating ISI to investigate PPI, may not detect differences in PPI.

The present experiment did not investigate the neurophysiologic mechanisms that underlie the prenatal nicotine-induced alterations in sensorimotor gating, however, the neurocircuitry that mediates PPI is well defined (Fendt et al., 2001), and speculation regarding the origins of these effects can be discussed. According to this model, the startle stimulus is detected by cochlear nuclei (CN) and is then processed by the caudal pontine reticular nucleus (PnC). The output of the PnC projects to interneurons which then mediate the startle response. PPI occurs if an auditory prepulse stimulus is detected by the ascending auditory pathway prior to onset of a startle stimulus. In this context, the prepulse stimulus activates the CN, and the information is then processed in the inferior colliculus (IC), the auditory thalamus (i.e. medial geniculate nucleus of the thalamus) and then in the superior colliculus (SC). Information representing the prepulse stimulus is sent from the SC to the pedunculopontine tegmental nucleus (PPTg) and cholinergic projections from the PPTg innervate the PnC. Thus, for a prepulse stimulus to attenuate the ASR, the PnC first receives information about the acoustic prepulse and shortly thereafter the PnC processes information about the startle stimuli. PPI is exhibited if the ISI is within a particular temporal range (Hoffman and Searle, 1965; Li, Korngut, et al., 1998; Li, Priebe, et al., 1998). The substantia nigra and the locus coeruleus produce further modulatory effects on PPI by sending GABAergic and noradrenergic projections, respectively, to the PnC (Davis, 1980; Davis et al., 1982; Fendt et al., 1994; Fendt et al., 2001; Koch, 1999; Koch et al., 1993; Koch and Schnitzler, 1997; Li et al., 2009; Yeomans et al., 2006; Yeomans et al., 2002).

In relation to the aforementioned PPI circuitry, the results of the present experiment suggest that alterations in processing produced by prenatal nicotine may be mediated by altered processing of both the startle and the prepulse stimuli. Evidence that gestational nicotine exposure altered processing of the startle stimulus comes from experiment 1. In that experiment, prenatal nicotine produced hypersensitivity to the startle stimulus across all ISIs, and the upward shift in the inhibition curve indicates that rats exhibited a generalized alteration in information processing. Thus it appears that prenatal nicotine exposure altered processing of the startle stimulus in preweanling animals. Offspring did not show a deficit in startle stimulus detection because gestational nicotine rats exhibited a greater magnitude of startle in the absence of prepulse stimuli, relative to controls (Figure 3). Thus, the findings

from the control trials suggests that processing deficits in the cochlear nuclei are not likely responsible for the detrimental effects of prenatal IV nicotine exposure on the ASR. This is similar to previous findings reporting that gestationally exposed animals do not exhibit a problem detecting an auditory cue used in a shock mediated passive avoidance procedure (Liang et al., 2006; see also Trammer et al., 1992). Rather, the prenatal nicotine-induced alteration in the ASR observed in the present experiment may be mediated by processing deficits in the PnC, and perhaps through gestational nicotine-induced changes in the cholinergic system that innervates the PPTg and PnC (Koch et al., 1993). Evidence that gestational nicotine exposure altered processing of the prepulse stimulus was observed in the second experiment. Specifically, adult females gestationally exposed to nicotine exhibited a lack of sensitivity to changes in ISI, rather than a general upward shift in the inhibition curve observed with the preweanlings. The difference in the pattern of the inhibition curves between prenatally exposed females and the other adults suggests that the decreased sensitivity to ISI was not a deficit of the cochlear nuclei detecting the prepulse stimulus because these animals exhibited inhibition of the ASR. The relative insensitivity to ISI may have been due to processing impairments in the ascending auditory pathway, which processes information about the prepulse stimulus. Thus, according to the data in experiment 2, prenatal nicotine-induced processing deficits in the auditory thalamus or the auditory cortex may be related to the alterations in PPI. The effects of IV gestational nicotine on the expression of nicotinic receptors were not measured in the present experiments and therefore comments on changes in the cholinergic system, both at the level of the PPTg/PnC and auditory thalamus/auditory cortex, would be speculative. Previous research, however, indicates that neonatal nicotine exposure produces changes in the expression of nAChR in the auditory thalamus and auditory cortex, and that these changes may alter auditory processing (Aramakis et al., 2000). Alterations in ASR observed on trials with auditory, but not tactile, prepulses on PND 18 suggest that specific disruption of nAChRs in the auditory pathway produced the findings of experiment 1. Future experiments will be necessary to determine if altered sensorimotor gating produced by gestational IV nicotine exposure are related to changes in nAChR expression.

The present study is the first to investigate the effects of IV gestational nicotine exposure on the preattentive processes involved in sensorimotor gating. The IV nicotine exposure method was used because this route of administration mimics the pharmacokinetics of nicotine when self-administered through the route of inhalation. Thus, the IV route of administration can be used to approximate levels of drug bioavailability and distribution half-life characteristic of human nicotine use (Benowitz et al., 2009; Booze et al., 1999). This method of nicotine exposure did not affect the total number of pups born to dams, the ratio of male to female pups, pup weights on PND 1, or pup weight gain, suggesting that the effects of 3x/day was not sufficient to induce hypoxic/ischemic stress via placental blood flow restriction (Birnbaum et al., 1994). Furthermore, Birnbaum (1994) utilized a continuous nicotine exposure model and reported a ~40% reduction in uterine and placental blood flow with doses at least 15-fold higher than the dose in the present model. Lastly, even if a mild transient hypoxic episode was somehow achieved in the current procedure, the fetus is not completely unprotected under such conditions. There are several compensatory mechanisms by which the fetus is protected against fetal brain hypoxia. These include lowering of oxygen tension in the umbilical cord, increased umbilical blood flow, blood flow redistribution within the fetus itself to protect the brain, and the use of anaerobic glycolysis (Mactutus and Fechter, 1986). Therefore, given the route and dose at which nicotine was administered in the current experiments it is unlikely that hypoxic stress contributed significantly to the differences seen between nicotine exposed and control animals.

It has been suggested that continuous levels of nicotine delivered via the transdermal patch may have adverse effects on the neural development of offspring, and therefore, nicotine replacement therapy may be detrimental to the health of offspring (see Pauly and Slotkin, 2008). That a low daily dose of IV nicotine, which closely mimics the bioavailability and absorption of nicotine induced by tobacco smoking, produced impairments in auditory sensorimotor gating indicates that smoking a “low” number of daily cigarettes while trying to achieve full smoking cessation will impart negative health consequences on the offspring, specifically in the domain of auditory sensorimotor gating. The present findings support the argument that self-administering low doses of nicotine, either through nicotine replacement therapy or reduced smoking (“chipping”) during pregnancy, likely will contribute to neurodevelopmental disorders in offspring.

In conclusion, the present findings demonstrate that gestational IV nicotine produced long-term alterations in sensorimotor gating among adult animals. This effect that was not readily identified in preweanlings as the effect of gestational nicotine was associated with general sensorimotor hyperresponsivity at PND 14 and this responsiveness was found to be specific to auditory prepulses on PND 18. These results are in accord with previous research reporting that prenatal nicotine exposure alters auditory processing in experimental animals and humans exposed to nicotine *in utero* (Fried et al., 1998, 2003; Jacobsen et al., 2007; McCartney et al., 1994; Picone et al., 1982; Popke et al., 1997). The alterations observed in the present experiments were produced by 3x/day IV nicotine exposures during GD 8–21. Currently, it is unknown if there is a critical developmental period for IV nicotine exposure to alter sensorimotor gating in offspring. Given that the IV exposure method allows the experimenter to deliver a rapidly absorbed bolus of nicotine multiple times per day, research directed at identifying the specific developmental period of vulnerability for nicotine-induced disruption of sensorimotor processing will be important to fully characterize the teratogenic effects of gestational IV nicotine.

Acknowledgments

The authors are grateful for the technical assistance offered by Rachel Singleton and Lauren Ballina. This research was made possible by NIDA grant DA 021287 (SBH) and by a Research Productivity Scholar award (K-21) granted by the University of South Carolina (SBH).

Abbreviations

ASR	acoustic startle response
CN	cochlear nuclei
GD	gestational day
IC	inferior colliculus
ISI	interstimulus interval
IV	intravenous
nAChRs	nicotinic acetylcholine receptor
PnC	caudal pontine reticular nucleus
PND	postnatal day
PPI	prepulse inhibition
PPTg	pedunculopontine tegmental nucleus
SC	superior colliculus

SUD substance abuse disorder

References

- Aramakis VB, Hsieh CY, Leslie FM, Metherate R. A critical period for nicotine-induced disruption of synaptic development in rat auditory cortex. *J Neurosci*. 2000; 20(16):6106–6116. [PubMed: 10934260]
- Azam L, Chen Y, Leslie FM. Developmental regulation of nicotinic acetylcholine receptors within midbrain dopamine neurons. *Neuroscience*. 2007; 144(4):1347–1360. [PubMed: 17197101]
- Beninato M, Spencer RF. A cholinergic projection to the rat superior colliculus demonstrated by retrograde transport of horseradish peroxidase and choline acetyltransferase immunohistochemistry. *J Comp Neurol*. 1986; 253(4):525–538. [PubMed: 3540040]
- Benowitz NL, Hukkanen J, Jacob P 3rd. Nicotine chemistry, metabolism, kinetics and biomarkers. *Handb Exp Pharmacol*. 2009; (192):29–60. [PubMed: 19184645]
- Birnbaum SC, Kien N, Martucci RW, Gelzleichter TR, Witschi H, Hendrickx AG, et al. Nicotine- or epinephrine-induced uteroplacental vasoconstriction and fetal growth in the rat. *Toxicology*. 1994; 94(1–3):69–80. [PubMed: 7801331]
- Booze RM, Welch MA, Wood ML, Billings KA, Apple SR, Mactutus CF. Behavioral sensitization following repeated intravenous nicotine administration: gender differences and gonadal hormones. *Pharmacol Biochem Behav*. 1999; 64(4):827–839. [PubMed: 10593207]
- Brennan PA, Grekin ER, Mortensen EL, Mednick SA. Relationship of maternal smoking during pregnancy with criminal arrest and hospitalization for substance abuse in male and female adult offspring. *Am J Psychiatry*. 2002; 159(1):48–54. [PubMed: 11772689]
- Broide RS, O'Connor LT, Smith MA, Smith JA, Leslie FM. Developmental expression of alpha 7 neuronal nicotinic receptor messenger RNA in rat sensory cortex and thalamus. *Neuroscience*. 1995; 67(1):83–94. [PubMed: 7477913]
- Broide RS, Robertson RT, Leslie FM. Regulation of alpha7 nicotinic acetylcholine receptors in the developing rat somatosensory cortex by thalamocortical afferents. *J Neurosci*. 1996; 16(9):2956–2971. [PubMed: 8622126]
- Bubenikova V, Votava M, Horacek J, Palenicek T. Relation of sex and estrous phase to deficits in prepulse inhibition of the startle response induced by ecstasy (MDMA). *Behav Pharmacol*. 2005; 16(2):127–130. [PubMed: 15767849]
- Buka SL, Shenassa ED, Niaura R. Elevated risk of tobacco dependence among offspring of mothers who smoked during pregnancy: a 30-year prospective study. *Am J Psychiatry*. 2003; 160(11):1978–1984. [PubMed: 14594744]
- Button TM, Maughan B, McGuffin P. The relationship of maternal smoking to psychological problems in the offspring. *Early Hum Dev*. 2007; 83(11):727–732. [PubMed: 17888593]
- Button TM, Thapar A, McGuffin P. Relationship between antisocial behaviour, attention-deficit hyperactivity disorder and maternal prenatal smoking. *Br J Psychiatry*. 2005; 187:155–160. [PubMed: 16055827]
- Cornelius MD, Goldschmidt L, DeGenna N, Day NL. Smoking during teenage pregnancies: effects on behavioral problems in offspring. *Nicotine Tob Res*. 2007; 9(7):739–750. [PubMed: 17577803]
- Corrigall WA, Coen KM. Nicotine maintains robust self-administration in rats on a limited-access schedule. *Psychopharmacology (Berl)*. 1989; 99(4):473–478. [PubMed: 2594913]
- Davis M. Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. *Neurosci Biobehav Rev*. 1980; 4(2):241–263. [PubMed: 6106916]
- Davis M, Gendelman DS, Tischler MD, Gendelman PM. A primary acoustic startle circuit: lesion and stimulation studies. *J Neurosci*. 1982; 2(6):791–805. [PubMed: 7086484]
- Dwyer JB, Broide RS, Leslie FM. Nicotine and brain development. *Birth Defects Res C Embryo Today*. 2008; 84(1):30–44. [PubMed: 18383130]
- Fendt M, Koch M, Schnitzler HU. Sensorimotor gating deficit after lesions of the superior colliculus. *Neuroreport*. 1994; 5(14):1725–1728. [PubMed: 7827317]

- Fendt M, Li L, Yeomans JS. Brain stem circuits mediating prepulse inhibition of the startle reflex. *Psychopharmacology (Berl)*. 2001; 156(2–3):216–224. [PubMed: 11549224]
- Fergusson DM, Woodward LJ, Horwood LJ. Maternal smoking during pregnancy and psychiatric adjustment in late adolescence. *Arch Gen Psychiatry*. 1998; 55(8):721–727. [PubMed: 9707383]
- Fitting S, Booze RM, Hasselrot U, Mactutus CF. Intrahippocampal injections of Tat: effects on prepulse inhibition of the auditory startle response in adult male rats. *Pharmacol Biochem Behav*. 2006; 84(2):189–196. [PubMed: 16790267]
- Fitting S, Booze RM, Mactutus CF. Neonatal hippocampal Tat injections: developmental effects on prepulse inhibition (PPI) of the auditory startle response. *Int J Dev Neurosci*. 2006a; 24(4):275–283. [PubMed: 16530999]
- Fitting S, Booze RM, Mactutus CF. Neonatal intrahippocampal glycoprotein 120 injection: the role of dopaminergic alterations in prepulse inhibition in adult rats. *J Pharmacol Exp Ther*. 2006b; 318(3):1352–1358. [PubMed: 16785316]
- Fleming P, Blair PS. Sudden Infant Death Syndrome and parental smoking. *Early Hum Dev*. 2007; 83(11):721–725. [PubMed: 17881163]
- Franke RM, Park M, Belluzzi JD, Leslie FM. Prenatal nicotine exposure changes natural and drug-induced reinforcement in adolescent male rats. *Eur J Neurosci*. 2008; 27(11):2952–2961. [PubMed: 18588535]
- Fried PA, Watkinson B, Gray R. Differential effects on cognitive functioning in 9- to 12-year olds prenatally exposed to cigarettes and marihuana. *Neurotoxicol Teratol*. 1998; 20(3):293–306. [PubMed: 9638687]
- Fried PA, Watkinson B, Gray R. Differential effects on cognitive functioning in 13- to 16-year-olds prenatally exposed to cigarettes and marihuana. *Neurotoxicol Teratol*. 2003; 25(4):427–436. [PubMed: 12798960]
- Geyer, MA.; Swerdlow, NR. *Curr Protoc Neurosci*. Vol. Chapter 8. 2001. Measurement of startle response, prepulse inhibition, and habituation; p. 7
- Gulinello M, Orman R, Smith SS. Sex differences in anxiety, sensorimotor gating and expression of the alpha4 subunit of the GABAA receptor in the amygdala after progesterone withdrawal. *Eur J Neurosci*. 2003; 17(3):641–648. [PubMed: 12581182]
- Happe HK, Morley BJ. Distribution and postnatal development of alpha 7 nicotinic acetylcholine receptors in the rodent lower auditory brainstem. *Brain Res Dev Brain Res*. 2004; 153(1):29–37.
- Heath CJ, Picciotto MR. Nicotine-induced plasticity during development: modulation of the cholinergic system and long-term consequences for circuits involved in attention and sensory processing. *Neuropharmacology*. 2009; 56(Suppl 1):254–262. [PubMed: 18692078]
- Hoffman HS, Flesher M. Startle Reaction: Modification by Background Acoustic Stimulation. *Science*. 1963; 141:928–930. [PubMed: 14043340]
- Hoffman HS, Searle JL. Acoustic Variables in the Modification of Startle Reaction in the Rat. *J Comp Physiol Psychol*. 1965; 60:53–58. [PubMed: 14334243]
- Holson RR, Pearce B. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicol Teratol*. 1992; 14(3):221–228. [PubMed: 1635542]
- Hord LL, Moran LM, Booze RM, Harrod SB, Mactutus CF. Ontogeny of prepulse inhibition: the role of prepulse sensory modality. *Int J Dev Neurosci*. submitted.
- Hukkanen J, Jacob P 3rd, Benowitz NL. Metabolism and disposition kinetics of nicotine. *Pharmacol Rev*. 2005; 57(1):79–115. [PubMed: 15734728]
- Ison JR. Reflex modification as an objective test for sensory processing following toxicant exposure. *Neurobehav Toxicol Teratol*. 1984; 6(6):437–445. [PubMed: 6531049]
- Jacobsen LK, Slotkin TA, Mencl WE, Frost SJ, Pugh KR. Gender-specific effects of prenatal and adolescent exposure to tobacco smoke on auditory and visual attention. *Neuropsychopharmacology*. 2007; 32(12):2453–2464. [PubMed: 17375135]
- Jacobsen LK, Slotkin TA, Westerveld M, Mencl WE, Pugh KR. Visuospatial memory deficits emerging during nicotine withdrawal in adolescents with prenatal exposure to active maternal smoking. *Neuropsychopharmacology*. 2006; 31(7):1550–1561. [PubMed: 16341023]

- Jovanovic T, Szilagyi S, Chakravorty S, Fiallos AM, Lewison BJ, Parwani A, et al. Menstrual cycle phase effects on prepulse inhibition of acoustic startle. *Psychophysiology*. 2004; 41(3):401–406. [PubMed: 15102125]
- Koch M. Sensorimotor gating changes across the estrous cycle in female rats. *Physiol Behav*. 1998; 64(5):625–628. [PubMed: 9817573]
- Koch M. The neurobiology of startle. *Progress in Neurobiology*. 1999; 59(2):107–128. [PubMed: 10463792]
- Koch M, Kungel M, Herbert H. Cholinergic neurons in the pedunculo pontine tegmental nucleus are involved in the mediation of prepulse inhibition of the acoustic startle response in the rat. *Exp Brain Res*. 1993; 97(1):71–82. [PubMed: 8131833]
- Koch M, Schnitzler HU. The acoustic startle response in rats—circuits mediating evocation, inhibition and potentiation. *Behav Brain Res*. 1997; 89(1–2):35–49. [PubMed: 9475613]
- Kramer MS. Intrauterine growth and gestational duration determinants. *Pediatrics*. 1987; 80(4):502–511. [PubMed: 3658568]
- Kramer MS, Olivier M, McLean FH, Dougherty GE, Willis DM, Usher RH. Determinants of fetal growth and body proportionality. *Pediatrics*. 1990; 86(1):18–26. [PubMed: 2359680]
- LeSage MG, Gustaf E, Dufek MB, Pentel PR. Effects of maternal intravenous nicotine administration on locomotor behavior in pre-weanling rats. *Pharmacol Biochem Behav*. 2006; 85(3):575–583. [PubMed: 17141848]
- LeSage MG, Keyler DE, Collins G, Pentel PR. Effects of continuous nicotine infusion on nicotine self-administration in rats: relationship between continuously infused and self-administered nicotine doses and serum concentrations. *Psychopharmacology (Berl)*. 2003; 170(3):278–286. [PubMed: 12898121]
- Levin ED, Lawrence S, Petro A, Horton K, Seidler FJ, Slotkin TA. Increased nicotine self-administration following prenatal exposure in female rats. *Pharmacol Biochem Behav*. 2006; 85(3):669–674. [PubMed: 17196243]
- Li L, Du Y, Li N, Wu X, Wu Y. Top-down modulation of prepulse inhibition of the startle reflex in humans and rats. *Neurosci Biobehav Rev*. 2009; 33(8):1157–1167. [PubMed: 19747594]
- Li L, Korngut LM, Frost BJ, Beninger RJ. Prepulse inhibition following lesions of the inferior colliculus: prepulse intensity functions. *Physiol Behav*. 1998; 65(1):133–139. [PubMed: 9811375]
- Li L, Priebe RP, Yeomans JS. Prepulse inhibition of acoustic or trigeminal startle of rats by unilateral electrical stimulation of the inferior colliculus. *Behav Neurosci*. 1998; 112(5):1187–1198. [PubMed: 9829796]
- Liang K, Poytress BS, Chen Y, Leslie FM, Weinberger NM, Metherate R. Neonatal nicotine exposure impairs nicotinic enhancement of central auditory processing and auditory learning in adult rats. *Eur J Neurosci*. 2006; 24(3):857–866. [PubMed: 16848798]
- Linnet KM, Dalsgaard S, Obel C, Wisborg K, Henriksen TB, Rodriguez A, et al. Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence. *Am J Psychiatry*. 2003; 160(6):1028–1040. [PubMed: 12777257]
- Livingstone PD, Wonnacott S. Nicotinic acetylcholine receptors and the ascending dopamine pathways. *Biochem Pharmacol*. 2009; 78(7):744–755. [PubMed: 19523928]
- Mactutus CF. Developmental neurotoxicity of nicotine, carbon monoxide, and other tobacco smoke constituents. *Ann N Y Acad Sci*. 1989; 562:105–122. [PubMed: 2662858]
- Mactutus, CF.; Fechter, LD. Perinatal hypoxia: Implications for mammalian development. In: Riley, EP.; Vorhees, CV., editors. *Handbook of behavioral teratology*. New York, NY US: Plenum Press; 1986. p. 427–470.
- Mactutus CF, Herman AS, Booze RM. Chronic intravenous model for studies of drug (Ab)use in the pregnant and/or group-housed rat: an initial study with cocaine. *Neurotoxicol Teratol*. 1994; 16(2):183–191. [PubMed: 8052193]
- Martin JA, Kung HC, Mathews TJ, Hoyert DL, Strobino DM, Guyer B, et al. Annual summary of vital statistics: 2006. *Pediatrics*. 2008; 121(4):788–801. [PubMed: 18381544]
- McCartney JS, Fried PA, Watkinson B. Central auditory processing in school-age children prenatally exposed to cigarette smoke. *Neurotoxicol Teratol*. 1994; 16(3):269–276. [PubMed: 7935260]

- Meyer MB, Comstock GW. Maternal cigarette smoking and perinatal mortality. *Am J Epidemiol*. 1972; 96(1):1–10. [PubMed: 5039725]
- Navarro HA, Seidler FJ, Eylers JP, Baker FE, Dobbins SS, Lappi SE, et al. Effects of prenatal nicotine exposure on development of central and peripheral cholinergic neurotransmitter systems. Evidence for cholinergic trophic influences in developing brain. *J Pharmacol Exp Ther*. 1989; 251(3):894–900. [PubMed: 2600820]
- Parisi T, Ison JR. Development of the acoustic startle response in the rat: ontogenetic changes in the magnitude of inhibition by prepulse stimulation. *Dev Psychobiol*. 1979; 12(3):219–230. [PubMed: 437361]
- Pauly JR, Slotkin TA. Maternal tobacco smoking, nicotine replacement and neurobehavioural development. *Acta Paediatr*. 2008; 97(10):1331–1337. [PubMed: 18554275]
- Pauly JR, Sparks JA, Hauser KF, Pauly TH. In utero nicotine exposure causes persistent, gender-dependant changes in locomotor activity and sensitivity to nicotine in C57Bl/6 mice. *Int J Dev Neurosci*. 2004; 22(5–6):329–337. [PubMed: 15380832]
- Paz R, Barsness B, Martenson T, Tanner D, Allan AM. Behavioral teratogenicity induced by nonforced maternal nicotine consumption. *Neuropsychopharmacology*. 2007; 32(3):693–699. [PubMed: 16554741]
- Picone TA, Allen LH, Olsen PN, Ferris ME. Pregnancy outcome in North American women. II. Effects of diet, cigarette smoking, stress, and weight gain on placentas, and on neonatal physical and behavioral characteristics. *Am J Clin Nutr*. 1982; 36(6):1214–1224. [PubMed: 7148740]
- Plappert CF, Rodenbucher AM, Pilz PK. Effects of sex and estrous cycle on modulation of the acoustic startle response in mice. *Physiol Behav*. 2005; 84(4):585–594. [PubMed: 15811394]
- Popke EJ, Tizabi Y, Rahman MA, Nespor SM, Grunberg NE. Prenatal exposure to nicotine: effects on prepulse inhibition and central nicotinic receptors. *Pharmacol Biochem Behav*. 1997; 58(4):843–849. [PubMed: 9408185]
- Russell MA, Feyerabend C. Cigarette smoking: a dependence on high-nicotine boli. *Drug Metab Rev*. 1978; 8(1):29–57. [PubMed: 31270]
- Saxton DW. The behaviour of infants whose mothers smoke in pregnancy. *Early Hum Dev*. 1978; 2(4):363–369. [PubMed: 750194]
- Sexton M, Fox NL, Hebel JR. Prenatal exposure to tobacco: II. Effects on cognitive functioning at age three. *Int J Epidemiol*. 1990; 19(1):72–77. [PubMed: 2351527]
- Simpson WJ. A preliminary report on cigarette smoking and the incidence of prematurity. *Am J Obstet Gynecol*. 1957; 73(4):807–815. [PubMed: 13411046]
- Slikker W Jr, Xu ZA, Levin ED, Slotkin TA. Mode of action: disruption of brain cell replication, second messenger, and neurotransmitter systems during development leading to cognitive dysfunction—developmental neurotoxicity of nicotine. *Crit Rev Toxicol*. 2005; 35(8–9):703–711. [PubMed: 16417037]
- Slotkin TA. Fetal nicotine or cocaine exposure: which one is worse? *J Pharmacol Exp Ther*. 1998; 285(3):931–945. [PubMed: 9618392]
- Slotkin TA. Cholinergic systems in brain development and disruption by neurotoxicants: nicotine, environmental tobacco smoke, organophosphates. *Toxicol Appl Pharmacol*. 2004; 198(2):132–151. [PubMed: 15236950]
- Slotkin TA. If nicotine is a developmental neurotoxicant in animal studies, dare we recommend nicotine replacement therapy in pregnant women and adolescents? *Neurotoxicol Teratol*. 2008; 30(1):1–19. [PubMed: 18380035]
- Slotkin TA, Orband-Miller L, Queen KL, Whitmore WL, Seidler FJ. Effects of prenatal nicotine exposure on biochemical development of rat brain regions: maternal drug infusions via osmotic minipumps. *J Pharmacol Exp Ther*. 1987; 240(2):602–611. [PubMed: 2433431]
- Stene-Larsen K, Borge AI, Vollrath ME. Maternal smoking in pregnancy and externalizing behavior in 18-month-old children: results from a population-based prospective study. *J Am Acad Child Adolesc Psychiatry*. 2009; 48(3):283–289. [PubMed: 19242291]
- Swerdlow NR, Braff DL, Taaid N, Geyer MA. Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. *Arch Gen Psychiatry*. 1994; 51(2):139–154. [PubMed: 8297213]

- Swerdlow NR, Hartman PL, Auerbach PP. Changes in sensorimotor inhibition across the menstrual cycle: implications for neuropsychiatric disorders. *Biol Psychiatry*. 1997; 41(4):452–460. [PubMed: 9034539]
- Thapar A, Fowler T, Rice F, Scourfield J, van den Bree M, Thomas H, et al. Maternal smoking during pregnancy and attention deficit hyperactivity disorder symptoms in offspring. *Am J Psychiatry*. 2003; 160(11):1985–1989. [PubMed: 14594745]
- Tizabi Y, Perry DC. Prenatal nicotine exposure is associated with an increase in [125I]epibatidine binding in discrete cortical regions in rats. *Pharmacol Biochem Behav*. 2000; 67(2):319–323. [PubMed: 11124396]
- Tong VT, Jones JR, Dietz PM, D'Angelo D, Bombard JM. Trends in smoking before, during, and after pregnancy - Pregnancy Risk Assessment Monitoring System (PRAMS), United States, 31 sites, 2000–2005. *MMWR Surveill Summ*. 2009; 58(4):1–29.
- Trammer RM, Aust G, Koster K, Obladen M. Narcotic and nicotine effects on the neonatal auditory system. *Acta Paediatr*. 1992; 81(12):962–965. [PubMed: 1290858]
- Varty GB, Hauger RL, Geyer MA. Aging Effects on the Startle Response and Startle Plasticity in Fischer F344 Rats. *Neurobiology of Aging*. 1998; 19(3):243–251. [PubMed: 9661999]
- Wakschlag LS, Pickett KE, Kasza KE, Loeber R. Is prenatal smoking associated with a developmental pattern of conduct problems in young boys? *J Am Acad Child Adolesc Psychiatry*. 2006; 45(4):461–467. [PubMed: 16601651]
- Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. *JAMA*. 2002; 287(2):195–202. [PubMed: 11779261]
- Ward C, Lewis S, Coleman T. Prevalence of maternal smoking and environmental tobacco smoke exposure during pregnancy and impact on birth weight: retrospective study using Millennium Cohort. *BMC Public Health*. 2007; 7:81. [PubMed: 17506887]
- Weissman MM, Warner V, Wickramaratne PJ, Kandel DB. Maternal smoking during pregnancy and psychopathology in offspring followed to adulthood. *J Am Acad Child Adolesc Psychiatry*. 1999; 38(7):892–899. [PubMed: 10405508]
- Yeomans JS, Lee J, Yeomans MH, Steidl S, Li L. Midbrain pathways for prepulse inhibition and startle activation in rat. *Neuroscience*. 2006; 142(4):921–929. [PubMed: 16996220]
- Yeomans JS, Li L, Scott BW, Frankland PW. Tactile, acoustic and vestibular systems sum to elicit the startle reflex. *Neurosci Biobehav Rev*. 2002; 26(1):1–11. [PubMed: 11835980]
- Young JW, Wallace CK, Geyer MA, Risbrough VB. Age-associated improvements in cross-modal prepulse inhibition in mice. *Behav Neurosci*. 2010; 124(1):133–140. [PubMed: 20141288]

Figure 1.

The mean peak ASR amplitude data across ISI for PPI trials collected on PND 14 presented by gestational treatment. Analyses revealed significant main effects of Trt [$F(1,32) = 7.568$, $P < .05$] and ISI [$F(1,32) = 12.237$, $P < .01$, quadratic]. This suggests a parallel, upward shift in the inhibition response curve for animals prenatally exposed to nicotine compared to controls.

Figure 2.

The mean peak ASR amplitude data across ISI for PPI trials collected on PND 18. As shown in panel A, the tactile prepulse data revealed no significant effects of gestational treatment. As shown in panel B, the auditory prepulse data revealed significant mean effects of ISI [$F(1,32) = 36.603$, $P < .001$, prominent quadratic] and Trt [$F(1, 32) = 4.270$, $P < .05$]. The parallel, upward shift in the inhibition response curve for the auditory prepulse data in animals exposed to nicotine during gestation suggests a generalized increase in startle responsiveness, with no alteration in inhibitory processes.

Figure 3.

The mean peak ASR amplitude data across ISI for control trials on PND 14 and 18 (averaged across both days). The significant main effect of gestational nicotine is presented, again suggesting an increased startle responsiveness, * $p. < .05$.

Figure 4.

The mean peak ASR amplitude data across ISI for PPI trials collected on PND 75. A significant interaction of Trt x Sex x ISI was noted, [$F(1,26) = 4.271$, $P < .05$, cubic]. Panel A shows that no significant sex effect was present in the GS animals, whereas panel B illustrates the differences between GN males and females, with females exhibiting a relative insensitivity to manipulation of ISI. The GN males displayed a pronounced peak of inhibition at the 40 ms ISI. This sex difference in the GN animals drives the overall three-way interaction of Trt x Sex x ISI.