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Variable Maternal Stress in Rats Alters Locomotor Activity, Social Behavior, and Recognition Memory in the Adult Offspring

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Abstract

Rats repeatedly exposed to variable prenatal stress (PNS) exhibit behavioral signs that are similar to those manifested in several neuropsychiatric disorders such as deficits in attention and inhibitory control, and impairments in memory-related task performance. The purpose of the study described here was to conduct a comprehensive battery of tests to further characterize the behavioral phenotype of PNS rats as well as to evaluate the sensitivity of the model to therapeutic interventions (i.e., to compounds previously shown to have therapeutic potential in neuropsychiatric disorders). The results of this study indicated that PNS in rats is associated with: 1) increased locomotor activity and stereotypic behaviors, 2) elevated sensitivity to the psychostimulant amphetamine, 3) increased aggressive behaviors toward both adult and juvenile rats and 4) delay-dependent deficits in recognition memory. There was no evidence that PNS rats exhibited deficits in other areas of motor function/learning, sensorimotor gating, spatial learning and memory, social withdrawal, or anhedonia. In addition, the results revealed that the second generation antipsychotic risperidone attenuated amphetamine-related increases in locomotor activity in PNS rats; however, the effect was not sustained over time. Furthermore, deficits in recognition memory in PNS rats were attenuated by the norepinephrine reuptake inhibitor, atomoxetine, but not by the $\alpha 7$ nicotinic acetylcholine receptor partial agonist, GTS-21. This study supports the supposition that important phenomenological similarities exist between rats exposed to PNS and patients afflicted with neuropsychiatric disorders thus further establishing the face validity of the model for evaluating potential therapeutic interventions.

Keywords

prenatal stress; cognition; amphetamine; atomoxetine; locomotor activity; recognition memory; aggression; schizophrenia

1. Introduction

Historically, studies of neuropsychiatric disorders (e.g., schizophrenia, bipolar affective disorder, major depression) have focused on the more overt (positive) symptoms (e.g., delusions, and hallucinations) or affective symptoms (e.g., depressed mood and anxiety). However, cognitive dysfunction is now viewed as equally important, playing a vital role in

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the long term outcome and quality of life of psychiatric patients (Kurtz, 2005; Barch, 2005; Young et al., 2009; Bidwell et al., 2011). Further, while the positive and affective symptoms of neuropsychiatric disorders are often improved by current therapeutic treatments, they have limited effects on cognitive symptoms and may even exacerbate these deficits (Wallace et al., 2011). Establishing valid animal models that exhibit cognitive impairments that have relevance to neuropsychiatric disorders is essential for the development and/or identification of novel therapeutic approaches.

A number of epidemiological studies have established correlations between prenatal stress and neuropsychiatric disorders including attention-deficit/hyperactivity disorder (ADHD) autism, depression, anxiety, and schizophrenia (Lewis and Levitt, 2002; Spauwen et al., 2004; Rice et al., 2007; Kinney et al., 2008; Davis and Sandman, 2012). During the prenatal period, the rapid growth of the central nervous system makes the fetus particularly vulnerable to insults and hormonal changes in its environment (reviewed, Markham and Koenig, 2011). Deviation from the crucial sequences of events which occur during brain development may lead to inappropriately timed neuronal birth, migration, and synaptogenesis, which could ultimately cause long lasting changes in both structure and function (Brunton and Russell, 2011). In fact, evidence of these developmental impairments has been identified in postmortem studies of human brain samples obtained from individuals afflicted with neuropsychiatric disorders, including schizophrenia (Rapoport et al., 2005). These observations have led to a growing interest in the development of prenatal stress models in animals for the purpose of elucidating neurobiological mechanisms of neuropsychiatric disorders as well as for evaluating novel therapeutic approaches (Wilson and Terry, 2010; Marco et al., 2011).

It has been suggested that repeated variable prenatal stress in rodents (henceforth referred to as PNS) might be an etiologically appropriate neurodevelopmental model for some components of schizophrenia (Koenig et al., 2005). Exposure to PNS results in altered behavioral signs that are similar to symptoms often associated with several neuropsychiatric disorders (e.g., social withdrawal, elevated amphetamine-induced locomotor activity, deficits in sensorimotor gating, etc., Koenig et al., 2005; Lee et al., 2007). In addition, PNS rats were found to exhibit impairments in the performance of several behavioral tasks of cognition including those designed to assess attention, spatial reference memory, and conditioned fear memory and extinction (Markham et al., 2010; Wilson et al., 2012a; Wilson et al., 2012b); deficits also associated with neuropsychiatric disorders (e.g., schizophrenia, attention deficit/hyperactivity disorder, depression) (Barch, 2005; Bidwell et al., 2011; Austin et al., 2001; Spieker et al., 2012; Quraishi and Frangou, 2002; Dere et al., 2010).

The purpose of the study described was to ascertain face validity (i.e., degree of phenomenological similarity between the animal model and the human condition it is meant to simulate) of the PNS model by conducting a comprehensive battery of tests frequently used to evaluate deficits associated with neuropsychiatric disorders. In addition, we evaluated the sensitivity of the model to therapeutic interventions (i.e., to compounds previously shown to have therapeutic potential in neuropsychiatric disorders) to further evaluate predictive validity (i.e., the degree to which the model can be used to predict efficacy of a new therapeutic agent in humans). The tasks we conducted included tests of motor function and exploratory activity (e.g., open field locomotor activity, rotarod, and grip strength) sensorimotor gating (prepulse inhibition of the auditory startle response), tests of social withdrawal and anhedonia (social interaction and sucrose preference test respectively) as well as tasks designed to assess learning and memory-related domains of cognition that are relevant to neuropsychiatric disorders (Morris water maze, radial arm maze, and novel object recognition).

2. Material and methods

2.1. Animals

Timed pregnant Sprague-Dawley female rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN, USA) arriving on day five of gestation were housed individually in a temperature-controlled (25°C) and light-controlled (12-h light/dark cycle) facility. Pregnant female dams were randomly assigned to either the control or the PNS group. Pregnant animals had free access to food (Teklad Rodent Diet 8604 pellets, Harlan, Madison, WI, USA) and water following their arrival. All procedure employed during this study were reviewed and approved by the Georgia Health Sciences University Institutional Animal Care and Use Committee and are consistent with the AAALAC guidelines. Measures were taken to minimize pain or discomfort in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Stress paradigm

The repeated variable prenatal stress paradigm used in this study was adapted from Koenig and colleagues (Kinnunen, 2003, Koenig et al., 2005). Pregnant rats were exposed to the paradigm beginning on day 14 of gestation until delivery of pups on gestational day 22 or 23. Stressors were applied during the light phase of the cycle unless otherwise stated. The stress paradigm consisted of: (1) restraint in Broome style rodent restrainers (PLAS Labs, Inc) (1 h); (2) exposure to a cold environment ($4 \pm 1^\circ\text{C}$, 6 h); (3) overnight food deprivation during the dark phase of the cycle; (4) forced swim in room temperature water (15 min); (5) reversal of the light–dark cycle (24 hr); and (6) social stress induced by overcrowded housing during the dark phase of the cycle (12 hr). Stressors were applied in a randomized manner to prevent accommodation with up to three stress sessions per day (Table 1). Pregnant control rats were exposed to normal animal care and maintenance procedures during this period. Following birth, all dams and pups were left undisturbed until weaning on postnatal day 22. Offspring were double housed with a same sex littermate, food and water was allowed *ad libitum*, and animals were left undisturbed except for normal care and maintenance. Beginning on postnatal day (PND) 56, animals were handled for one week prior to all testing and training to reduce human contact-related stress and anxiety-related behaviors. Study subjects were transferred (in their home cages) to the behavioral testing rooms each morning approximately 30 min prior to training and testing. Both male and female rats were tested in each behavioral task and testing began on PND 63 (Table 2).

2.3. Rotarod

Motor tasks (rotarod and grip strength) were tested on the same day with a 2 hr rest period between tasks. Motor coordination, balance, and motor learning were evaluated with an accelerating rotarod (Rotor-Rod System®, San Diego Instruments). Individual rats were assessed for their ability to maintain balance on a rotating bar that accelerated from 0 to 40 rpm over a five min period. The amount of time elapsed before each subject fell from the rod was recorded. Each test subject was given four trials per day for three consecutive days with an intertrial interval of 30 min for rest.

2.4. Grip strength

Forelimb grip strength was measured with a digital grip strength meter (Animal Grip Strength System®, San Diego Instruments) by holding the rat by the nape of the neck and by the base of the tail. The forelimbs were placed on the tension bar and the rat was pulled back gently until it released the bar to measure grip strength (measured in kg of resistance). Each subject was given three consecutive trials per day for three consecutive days.

2.5. Pre-Pulse Inhibition

To assess the effect of prenatal stress on sensorimotor gating, animals were tested using a pre-pulse inhibition (PPI) procedure we have published previously (Hohnadel et al., 2007). Tests were conducted in four standard startle chambers (San Diego Instruments, San Diego, CA), each consisting of a Plexiglas tube (diameter 8.2 cm, length 25 cm), placed in a sound attenuated chamber. The tube was mounted on a plastic frame, under which a piezoelectric accelerometer was mounted which recorded and transduced the motion of the tube. On day 1, the experimental rats were each placed in one of the startle test chambers for a period of 20 min (without any startle stimuli) as an initial period of acclimation to the apparatus. One day 2, the animals were again placed in the test chamber and then exposed to a 5 min habituation period during which 70dB background white noise was present. This period was followed by twelve startle stimuli and a set of predefined prepulse levels (i.e., 5, 10, and 15 dB above background noise). On day 3, PPI testing began by placing the experimental rats into the startle chamber followed by a 1 min habituation period. After this period, the rats received 12 no-stimulus trials, 12 startle trials, and 12 prepulse/startle trials of each of the pre-defined prepulse levels in a pseudo-random fashion for a total of 60 trials. The intertrial interval ranged from 10 to 30 s. During the no-stimulus trial, no startle noise was presented, but the movement of the rat was scored. This represented a control trial for detecting differences in overall activity. The startle trials consisted of single 120 dB white noise bursts lasting 20 ms. The prepulse/startle trials consisted of a prepulse (20 ms burst of white noise with intensities of 75, 80, or 85 dB) followed 100 ms later by a startle stimulus (120 dB, 20 ms white noise). The resulting movement of the rat in the startle chamber was measured during the 100 ms following the startle stimulus onset. Basal startle amplitude was determined as the mean amplitude of the 12 startle trials. PPI was calculated according to the formula $100 - 100\% \times (PPx/P120)$, in which PPx is the mean of the 12 prepulse inhibition trials (i.e., for each individual prepulse intensity), and p120 is the basal startle amplitude.

2.6. Amphetamine-Induced Locomotor Activity

Rat open field activity monitors (43.2 × 43.2 cm, Med Associates, St. Albans, VT, USA) were used for locomotor activity and amphetamine sensitivity experiments. The following parameters were recorded for the entire 3 hour test duration: horizontal activity (horizontal photobeam breaks or counts), number of stereotypical movements (repeated photobeam breaks), and vertical activity (vertical photobeam breaks). Thus, spontaneous locomotor activity, exploratory activity (rearing and sniffing movements) and stereotypical movements were assessed. Rats were habituated in the test chambers for 60 min allowing for analysis of both locomotor activity and habituation when exposed to a novel environment. Rats were then injected subcutaneously (s.c.) with 1.5 mg/kg amphetamine and monitored for an additional 120 min to determine locomotor activity sensitivity to the dopaminergic psychostimulant.

2.7. Social Interaction Test

To test social interaction of control and prenatally stressed rats, a black plastic (rectangular) open field box (65.5 × 41.0 × 36.5 cm) with normal rodent bedding material (Sani-Chips®, Harlan Teklad Madison, WI) covering the floor was used in dim lighting (70 lux). The day before testing, experimental rats as well as their same sex, weight-matched target rats (no previous interaction with experimental rats) were separately placed into the open field box for a 20 min habituation. On the day of testing, experimental rats were placed into the open field box for a 10 min period. When the 10 min session ended, a same sex, weight-matched target rat was introduced into the arena for an additional 10 min. Experimental and target rats were not used in this paradigm more than one time. The box was cleaned with 70% ethanol between each trial. All sessions were recorded via mounted overhead cameras. Social interaction, including both social (e.g., sniffing, grooming, following, crawling over/

under) and aggressive (e.g., chasing, boxing, biting, offensive kicking, pinning, and wrestling) behaviors towards the target rat, were scored for the entire session. % aggression = time displaying aggressive behaviors/time displaying social + aggressive behaviors * 100.

2.8. Sucrose Consumption

The sucrose consumption test was used as a measure of anhedonia. PNS and control animals were given, for 24 hr, a free choice between two bottles, one with a 1% sucrose solution and another with water. To prevent effects of side preference in drinking behavior, the position of the bottles was switched after 12 hr. No previous food or water deprivation was applied before the test. At the end of 24 hr, the amount of water or solution left in the bottles was measured. The preference for sucrose was calculated as a percentage of consumed sucrose solution of the total amount of liquid consumed.

2.9. Morris Water Maze (MWM) Task

2.9.1. Test apparatus—Water maze experiments were conducted as described previously (Terry et al., 2006). Water maze tasks were performed in a circular pool (diameter: 180 cm, height: 76 cm) and filled to a depth of 35 cm of water (maintained at 25.0 ± 1.0 °C). The pool was located in a large room with a number of extra-maze visual cues including geometric images (e.g., squares, triangles, circles). Swimming activity of each rat was monitored via a mounted overhead camera and video tracking system (Noldus EthoVision® Pro 3.1).

2.9.2. Hidden platform task—An invisible (black) 10 cm × 10 cm square platform was submerged approximately 1.0 cm below the surface of the water and placed in the center of a quadrant. Rats were tested two trials per day with a one min intertrial interval for six consecutive days. A trial was initiated by placing the rat into one of the four quadrants, pseudo-randomized such that all four quadrants were used once every two training days. Rats were given a maximum swim time of 90 s to find the platform. When successful, the trial was terminated and the rat was allowed a 30 s rest period on the platform, if unable to locate the platform within the time period allocated the animal was physically placed on the platform for a 30 s rest period. Latency to locate platform and distance traveled were recorded.

2.9.3. Probe trials—Twenty-four hours following day six of the hidden platform trials, probe trials (90 s in duration) were conducted in which the platform was removed from the pool. The number of crossings over the previous platform location and the % of time animal spent in the target quadrant were measured to assess spatial bias.

2.9.4. Visible platform task—Following probe trials, a visible platform task was performed as a general estimate of visual acuity. A highly visible (white) 12 cm × 12 cm cover with a flag was attached to the platform, raising the surface approximately 1.0 cm above the surface of the water. Probe trials included four trials with a one min intertrial interval. The platform was moved on each trial so that all four quadrants were tested. A trial was initiated by placing the rat in the quadrant diametrically opposite to the platform quadrant with a time limit of 90 s to locate platform. The latency to locate the platform was recorded.

2.10. Radial Arm Maze (RAM) Task

2.10.1. Test apparatus—The offspring of stressed and control dams were tested using radial arm maze (RAM) procedures we have previously published (Terry et al., 2008). Med-Associates (MED-RAM-1R) computer-automated eight-arm radial mazes were used

consisting of a central octagonal hub (arena) with automatic guillotine doors connected to aluminum arms radiating distally (45.7 cm long). IR-photo beam sensors were positioned at the entrance to each runway, and a food pellet receptacle and head entry detector were positioned at the end of each runway. The maze was positioned approximately 90 cm above the floor in a testing room with a number of extramaze cues (composed of large geometrical shapes). Beginning on PND 56–70, animals were handled for one week to reduce stress and anxiety in preparation for training and testing. During the second week animals began food restriction to reduce weight to 80% of normal body weight. On the last two days of the second week, animals were given a 15 min free exploration (habituation) session with reinforcement food pellets scattered randomly throughout the entire maze area to acquaint the animals to the RAM apparatus.

2.10.2. Acquisition (win–shift training)—Following habituation, animals were trained in a win–shift procedure. Trial was initiated by placing the rat into the central octagonal arena. After a 30 s delay, all guillotine doors raised allowing access to all of the eight arms. When the animal broke a photobeam in the pellet receptacle at the end of each runway, a reward pellet was delivered. When the rat moved back into the central arena all doors closed for 5 s and then reopened. All reentries into an arm that previously delivered a reward were scored as working memory errors. All rats were trained to meet the criterion of four consecutive days with two or less errors per day.

2.10.3. Delayed Non-Match to Position (DNMTP)—Once test animals reached win–shift criterion, DNMTP testing was initiated. Animals began testing with an information (forced 4) session in which four of the eight arms (pseudo-randomly chosen) were blocked (i.e., the animal could only investigate the four remaining open arms). This information session ended when all four arms were visited or when the trial timed out (15 min). After a predetermined delay interval, all eight arms were accessible during the retention (free 8) session. Food reinforcement occurred only in arms not previously visited. The test session ended when all four correct arms were visited or when the trial timed out (15 min). Entry into arms previously entered during the information session or repeat visits to any arm during the retention session was recorded as an error. Animals were trained at a 15 min delay interval to meet the criterion of four consecutive days with one or less errors per day. Once criteria were reached, animals were tested with longer delays of 1, 3, and 6 hours, presented twice in a pseudorandom fashion, along with 15 min delays intervals.

2.11. Spontaneous Novel Object Recognition (NOR) Test

The NOR task was conducted using a procedure we have previously published (Terry et al., 2007). The offspring of stressed and control dams were tested using an apparatus consisting of black plastic (rectangular) open field box (length $\times$ width $\times$ height = 65.5 $\times$ 41.0 $\times$ 36.5 cm, respectively) with normal rodent bedding material (Sani-Chips®, Harlan Teklad Madison, WI) covering the floor. The objects discriminated were made of glass, ceramic, clay, or plastic. Each object was available in four identical copies, and the weights were such that the rat could not move the objects. Objects varied in height from 9.5 to 15 cm and in width from 6 to 10 cm. Habituation to the test apparatus consisted of two daily 10 min sessions in which the animals were allowed to freely explore the open field box. Video-recorded OR testing began on the third day and ended on day five.

Each test day began with a 3 min (A/A) information session, in which each rat was placed in the box and exposed to two identical sample stimuli. The objects were placed near two adjacent corners (i.e., 10 cm from each wall) of the box and the rat was placed into the box equidistant from each object during a given test session. The (A/A) information session was followed by a 15 min, 1hr or 3 hr delay period (administered in a pseudorandom order for

the test subjects), during which time the animal was returned to its home cage. Subsequently, a 3 min dissimilar stimuli (A/B) session followed, in which the rat was placed into the box in the same location as in the previous session (bedding material on floor was rearranged to disrupt olfactory cues) and exposed to the familiar object (A) and a novel object (B). These objects were placed in the same locales as the two objects in the previous session. The total amount of time the animal spent with each object was recorded, where “time spent” was operationally defined as the animal directing its nose to the object at a distance of less than 2 cm and/or by the animal touching the object with its nose or mouth. The proportion of the total exploration time that the animal spent investigating the novel object was the index of recognition memory. A discrimination index (d2) was calculated on each A/B trial and was defined as the difference in time spent exploring the novel and familiar objects divided by the total exploration time for both objects: $d2 \text{ index} = (\text{novel} - \text{familiar}) / (\text{novel} + \text{familiar})$. This measure is considered an index of recognition memory and takes into account individual differences in the total amount of object exploration time.

2.12. Evaluation of Potential Therapeutic Interventions

Following behavioral performance assessments, both PNS and control animals were treated acutely with the second generation antipsychotic, risperidone (4-[2-[4-(6-fluorobenzo[d]isoxazol-3-yl)-1-piperidyl]ethyl]-3-methyl-2,6-diazabicyclo[4.4.0]deca-1,3-dien-5-one), to determine its ability to attenuate amphetamine-induced locomotor activity. A second cohort of animals were treated acutely with the norepinephrine reuptake inhibitor, atomoxetine ((3*R*)-*N*-methyl-3-(2-methylphenoxy)-3-phenylpropan-1-amine), or the partial α -7 nicotinic receptor agonist, DMXB-A (3-[(3*E*)-3-[(2,4-dimethoxyphenyl)methylidene]-5,6-dihydro-4*H*-pyridin-2-yl]pyridine]; code name GTS-21), to determine their ability to improve recognition memory during performance of the NOR task.

For amphetamine-induced locomotor activity, subjects were injected intraperitoneally (i.p.) with risperidone (0.3 mg/kg; A&A Pharmachem, Ottawa, Ontario Canada) or vehicle (0.1 M acetic acid in saline; pH = 5.0) 30 min prior to injection of 1.5 mg/kg amphetamine and monitored as previously described. The dose of risperidone was chosen based on efficacy with limited possibility of side effects such as sedation (Ahnaou et al., 2003; Natesan et al., 2008; Batool et al., 2010).

For atomoxetine NOR studies, animals were injected (i.p.) with vehicle (0.01 mol/l PBS), 1, or 3 mg/kg atomoxetine hydrochloride (Pfizer, Ann Arbor, MI) 30 min prior to testing of the (A/A) information session followed by a 3 hr delay period. Doses were chosen based on efficacy in behavioral experiments in a previously published study (Wilson et al., 2012). Atomoxetine and vehicle were administered in a pseudorandom manner to obviate any effects associated with the order of drug administration.

For GTS-21 NOR studies, animals were injected (i.p.) with vehicle (0.9% NaCl), 1, or 3 mg/kg GTS-21 (Memory Pharmaceuticals, Montvale, NJ) 30 min prior to testing of the (A/A) information session followed by a 3 hr delay period. Doses were chosen based on efficacy in previously published studies (Arendash et al., 1995; Nanri et al., 1998). GTS-21 and vehicle were also administered in a pseudorandom manner.

2.13. Statistics

Study results are expressed as means $\pm$ standard error of the mean (SEM) of the individual values of rats from each group. For single factor comparisons between the study groups, unpaired two-tailed t-tests or Mann-Whitney sum tests were performed. Statistical comparisons of groups with two-factors were conducted using a two way repeated measures ANOVA followed by post hoc analysis using the Student–Newman–Keuls multiple

comparison method (SigmaStat 2.03, SPSS Inc., Chicago, IL, USA). Statistical comparison of groups with three-factors were conducting using a three way repeated measures ANOVA followed by post hoc analysis with Bonferroni correction (SAS, JMP statistical software, Cary, NC, USA). Statistical significance was assessed using an alpha level of 0.05. Trends toward significance were considered at the $p < 0.10$. When gender differences were not statistically significant, analyses were collapsed across gender.

3. Results

3.1. Litter Characteristics

Table 3 illustrates the effects of variable PNS on litter characteristics. Exposure to prenatal stress did not significantly affect the litter size or the female to male ratio of the litters, however, it did result in a significantly lower weights for both females (Mann-Whitney $U = 873$, $n_1=n_2=48$, $p<0.05$) and males (Mann-Whitney $U = 866$, $n_1=n_2=48$, $p<0.05$) at weaning, postnatal day 22. However, by postnatal day 56, when behavioral testing began, there was no longer a significant difference between weights of the PNS and control animals in either the female or male groups. Exposure to the prenatal stress procedure did not result in changes in the number of live born pups or the latency to parturition (data not shown).

3.2. Motor Function

3.2.1. Grip Strength—Statistical analysis of forelimb grip strength revealed a significant effect of the day of testing $F(2,71) = 24.07$, $p<0.001$, without a main effect of group $F(1,44) = 0.19$, $p=0.67$ or a group by day interaction $F(2,71) = 0.40$, $p=0.67$. Post hoc analysis indicated that grip strength increased in both groups on day 2 compared to the first day of testing, however there were no significant differences between PNS and control rats (data not shown).

3.2.2. Rotarod—Statistical analysis of performance of the rotarod task similarly revealed a significant effect of the day of testing $F(2,71) = 10.82$, $p<0.001$, without a main effect of group $F(1,44) = 1.03$, $p=0.32$ or a group by day interaction $F(2,71) = 0.81$, $p=0.45$. Post hoc analysis indicated that performance on the rotarod improved in both groups on day 2 compared to the first day of testing, however there were no significant differences between PNS and control rats (data not shown).

3.3. Prepulse Inhibition

Fig. 1 illustrates the effects of variable PNS on startle responses during a prepulse inhibition task (PPI). Statistical analysis of % prepulse inhibition at 75, 80, and 85 dB prepulse levels (i.e., 5, 10, and 15 db above background white noise) (Fig. 1A) indicated a significant effect of prepulse levels $F(2,143) = 52.75$, $p<0.001$, without a main effect of group $F(1,90) = 0.46$, $p=0.50$ or a group by prepulse level interaction $F(2,143) = 0.63$, $p=0.54$. Post hoc analysis indicated a significant reduction in startle response that was dependent on the magnitude of the prepulse stimulus. There were no significant differences between PNS and control animals on startle amplitude (Fig. 1B) or overall % prepulse inhibition (i.e., averaged across prepulse level, Fig. 1C). In a second cohort of test subjects, prepulse levels of 73, 76, and 82 dB (i.e., 3, 6, and 12 dB above background white noise) were evaluated and similar results were obtained (i.e., no differences between PNS subjects and non-stressed controls, data not shown).

3.4. Amphetamine-Induced Locomotor Activity

Fig. 2 illustrates the effects of variable PNS on amphetamine-induced locomotor activity. Statistical analysis of ambulatory counts (spontaneous locomotor activity) (Fig. 2A)

revealed highly significant main effect of group $F(1,560) = 16.88$, $p < 0.001$, effect of time period $F(35,647) = 22.38$, $p < 0.001$, and a group by time period interaction $F(35,647) = 2.61$, $p < 0.001$. Post hoc analysis indicated that locomotor activity decreased in both groups as a function of time during the 60 min habituation period. Moreover, after injection with amphetamine (1.5 mg/kg), there was a significant increase in locomotor activity in both groups compared to baseline that continued until the last 20 min of recording. Post hoc analysis also indicated that exposure to PNS was associated with higher levels of locomotor activity (compared to non-stressed controls) during the first 40 min of habituation, as well as during the first 90 min after injection with amphetamine. Statistical analysis of total ambulatory counts (averaged across time), further supported the observation that exposure to PNS in rats (compared to non-stressed control animals) resulted in elevated locomotor activity, both during the habituation phase (Fig. 2B) to the novel environment ($t = 4.56$, $df = 16$, $p < 0.001$) and following the amphetamine injection (Fig. 2C) exposure ($t = 3.97$, $df = 16$, $p < 0.001$).

Statistical analysis also indicated that PNS animals had a significant increase in stereotypic-like behavior (i.e., elevated stereotypic counts) during both habituation (Fig. 2D) (Mann-Whitney $U = 0$, $n_1 = n_2 = 9$, $p < 0.001$) and following injection with amphetamine (Fig. 2E) ($t = 4.66$, $df = 16$, $p < 0.001$) compared to control animals. There was also a significant decrease in vertical activity (vertical counts) in PNS animals compared to controls ($t = 4.66$, $df = 16$, $p < 0.001$) during habituation, but there were no differences between the two groups following amphetamine exposure (data not shown).

3.5. Social Interaction

Fig. 3 illustrates the effects of variable PNS on social interaction with same sex/aged matched rats as well as same sex juveniles. Analysis of social interaction with same sex/aged matched target rats (Fig. 3A) revealed a significant effect of the time period $F(3,95) = 227.90$, $p < 0.001$, without a main effect of group $F(1,66) = 0.14$, $p = 0.71$ or group by time period interaction $F(3,95) = 0.52$, $p = 0.67$. Post hoc analysis indicated a significant reduction in social interaction as the 10 min time period progressed in both groups, however there were no differences between PNS and control animals. Interestingly however, PNS subjects exhibited significantly increased aggressive behaviors (Fig. 3B) compared to controls ($t = 4.33$, $df = 22$, $p < 0.001$) toward the gender and aged-matched target subjects.

Statistical analysis of social interaction with same sex juveniles (Fig. 3C) also revealed a significant effect of the time period $F(3,95) = 185.21$, $p < 0.001$, without a main effect of group $F(1,66) = 0.03$, $p = 0.87$ or a group by time period interaction $F(3,95) = 0.21$, $p = 0.88$. Similar to the results with age and gender-matched target rats, post hoc analysis showed a significant reduction in social interaction over time for both groups, but no differences between PNS and control animals in their social interaction with juveniles. As in the case of interactions with adult target rats (described above), exposure to PNS (compared to controls) significantly increased aggressive behaviors (Fig. 3D) toward juvenile target rats ($t = 5.43$, $df = 22$, $p < 0.001$).

3.6. Sucrose Consumption

There were no statistically significant differences in sucrose preference between PNS and control animals. Both groups had an average sucrose preference [(sucrose consumed / sucrose + water consumed * 100) over a 24 hr period] of 87% (data not shown). Further, there were no differences between the total volumes of liquids consumed during the 24 hr period.

3.7. Morris Water Maze Task

Fig. 4 illustrates effects of PNS on performance of the Morris Water Maze (MWM) task. The following statistical results were obtained from analysis of platform latencies (s) (Fig. 4A) revealed a significant effect of the day of testing $F(5,143) = 12.26$, $p < 0.001$, without a main effect of group $F(1,110) = 0.11$, $p = 0.75$ or a group by day interaction $F(5,143) = 0.80$, $p = 0.55$. Post hoc analysis revealed that both groups had increasing levels of efficiency to locate the hidden platform over the course of the six days of testing of the hidden platform task as indicated by the decreasing slope of the acquisition curves. There were no significant differences between PNS and control animals in latencies to locate the platform, however. Analysis of swim speeds (Fig. 4B) revealed no significant main effect of group, effect of the day of testing, or group by day interaction. Interestingly, in the probe trials analysis, exposure to PNS was actually associated with an increase in the % of time spent in the target quadrant (Fig. 4C) ($t = -2.47$, $df = 22$, $p < 0.05$) and in the number of crossings over the previous platform location (Fig. 4D) (Mann-Whitney $U = 35$, $n_1 = n_2 = 12$, $p < 0.05$) compared to controls. There were no significant differences in platform latencies of PNS and control animals during visible platform tests.

3.8. Radial Arm Maze (RAM)

Fig. 5 illustrates the effect of variable PNS on performance of a radial arm maze (RAM) task. In the win-shift task (Fig. 5A), statistical analysis of the % correct of the first 8 arms entered over 10 days of training revealed a significant effect of the day of testing $F(9,479) = 27.24$, $p < 0.001$, without a main effect of group $F(1,414) = 0.17$, $p = 0.69$ or a group by day interaction $F(9,479) = 0.55$, $p = 0.84$. Similar results were obtained from the analysis of the number of errors per session (Fig. 5B) over 10 days of win-shift training, effect of day $F(9,479) = 29.83$, $p < 0.001$, group $F(1,414) = 0.17$, $p = 0.69$, group by day interaction $F(9,479) = 0.52$, $p = 0.86$. Post hoc analysis indicated that significant learning occurred in both groups over the 10-day training period (i.e., increase in % correct and decrease number of errors). There were no significant differences in the number of sessions it took PNS and control animals to meet the predetermined training criteria (2 errors per session) during win-shift training.

In the delayed non-match to position (DNMTP) training sessions in the RAM (Fig. 5C), analysis of the % correct of first 4 arms entered (15 min delay intervals) revealed a significant effect of the day of testing $F(9,479) = 5.50$, $p < 0.001$, without a main effect of group $F(1,414) = 0.37$, $p = 0.54$ or a group by day interaction $F(9,479) = 0.39$, $p = 0.94$. Similar (non-significant results) were obtained from the analysis of the number of errors per session (data not shown) over 10 days of DNMTP training. Similar to the win-shift task, post hoc analysis also indicated that significant learning occurred in both groups as indicated by a significant increase in % correct and a significant decrease in the number of errors over the 10 days of DNMTP training. Further, the number of sessions required to meet the predetermined training criteria (1 error per day; data not shown) were not significantly different between the groups. In addition, statistical analysis of % correct of the first 4 arms entered (Fig. 5D) during DNMTP at 15 min, 1 hr, 3 hr, and 6 hr delay intervals revealed a main effect of delay $F(3,183) = 27.13$, $p < 0.001$, without a main effect of group $F(1,132) = 0.81$, $p = 0.73$ or group by delay interaction $F(3,183) = 0.93$, $p = 0.43$. Post hoc analysis indicated a significant decrease in % correct as the delay intervals were increased. This was further supported as statistical analysis of the number of errors per session (data not shown) revealed a significant effect of delay $F(3,183) = 28.06$, $p < 0.001$, without a main effect of group $F(1,132) = 0.15$, $p = 0.70$ or group by delay interaction $F(3,183) = 1.06$, $p = 0.37$.

3.9. Spontaneous Novel Object Recognition Test (NOR)

Fig. 6 illustrates the effects of variable PNS on performance of a novel object recognition (NOR) task across 3 different delay intervals, 15 min (Fig. 6A), 1 hr (Fig. 6B), and 3 hr (Fig. 6C). The effects of group (i.e., PNS versus non-stressed controls), object type (familiar versus novel), delay (15 min, 1 hr, 3 hr) and all crosswise interactions were assessed. Statistical analysis revealed a significant effect of the object type $F(1,220) = 135.28$, $p < 0.001$, delay $F(2,220) = 8.46$, $p < 0.001$ and a significant group by delay interaction $F(6,220) = 2.35$, $p < 0.05$; however there was no indication of a main effect of group $F(3,220) = 1.06$, $p = 0.19$, group by object type interaction $F(3,220) = 1.51$, $p = 0.21$ or group by object by delay interaction $F(6,220) = 1.62$, $p = 0.14$. Post hoc analysis indicated a significant preference for the novel object at all delays for both control males and females. However, while both PNS males and females showed a preference for the novel object at the 15 min delay, the preference was lost in the PNS males at the 3 hr delay and PNS females at both 1 hr and 3 hr delay intervals. Statistical analysis of discrimination indices (d_2 , see formula in the Methods section; see insets to Fig. 6A, B, and C) revealed a significant main effect of group $F(3,88) = 2.93$, $p < 0.05$, delay $F(2,143) = 10.16$, $p < 0.001$, and a group by delay interaction $F(2,143) = 2.42$, $p < 0.05$. Post hoc analysis indicated that PNS was associated with a significant decrease in discrimination index at the 3 hr delay in males, and at both 1 hr and 3 hr delays in females. There were no significant differences in total exploration between the information (A/A) and test (A/B) session in either groups, both groups had similar total exploration times (data not shown).

3.10. Evaluation of Potential Therapeutic Interventions

3.10.1. Effects of Risperidone on Amphetamine-Induced Locomotor Activity—

Fig. 7 illustrates the effects of risperidone (0.3 mg/kg), on amphetamine-induced locomotor activity. Similar to the locomotor activity results reported above, in this later series of experiments, statistical analysis of ambulatory counts (Fig. 7A) revealed a significant main effect of group $F(3,1120) = 16.09$, $p < 0.001$, significant effect of the time period $F(35,1295) = 37.51$, $p < 0.001$, and a significant group by time period interaction $F(35,1295) = 4.68$, $p < 0.001$. Post hoc analysis revealed that while the PNS groups were significantly more active during the first 20 min of testing, there was a significant reduction in ambulatory counts in all groups over the course of the 60 min habituation period. Further, there was a significant increase in ambulatory counts for approximately 90 min following treatment with amphetamine (1.5 mg/kg) in PNS and controls animals receiving vehicle. Interestingly, in the PNS subjects, the increase in ambulatory counts induced by amphetamine animals was delayed by risperidone for approximately 35 min, but not sustained afterwards. Risperidone was effective in attenuating the effects of amphetamine over the full 90 min in the control subjects; however risperidone was effective only temporarily (i.e., 35 min) on attenuating the effects of amphetamine in PNS animals.

Statistical analysis of total ambulatory counts (averaged across the experiment) following treatment with amphetamine (Fig. 7B) revealed a significant main effect of group $F(1,32) = 34.83$, $p < 0.001$ and a significant effect of dose $F(1,35) = 4.62$, $p < 0.05$, without a group by dose interaction $F(1,35) = 1.44$, $p = 0.24$. Post hoc analysis indicated that exposure to PNS resulted in increased amphetamine-induced locomotor activity, whether treated with vehicle or risperidone, compared to controls. Additionally, treatment with risperidone was associated with a reduction in ambulatory counts in control animals, but not in PNS animals. Statistical analysis of total vertical counts (Fig. 7C) following treatment with amphetamine indicated a significant main effect of group $F(1,32) = 2.66$, $p = 0.11$ and a significant effect of dose $F(1,35) = 5.53$, $p < 0.05$, without a group by dose interaction $F(1,35) = 0.04$, $p = 0.84$. Post hoc analysis revealed a significant reduction in vertical activity in both PNS and control animals treated with risperidone, compared to vehicle treated animals. Similar to the

aforementioned experiments described above, statistical analysis of stereotypic counts in this series of experiments revealed significant differences between PNS and control subjects, with a significant main effect of group $F(1,32) = 61.95$, $p < 0.001$, without an effect of dose $F(1,35) = 0.84$, $p = 0.37$ or a group by dose interaction $F(1,35) = 0.52$, $p = 0.48$. Post hoc analysis indicated that PNS animals had significantly higher stereotypic activity compared to controls, but the effect was not altered by risperidone (data not shown).

3.10.2. Effect of Atomoxetine and GTS-21 on NOR—Fig. 8 illustrates the effects of atomoxetine and GTS-21 on NOR performance (as indicated by the d2 index) after a 3 hr delay. In the case of atomoxetine (Fig. 8A) statistical analysis indicated a significant effect of dose $F(2,71) = 6.44$, $p < 0.05$ and a significant group by dose interaction $F(2,71) = 5.87$, $p < 0.05$, without a main effect of group $F(1,44) = 2.33$, $p = 0.14$. Post hoc analysis indicated that both doses of atomoxetine (compared to vehicle) were associated with significant improvements in the discrimination index in PNS animals, but not controls. In addition, in the between group comparisons (i.e., PNS subjects versus controls), the vehicle-associated performance was significantly different, however, PNS performance at each dose of atomoxetine was not significantly different from the control animals administered vehicle (i.e., further establishing that atomoxetine reverses NOR deficits following PNS).

The effects of GTS-21 on NOR in PNS and control subjects after a 3 hr delay are illustrated in Fig. 8B. In these experiments, statistical analysis revealed a significant main effect of group $F(1,44) = 22.2$, $p < 0.001$, without a significant effect of dose $F(2,71) = 0.29$, $p = 0.75$, or a group by dose interaction $F(2,71) = 0.45$, $p = 0.64$. Post hoc analysis indicated that PNS subjects had a significantly lower discrimination index compared to control, irrespective of treatment.

4. Discussion

The results of this study can be summarized as follows: exposure to variable PNS in rats was associated with: 1) increased locomotor activity and stereotypic behaviors, 2) elevated sensitivity to the psychostimulant amphetamine, 2) increased aggressive behaviors toward both adult and juvenile rats of the same gender and 3) delay-dependent deficits in recognition memory. There was no indication that PNS rats had deficits in other forms of motor function or motor learning (i.e., grip strength or rotarod performance), sensorimotor gating, spatial learning and memory, or that they exhibited social withdrawal or anhedonia (e.g., as would be indicated avoidance of other rats or a decrease in sucrose preference, respectively). In experiments designed to evaluate PNS in rats as a model system for assessing potential therapeutic interventions: 1) the second generation antipsychotic risperidone attenuated amphetamine-related increases in locomotor activity, however, the effect was not sustained over time, and 2) deficits in recognition memory were attenuated by the norepinephrine reuptake inhibitor, atomoxetine, but not by the $\alpha 7$ nicotinic acetylcholine receptor partial agonist, GTS-21.

In this study, a number of behavioral tasks often used to evaluate potential animal models of neuropsychiatric disorders (i.e., sensorimotor gating, sensitivity to psychostimulants, social interaction, anhedonia) were performed to determine the effects of PNS in rats. For instance, PNS rats were evaluated in the amphetamine-induced hyperlocomotion test which is commonly used as a pharmacological (i.e., hyperdopaminergic) model of psychosis in animals (Sharp et al., 1987; Geyer and Ellenbroek, 2003). Amphetamine (a psychomotor stimulant), is known to exacerbate the symptoms of schizophrenia, as well as induce paranoid-hallucinatory psychosis when given in high doses in normal patients (Yui et al., 1999). In the current study, acute treatment with amphetamine (1.5 mg/kg) produced hyperlocomotion and increased stereotypic behaviors in both control and PNS rats.

However, the magnitude of the amphetamine effect in PNS subjects was considerably higher than in controls. These results of this study are similar to those previously published by Koenig and colleagues, although their study lacked measures of vertical and stereotypic activity (Koenig et al., 2005). The elevations in stereotypic movements in PNS subjects (compared to controls) in the current study is important to note given that stereotypies (defined as repetitive and functionless motor behaviors) are a common feature of neuropsychiatric disorders including schizophrenia and autism (see Garner et al., 2003; Morrens et al., 2006)

Prepulse inhibition (PPI) experiments were conducted in this study for the purpose of evaluating sensorimotor gating in PNS rats. Deficits in PPI are often observed in schizophrenia patients as well as in schizophrenia-related animal models (reviewed, Wilson and Terry, 2010). PPI (i.e., the reduction in startle response produced by a low-intensity stimulus presented before a high-intensity, startle-producing stimulus; see Graham et al., 1975) has been widely used as a neurophysiological measure of the pre-attentive stages of information processing (Braff and Geyer, 1990). It has been suggested that the sensory gating deficits associated with neuropsychiatric disorders may contribute to the deficits in attention as well as the positive symptoms (e.g., hallucinations) often exhibited by patients. Surprisingly, in the present study, there were no impairments in PPI associated with exposure to variable PNS. These results contrast to those previously published by Koenig et al., 2005, who found that exposure to variable PNS resulted in PPI deficits at 1 and 3 dB above background. The basis for the different results of the two studies is unclear. The age of the PNS animals in this study (PND 61 and PND 68) were similar to those in Koenig's study (PND 54) during performance of the PPI task, thus it is unlikely that the difference between the two studies was due to the age of test subjects. We did evaluate prepulse levels as low as 3 dB above baseline in our studies, but have not been able to reliably observe PPI in rats with lower prepulse levels using SR labs instrumentation in our lab; therefore it is possible that exposure to PNS results in very modest effects on PPI that could not be detected in our experiments. It is also important to note that there were considerable differences between the two protocols used. Most noteworthy was the fact that in our study, animals were exposed to the apparatus for two days of acclimation prior to testing, while it appears Koenig's group only allowed for a five minute acclimation period immediately prior to testing.

A decrease in social interaction with other rodents has been interpreted as a behavioral deficit that mimics the avoidance behaviors observed in some neuropsychiatric disorders (Sams-Dodd, 1999). It was previously reported (Lee et al., 2007) that male rats exposed to variable PNS interacted with a weight-matched, same-sex peer rats much less than non-stressed controls (i.e., evidence of social withdrawal). In the current study, there was no indication of significant differences in the amount of time male rats spent socially interacting with weight/age-matched or juvenile target rats. Interestingly though, the percentage of time (during the social interactions) that PNS rats displayed aggressive behaviors toward age-matched and juvenile target rats (e.g., chasing, biting, pinning) were significantly increased (compared to controls). It is important to note, that aggressive behaviors are evident in patients diagnosed with several neuropsychiatric disorders such as attention deficit/hyperactivity disorder (ADHD), borderline personality disorder, and schizophrenia (King and Waschbusch, 2010; Látalová and Prasko, 2010; Soyka, 2011). The cause for disparity among the two studies is unknown, although one factor may include differences in the lighting conditions (i.e., 70 lux in the current study, 150 lux in Lee's study) which are well known to effect behavioral responses in rats. Furthermore in Lee's study, behaviors were only recorded as aggressive if the behavior led to injury (e.g., biting to injury). While the results of the two studies may not parallel each other, both studies provide evidence that exposure to PNS results in altered social interactions.

A decrease of sucrose intake when both sucrose and water are available to the animal has often been interpreted as an anhedonia-like behavior (Katz, 1982). Exposure to PNS did not alter sucrose preference over a 24 hr period in the current study. Further, the measure of sucrose preference was not influenced by variations in total liquid intake as there were no significant differences between PNS and control animals in the total amount of liquid consumed. This would suggest that PNS does not result in anhedonic-like behaviors, although there have been a few studies that suggest that saccharin/sucrose intake may not be a consistent measure anhedonia (see Hatcher et al., 1997; Willner, 1997).

As previously discussed in the introduction, cognitive dysfunction is a common feature of neuropsychiatric disorders and it is thought to play a central role in the quality of life of the patient, yet few viable treatment options are available. Animal models exhibiting deficits in one or more of the relevant domains of cognition would be useful for evaluating potential treatments. In this study, a number of behavioral tasks often used to evaluate the cognitive domains associated with neuropsychiatric disorders (i.e., MWM, RAM, and NOR) were also conducted to determine the effects of PNS on cognition. Accordingly, in the current study, we employed the fixed platform version of the Morris water maze (MWM) task (Morris, 1981), to assess spatial reference learning and memory and the radial arm maze (RAM) task (Olton and Papas, 1979) to assess spatial working and short term memory (with the win-shift and delayed non-match to position tasks, respectively). Surprisingly, exposure to PNS did not alter the acquisition of the hidden platform task in the MWM or the win-shift task in the RAM. Likewise, the delayed non-match to position task was unaffected by PNS.

Interestingly, however, PNS rats demonstrated superior performance in the MWM probe trials compared to control rats as indicated by a significant increase in the amount of time spent in the previous target quadrant and an increase in number of platform area crossings. These results could be interpreted as either superior spatial recall or enhanced perseveration (Young et al., 2009) in PNS subjects. Interestingly, a previous study found that exposure to prenatal restraint stress resulted in improved performance of the MWM in female rats, suggesting that exposure to stress during early developmental stages may enhance spatial learning and memory in rats (Zuena et al., 2008). While it is possible that exposure to PNS may in some manner aid the animals in adapting to the stressful environment of the MWM in the current study, other than in probe trials, there were no indications of improved performance of any of the other measures of MWM or of the RAM task. Additionally, we previously reported that exposure to variable PNS resulted in behaviors associated with increased perseveration during a 5-choice serial reaction time task, therefore we would argue that the enhanced performance during probe trials more likely reflects an increase in perseveration than enhanced spatial recall (Wilson et al., 2012). The MWM findings are in contrast to a previous study (Markham et al., 2010) where exposure to variable PNS resulted in deficits of spatial learning in male rats; although the differences between the two studies may be due to differences in the testing protocol (i.e., in the current study rats were tested with two trials per day for six consecutive days, using four quadrants with a maximum swim time of 90 s to find the platform with no periods of habituation to the apparatus, while in the Markham study rats were tested with four trial per day for three consecutive days, using three quadrants with a maximum swim time of 120 s with two days of habituation to the apparatus prior to testing).

We employed the spontaneous novel object recognition (NOR) procedure as a non-spatial recognition memory task that exploits the animals natural desire to explore novel objects (Ennaceur and Delacour, 1988). Recognition memory consists of two components, a recollective (episodic) component and a familiarity component (Squire, 2004). Importantly, several neuropsychiatric disorders (e.g., post-traumatic stress disorder, schizophrenia and major depression) are associated with episodic memory deficits (Dere et al., 2010). Exposure to PNS resulted in deficits in recognition indices at the 3 hr delay (but not the 15

min delay) in both female and male rats, suggesting that PNS results in deficits in recognition memory that are delay dependent. Further, PNS females also exhibited significant deficits at the 1 hr delay, suggesting that female rats may be more sensitive to the PNS-associated deficits in recognition memory. Interestingly, previous reports suggest that stress-related disorders are often more prevalent in women than in men (Weissman et al., 1996). Additionally, there have been several reports that female rats may be more sensitive to stress induced cognitive deficits and that estrogen may play an essential role in this sensitivity (Toufexis et al., 2007; Shansky et al., 2010). Our NOR results in male rats exposed to PNS support those of a previously published study (Markham et al., 2010).

In the final phase of experiments, we evaluated the sensitivity of the PNS model to therapeutic interventions (i.e., to compounds that are either currently used for the treatment of neuropsychiatric disorders or that have shown potential in these illnesses) to begin assessing the predictive validity of the PNS model. Drugs were evaluated for effects in the tasks where differences in performance were observed between PNS and control subjects. Specifically, the second generation antipsychotic (i.e. risperidone), was evaluated in the amphetamine-locomotor activity assay and the norepinephrine reuptake inhibitor atomoxetine and a $\alpha 7$ nicotinic acetylcholine receptor partial agonist GTS-21 were evaluated in the NOR task.

In the amphetamine-locomotor activity experiments risperidone was evaluated since it is a second generation antipsychotic that is commonly used for the treatment of schizophrenia, as well as other neuropsychiatric disorders such as bipolar disorder and ADHD (Zupancic, 2011; Seida et al., 2012; Bryois, 1994). As expected, risperidone (0.3 mg/kg) fully attenuated the hyperlocomotion associated with amphetamine in the control rats over the course of the experiment; however, in PNS rats the effects were not sustained. The basis of this differential drug response is unclear, but could be related to dose. Amphetamine is known to increase the release of dopamine in the striatum, which is the proposed mechanism of hyperlocomotion in rats (Sharp et al., 1987; Laruelle et al., 1999). As PNS animals have much higher levels of locomotor activity in response to amphetamine compared to controls, one could hypothesize that the levels of dopamine released may also be significantly higher in PNS animals (thus requiring a higher dose of risperidone to block the effect). To support this argument, several studies of prenatal stress exposure (i.e., maternal restraint stress, prenatal corticosterone exposure, prenatal dexamethasone exposure) reported significantly higher basal and amphetamine-stimulated extracellular dopamine striatal output in adolescent and young adult rats compared to controls, as well as elevated locomotor activity (Diaz et al., 1995; Muneoka et al., 1997; Silvagni et al., 2008). The exact mechanism by which PNS results in increased basal dopamine levels is unknown; however elevated glucocorticoid levels observed in PNS rats may be responsible as it was previously found that corticosterone peripherally administered to rats in a dose selected to approximate stress-induced plasma concentrations, increased extracellular dopamine and stimulated locomotor activity (Piazza et al., 1996). As risperidone has a relatively long half life ($t_{1/2}$ = 13.3 hr) in brain tissue of rats, it is unlikely that the pharmacokinetics of risperidone were responsible for the lack of sustained effects in these animals (Avaragiri and Marder, 2002).

Atomoxetine, which inhibits the norepinephrine transporter, is the first marketed non-stimulant ADHD medication. Atomoxetine has been shown to enhance attention and to attenuate impulsivity in both children and adults with ADHD as well as patients diagnosed with autism, bipolar disorder, and depression (Michelson et al., 2003; Chamberlain et al., 2007; Bangs et al., 2007; Chang et al., 2009; Zeiner et al., 2011). Animal studies have shown that the brain norepinephrine system is rapidly activated by stressors resulting in an increase in norepinephrine release in several brain regions (e.g., PFC, hippocampus) and attenuation of this elevated neurotransmission may be essential for optimum behavioral

function. Importantly, cognitive function is enhanced by moderate increases in the levels of norepinephrine, while higher levels or depletion leads to cognition impairment (Ramos and Arnsten, 2007). In other studies, dysregulation of norepinephrine neurotransmission has been associated with deficits in attention and memory as well as increased aggressive behaviors (Comai et al., 2012). In a previous study, we found that acute treatment with atomoxetine (0.3-0.3 mg/kg) attenuated deficits of attention and inhibitory control associated with PNS during a 5-choice serial reaction time (Wilson et al., 2012). In the current study, atomoxetine (1.0–3.0 mg/kg) was found to significantly improve recognition memory the PNS animals. These results suggest a new (i.e. previously unpublished) indication for atomoxetine (recognition memory deficits) and that the PNS rat model is useful for evaluating norepinephrine reuptake inhibitors for such an indication.

We evaluated the $\alpha 7$ nicotinic partial agonist, GTS-21, in the PNS rat model due to the well-established role of $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChRs) as therapeutic targets for the cognitive dysfunction of schizophrenia (Leonard et al., 1996; Leiser et al., 2009). It is relatively well established that $\alpha 7$ nAChRs play an important role in cognitive function (e.g., attention, working memory) and they are known to be regulated by corticosteroids (Leiser et al., 2009). GTS-21, also known as DMXBA, has been found to improve learning and memory in a number of behavior tasks in rats. (Arendash et al, 2005; Meyer et al., 1997). In addition, GTS-21 was also found to have positive effects on cognition in a small (N=12) proof-of-concept clinical trial in patients with schizophrenia (Olincy et al., 2006). However, in a larger Phase 2 clinical trial (N=31) performance on the six domains of the MATRICS Consensus Cognitive Battery did not differ between placebo and GTS-21 (Freedman et al., 2008). In the present study, GTS-21 had no significant effect on PNS-associated deficits in recognition memory at either dose administered (1.0 and 3.0 mg/kg). This suggests that the deficits in recognition memory associated with exposure to variable PNS may not be related to alterations of $\alpha 7$ nicotinic receptors.

As noted in the preceding paragraphs, there were both similarities (elevated amphetamine-induced locomotor activity and deficits in recognition memory) and differences (lack of deficits in sensorimotor gating, social withdrawal and spatial learning and memory) in our behavioral results in PNS rats compared to those of other investigators (Koenig et al., 2005; Lee et al., 2007; Markham et al., 2010). While it is possible that variations in the behavioral test protocols, environmental factors, amount of handling, etc., may have contributed to the disparate findings; variations may also be due to what has been described as “substrain” effects. We obtained timed-pregnant rats from a different vendor than the investigators cited above (Charles River Laboratories, Wilmington, MA, versus Harlan Sprague-Dawley Indianapolis, IN). Interestingly, Sprague-Dawley rats from these two vendors were previously found to have different hypertensive responses to chronic NO synthase (Pollock and Rekito, 1998). Further, it was previously found that two sources of outbred Sprague-Dawley rats had differential sensitivity to the adrenocorticotrophic hormone (Matthys et al, 1998). Thus, exposure to variable PNS may differentially affect “substrains” of Sprague-Dawley. It is important to note that it has previously been reported that exposure to variable PNS produces different outcomes in several strains of rats (Neeley et al, 2011).

There were additional (non- behavioral) observations in this study that are important to note. In previous studies, exposure to prenatal stress has often been associated with adverse birth outcomes such as preterm delivery of offspring and low birth weight, both in humans and rodents (Wadhwa et al., 1993; Weinstock, 2005; Class et al., 2011). In the current study, exposure to variable PNS did not result in preterm labor or an increase in stillborn pups. Further, there was no indication that PNS affected the female to male ratio or size of the litter. However, at postnatal day 22 (weaning), female and male rats exposed to PNS were significantly smaller compared to control rats, possibly due to low birth weights as there was

no indication of altered care by mother. In spite of this, by testing (postnatal day 56) there were no longer any differences in weight between the PNS and control animals. While low birth weight and prenatal stress exposure in rats have been associated with motor disabilities which could affect performance of behavioral tasks (Knoches and Doyle, 1993), there was no indication that exposure to variable PNS resulted in deficits in motor function (rotarod and grip strength) in this study. Therefore it is unlikely that PNS resulted in deficits in physical developmental that could have influenced behavioral results.

CONCLUSION

In conclusion, the results of this study suggest that exposure to variable PNS in rats results in elevated sensitivity to psychostimulants, increased aggressive behaviors, and deficits in recognition memory, and that some of the deficits are responsive to pharmacologic intervention. These observations combined with previous reports of impairments in attention, inhibitory control, fear conditioning and extinction (Wilson et al., 2012a; Wilson et al., 2012b) and other domains of cognition (see Koenig et al., 2007; Markham et al., 2010) suggest that important phenomenological similarities exist between prenatally stressed rats and patients afflicted with neuropsychiatric disorders. These observations further support the face validity of this model system for studying some aspects of these conditions as well for evaluating potential treatments (predictive validity).

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Abbreviations

ADHD	attention-deficit/hyperactivity disorder
DNMTP	delayed non-match to position
MWM	Morris water maze
NOR	novel object recognition
PNS	variable prenatal stress
PPI	pre-pulse inhibition
RAM	radial arm maze

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Highlights

- Prenatal stress leads to elevated locomotor activity
- Prenatal stress leads to increased aggression
- Prenatal stress leads to recognition memory deficits

Fig. 1. (A) Effects of exposure to PNS on the percentage of prepulse inhibition (PPI) in rats for three prepulse levels (5, 10, and 15 db above background noise). (B) PNS effects on the mean startle amplitude to a 120 db, 20 ms noise burst. (C) PNS effects on the percentage of PPI averaged across the three prepulse levels. Data are expressed as the mean $\pm$ SEM for each test group. PNS = prenatally stressed; CON = non-stressed controls. N = 24 per group.

Fig 2.

Effects of PNS on amphetamine-induced locomotor activity during an open field task. Illustrates (A) ambulatory counts (spontaneous locomotor activity) over a 3 hr time period. Animals were injected (i.p.) with 1.5 mg/kg amphetamine after 1 hour of habituation to the novel environment. (B) Total ambulatory counts during habituation and (C) amphetamine exposure. (D) Total stereotypic counts during habituation and (E) amphetamine exposure. Data are expressed as the mean $\pm$ SEM for each test group. * represents a significant difference ($p < 0.05$) between activity of PNS and control animals. PNS = prenatally stressed; CON = non-stressed controls. N = 9 per group.

Fig. 3.

Effects of exposure to PNS on social interaction. (A) The amount of time (s) that animals spent socially interacting with the same sex/weight matched target rats throughout a 10 min time period. (B) % time of social interaction that animals displayed aggressive behaviors (e.g., chasing, boxing, biting, offensive kicking, pinning, and wrestling) toward same sex/weight matched target rats. (C) The amount of time that animals spent socially interacting with the same sex juvenile target rat. (D) % time of social interaction that animals displayed aggressive behaviors toward same sex juvenile target rats. Data are expressed as the mean $\pm$ SEM for each test group. * represents a significant difference ($p < 0.05$) between PNS and control rats. PNS = prenatally stressed; CON = non-stressed controls. N = 12 per group.

Fig. 4.

Effects of PNS on performance of a Morris Water Maze (MWM) task. (A) Illustrates the latencies to platform (s) and (B) average swim speeds (cm/s) in the hidden platform test over six consecutive days of testing. (C) % time in target quadrant and (D) number of platform crossings during probe trails. (E) Latencies to platform during visible platform test. Data are expressed as the mean $\pm$ SEM for each test group. * represents a significant difference ($p < 0.05$) in performance between PNS and control animals. PNS = prenatally stressed; CON = non-stressed controls. N = 12 per group.

Fig. 5. Effects of PNS on performance of a radial arm maze (RAM) task. (A) Win-shift acquisition over 10 days of testing as assessed by the % correct of the first 8 arms entered and (B) number of errors per session. Inset = number of sessions animals took to reach criteria (2 errors per day) during win-shift training. (C) Delayed non-match to position (DNMT) acquisition over ten days of testing as assessed by % correct of the first 4 arms entered. (D) % correct of first 4 arms entered at 15 min, 1 hr, 3 hr, and 6 hr delay intervals. PNS = prenatally stressed; CON = non-stressed controls. N = 24 per group.

Fig. 6. Effects of PNS on the performance of a spontaneous novel object recognition task. The preference for the novel objects compared with the familiar objects is illustrated at the (A) 15 min, (B) 1 hr, and (C) 3 hr delay. Insets illustrate the “discrimination index” (d2), which refers to the proportion of the total exploration time the animal spent investigating the novel object [$d2 \text{ index} = (\text{novel} - \text{familiar}) / (\text{novel} + \text{familiar})$]. Data are expressed as the mean $\pm$ SEM for each test group. * represents a significant difference ($p < 0.05$) in preference for novel object. # represents a significant difference ($p < 0.05$) in indices compared to the 15 min delay performance. PNS = prenatally stressed; CON = non-stressed controls; M = males; F = females. N = 24 per group.

Fig. 7.

Effects of the atypical antipsychotic, risperidone, on amphetamine-induced locomotor activity. (A) Illustrates the effects of risperidone (0.3 mg/kg; given 30 min prior to amphetamine treatment) on amphetamine-induced (1.5mg/kg) ambulatory activity during an open field task. (B) Illustrates the effects of risperidone on total ambulatory counts and (C) total vertical counts for a 2 hr duration following acute treatment of amphetamine. Data are expressed as the mean $\pm$ SEM for each test group. † represents a significant ($p < 0.05$) reduction in ambulatory counts of PNS animals treated with risperidone compared to PNS animals treated with vehicle. # significantly different compared to vehicle-associated performance. * represents a significant difference ($p < 0.05$) between performance of PNS

and control animals. PNS (S) = prenatally stressed; CON (C) = non-stressed controls, VEH (V) = vehicle, RISP (R) = risperidone. N = 9 per group.

Fig. 8. Effects of the norepinephrine reuptake inhibitor atomoxetine (A) and the partial α -7 nicotinic receptor agonist GTS 21 (B) on performance of a NOR task as determined by the discrimination index (d2) [d2 index = (novel – familiar)/(novel + familiar)]. Data are expressed as the mean $\pm$ SEM for each test group. # significantly different ($p < 0.05$) compared to vehicle-associated performance. * represents a significant difference ($p < 0.05$) between performance of PNS and control animals. PNS = prenatally stressed; CON = non-stressed controls. N = 12 per group.

Table 1

Gestational Day	Time of Day		
	AM	Noon	PM
14	Restraint (1 hr)	Swim (15 min)	Restraint (1 hr)
15	Cold Exposure (6 hr)		Food Deprivation (Overnight)
16	Swim (15 min)	Restraint (1 hr)	Swim (15 min)
17		Swim (15 min)	Light/Dark Reversal (24 hr)
18	Social Stress (12 hr)		
19	Restraint (1 hr)	Swim (15 min)	Restraint (1 hr)
20			Cold Exposure (6 hr)
21	Swim (15 min)	Restraint (1 hr)	Swim (15 min)

Schedule of the various stressors applied for the variable prenatal stress paradigm. Table adapted from Koenig et al., 2005.

Table 2

Cohort	Litters	N (2 M & 2 F per litter)	Treatment	Days Task Conducted					
				PND 56–60	PND 61–67	PND 68–75	PND 76–82	PND 83–89	PND 90–96
1	S1, S2, S3 C1, C2, C3	N=12 N=12	Stress Control	Handled	Grip Strength, Rota Rod, Open Field	Object Recognition	Morris Water Maze	-	-
2	S4, S5, S6 C4, C5, C6	N=12 N=12	Stress Control	Handled	Social Interaction (Adults)	PrePulse Inhibition	-	Open Field w/Amphetamine	-
3	S7, S8, S9 C7, C8, C9	N=12 N=12	Stress Control	Handled	Food Restriction Began	Radial Arm Maze	Radial Arm Maze	Radial Arm Maze	Radial Arm Maze
4	S10, S11, S12 C10, C11, C12	N=12 N=12	Stress Control	Handled	Object Recognition	-	Social Interaction (Juveniles)	Sucrose Consumption	-
5	S13, S14, S15 C13, C14 C15	N=12 N=12	Stress Control	Handled	Food Restriction Began	Radial Arm Maze	Radial Arm Maze	Radial Arm Maze	Radial Arm Maze
6	S16, S17, S18 C16, C17, C18	N=12 N=12	Stress Control	Handled	Prepulse Inhibition	-	Open Field w/Amphetamine & Risperidone	-	-
7	S19, S20, S21 C19, C20, C21	N=12 N=12	Stress Control	Handled	Object Recognition Atomoxetine	-	-	Object Recognition GTS-21	-

Age (i.e., postnatal day; PND) of rats, assigned litters, and order of behavioral tasks. M = male; F = female.

Table 3

Group	Litter Size	Female to Male Ratio	Male Weight at Weaning (g)	Female Weight at Weaning (g)	Male Weight at Testing (g)	Female Weight at Testing (g)
CON	11.1 ± 0.6	1.09 ± 0.1	59.4 ± 2.1	54.4 ± 1.9	330.8 ± 6.8	211.9 ± 3.7
PNS	12.1 ± 0.7	1.05 ± 0.1	53.3 ± 2.2 *	49.4 ± 1.9 *	321.3 ± 6.4	207.9 ± 3.7

Effects of exposure to the prenatal stress paradigm on litter characteristics including; litter size, female to male ratio, weight at weaning (postnatal day 22), and weight at testing (postnatal day 56). Data are presented as the mean ± SEM.

* represents a significant difference between control (CON) and PNS animals ($p < 0.05$). Litter N = 21 per group, Pups N = 48 per group.