The Influence Of Prenatal Stress On Behaviors Associated With Schizophrenia And Autism Spectrum Disorder.

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THE INFLUENCE OF PRENATAL STRESS ON BEHAVIORS ASSOCIATED WITH SCHIZOPHRENIA AND AUTISM SPECTRUM DISORDER.

A Thesis Presented

by

Harold Bauerle

to

The Faculty of the Graduate College

of

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for the Degree of Master of Arts
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ABSTRACT

Disorders such as schizophrenia (SCZ) and autism spectrum disorder (ASD) have long been associated with prenatal stress. In these three experiments, we attempted to correlate stress during gestation with behaviors considered to have good facial validity with SCZ and ASD in both juvenile and adult animals. To differentiate the effects of prenatal stress (PS) from the effects of early life stress due to a dam’s behavior (MS), half of offspring animals were cross fostered to dams treated in the alternative condition as the offspring during pregnancy in experiments 2 and 3. In experiment 1, but not in 2 or 3, maternal animals that did not receive stress during pregnancy retrieved pups later than those that did. Our results in experiment 1 indicate that movement in a novel open field is dependent upon PS in a manner influenced by animal sex. In experiment 2, where cross fostering was considered, PS was a significant influence in females, while MS had considerable effect in males. Additionally, in males, animals treated by both PS and MS moved more than other male animals. Experiment 3 showed distinctions in male animals due to MS, but in startle amplitude, not open field movement. Overall, these experiments show the influence of PS and MS upon animals in juveniles and adults, but effects may be somewhat occluded due to litter effects.
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CHAPTER 1: INTRODUCTION

Stress during the gestational period in human beings has been implicated in numerous adult and childhood psychiatric disorders such as schizophrenia (SCZ) and autism spectrum disorders (ASD). An early Finnish study compared adult subjects whose fathers had died before their births to those whom had lost their fathers in the first year of life and it was found that in this sample, composed of births from 1925 to 1957, rates of SCZ were greater in those that suffered the prenatal stressor of paternal loss rather than the same loss during early life (Huttunen & Niskanen, 1978). This study also identified that the second trimester and the period immediately prior to parturition were especially sensitive to emotional stressors. Furthermore, unwanted pregnancies, based on maternal self-report in the sixth or seventh month of pregnancy, had a higher incidence rate for adult SCZ when compared to wanted or wanted-but-mistimed pregnancies in a study of a 1966 Finland birth cohort (Myhrman et al., 1996). Prenatal stress (PS) has further been associated with SCZ in humans based on longitudinal cohort studies, with rates of SCZ being greater in birth cohorts who experienced stressors such as the German invasion of the Netherlands (Os & Selten, 1998), and an influenza epidemic in Helsinki, Finland while in utero during their second trimester (Watson, Mednick, Huttunen, & Wang, 1999). A more recent Finnish study determined that a familial history of SCZ in association with prenatal urinary tract infection (UTI) in the mother was a significant factor in whether or not offspring would develop
SCZ, while UTI during pregnancy was not a factor in control families with no history of SCZ (Clarke et al., 2009). In yet another study using the Finnish national database, there was an association with poliomyelitis infection in the second trimester of pregnancy and the offspring later developing SCZ (Suvisaari et al., 1999). Considered together, these studies form a strong argument for the involvement of PS in the determination of whether or not an individual with pre-existing familial vulnerability will develop SCZ.

Examination of birth cohorts that either experienced traumatic events or not during gestation determined that ASD had a dose-dependent relationship with the degree to which mothers were exposed to and affected by hurricanes or other extreme weather, with the prevalence being greatest when the exposure was in the later end of gestation (Kinney et al., 2008). A study that surveyed the mothers of children diagnosed with ASD and Down’s syndrome for stressors during pregnancy found that stressors during weeks 21-32 were associated with autism, with a peak at weeks 25-28, the end of the second trimester (Beversdorf et al., 2005). A very recent longitudinal study that examined maternal stress levels based on recent life events in a group of 2,900 pregnant women determined that this stress during gestation could explain about 1% of the variability in whether or not a child would develop ASD (Ronald, Pennell, & Whitehouse, 2011). However, a Danish study examined birth cohorts from 1978 to 2003 for incidences of maternal bereavement due to the loss of a loved one during or up to a year before pregnancy and did not find increased rates of ASD
in the offspring of so-bereaved, regardless of time frame (Li et al., 2009). While perhaps not as powerful an argument as that for the association of PS and SCZ, the above papers demonstrate that there’s a strong likelihood that PS controls some of the variability in the development of ASD.

While the human data presented above makes a compelling case for the association between PS and these two diseases, ethical manipulation of prenatal conditions requires the use of animals as a model. To this end, we must consider what testable behaviors may be used as a measure of the degree to which the animal’s behavior mimics that seen in SCZ and ASD. Enhanced movement in a field is typically used as a measure of SCZ-like behavior in rats (Lipska & Weinberger, 2000). In rats, NMDA receptor antagonism through MK-801 or ketamine, administration of which is typically considered to be an acceptable animal model of some aspects of schizophrenia (Kesby et al., 2006), results in the enhanced movement rate termed hyperlocomotion (Ma & Leung, 2006). Pregnant Wistar rats stressed by a restraint schedule similar to that used here produced male offspring that had increased locomotion compared to controls when placed within a novel environment (Deminière et al., 1992). A study that exposed pregnant mice to 6 hours of restraint stress for 11 days prior to parturition found increased locomotion in the 8-week old offspring in both a novel environment and on a wheel running task (Son et al., 2007); these studies suggest that PS is associated with increased locomotion in a novel open field compared to controls, an effect considered to be a measure of face validity to SCZ.
in animals. However, in another study examining strain differences in Fischer 344 and Lewis rats, a PS paradigm similar to that used here found no main effect for PS on movement in an open, elevated arena, though PS was associated with lower movement rates early in testing in Lewis rats (Stöhr et al., 1998).

Sprague-Dawley rats, stressed via 2 hour restraint from P15 till parturition, male offspring rats 90 to 110 days old had reduced locomotion and rearing compared to non-stressed males, while females remained non-significantly affected by PS compared to controls (Alonso, Arevalo, Afonso, & Rodríguez, 1991). In general, the effects of PS on locomotion seems to have a differential effect depending upon animal strain, with animals similar to those used in this study not traditionally showing a reduction in locomotion in response to PS in males.

Humans diagnosed with SCZ have been observed to have sensory gating deficits resulting in reduced prepulse inhibition (PPI; Grillon, Ameli, Charney, Krystal, & Braff, 1992; Braff, Swerdlow, & Geyer, 1999), another measure typically used in assessing animal models of schizophrenia. An interesting, perhaps illustrative study that used a PS paradigm similar to that used here and maternal separation for 6 hours of 4 days prior to weaning as an early life stressor found that there were significant effects of PS on animal weight in Wistar rat 5-month-old offspring, in which PS-treated male rats were lighter, and on PPI, where PS-treated animals had enhanced %PPI compared to controls, but animals treated by both PS and maternal separation had reduced %PPI (Lehmann, Stöhr, & Feldon, 2000). This same study found a reduction in
locomotion in a novel environment as a result of PS in female animals, but not male. A study using a similar manipulation with Sprague-Dawley rats found that PS animals had reduced PPI compared to controls at a variety of prepulse intensities (Koenig et al., 2005). Administration of dexamethasone (DEX), a high-affinity glucocorticoid receptor (GR) agonist that is frequently used to mimic the effects of stress, to Wistar rats during P15-21 of gestation produced offspring that showed attenuated weight gain compared to controls out to P80 (Hauser, Feldon, & Pryce, 2006). In this study, DEX administration during gestation, whether or not the animal was reared by a dam that received DEX treatment during pregnancy, was associated with increased %PPI with a prepulse of 84 dBs, but not at 72, 76 and 80 dBs.

Reduced social interaction is considered a major symptom of ASD in humans, and is typically used as a measure of face validity when considering an animal model of autism (Schneider & Przewłocki, 2004). Forty-five minutes of restraint stress once a day P11-22 as PS in Sprague-Dawley rats produced offspring that increased weight and reduced social interaction, an effect that was rescued by an enriched early life environment (Morley-Fletcher, Rea, Maccari, & Laviola, 2003). A PS schedule that used 8 days of variable stressors immediately prior to parturition produced offspring that, at P56-64, showed no difference in locomotion in a novel arena when compared to controls, but did show markedly reduced social interaction, an effect that was reduced or abolished by administration of oxytocin (OT) into the central amygdala in a dose dependent
manner (Lee et al., 2007). A PS procedure of placing the pregnant rat in a cage with a cat for two, 15 minute intervals on either the 10th, 14th or 19th gestational day found the P60 offspring found that offspring that experienced PS at either the E10 or E19 time points displayed significantly reduced social interaction compared to controls (Patin, Lordi, Vincent, & Caston, 2005). Interestingly, social interaction deficit was alleviated by administration of an NMDA agonist among both humans diagnosed with ASD (Posey et al., 2004) and in an animal model of autism (Moskal, Burgdorf, Kroes, Brudzynski, & Panksepp, 2011).

Consideration of the above literature demonstrates that hyperlocomotion in a novel open field and reduced %PPI are behaviors considered to have good face validity for SCZ in animal models. Similarly, reduced social interaction is considered to be a behavior anticipated in animal models of ASD. PS has been associated separately with both %PPI and social interaction in animals similar to those used in these experiments, but hyperlocomotion has not consistently been shown, and not traditionally observed in the Sprague-Dawley animals utilized here. Sometimes, when the effect of the rearing dam’s stress during gestation is considered in the experimental design, that can be shown to have an effect on the results beyond the variability controlled by PS. In these studies, PS was often shown to have the effect of reducing weight gain among treated animal compared to controls.

In this study, we will examine the relationship between PS and several behavioral outcomes associated with SCZ and ASD. As described above,
hyperlocomotion has been associated with SCZ, but its relationship with PS in
the literature is tenuous. Reduced PPI has been implicated in both SCZ and ASD,
has been associated with PS and DEX administration as well. Reduction in social
interaction is a key symptom of ASD, but is only allegorically associated with SCZ
(Corcoran, Mercer, & Frith, 1995). Indeed, in a very recent study, locomotion,
degree of PPI and social interaction have been compared using adult Wistar rats
that found that high PPI was associated with increased interaction with
treatment-paired animals, and also increased locomotion in a novel arena
(Goktalay, Kayir, Ulusoy, & Uzbay, 2014). We hypothesized that animals that
have been exposed to PS in utero would express reduction in prepulse inhibition,
social interaction and center field entries during a novel arena presentation, but
increase locomotion within the novel open field. In experiment 1, we will
examine how PS exposure affects early- and late-juvenile animals regarding
behaviors such as open field locomotion, PPI and social interaction. In
experiment 2, we will add cross fostering to examine the influence of the mother,
whom has either been stressed or not during pregnancy, has on her reared
animals, an effect we’ll term MS in this paper. Previously, cross fostering has
been shown to reverse the influence of PS on offspring animals on stress
hormone reactivity (Maccari et al., 1995). Additionally, rats of the Fischer and
Lewis strains behave differently in terms of open field behavior, startle
responsiveness and stress hormone levels if raised by a dam of their own strain
than if cross fostered to a dam of the other lineage (Gomez-Serrano, et al. 2001).
Examining cross fostering will serve to differentiate the prenatal environment created, in part, by PS treatment from the environment of the mother in early life. Finally, in experiment 3 we will examine animals both as early juveniles and as near adults on postpartum day 90 (P90), showing us the enduring influences of both PS and MS on offspring animals.

Figure 1: Depicts timeline of each of the 3 experiments, including differences in treatment leading up to tests. Experiment 1 includes behavioral testing for social interaction, open field and PPI, while Experiments 2 & 3 don’t include tests for social interaction.
CHAPTER 2: EXPERIMENT 1

Experiment 1 will test the association between PS and behaviors associated with SCZ and ASD, specifically locomotion in a novel open field, %PPI and social interaction. The effect of the PS manipulation will be validated by examination of animal weight. We hypothesized that we’ll see an increase of movement in the open field in animals that have received PS, associated with a reduction in %PPI and social interaction. An outline of all experiments is depicted in Figure 1.

2.1. Methods

2.1.1. Subjects

Primiparous Sprague Dawley rats were ordered time pregnant to day 7 of pregnancy (E7) from Charles River Canada. Animals were delivered double housed and maintained that way until immediately prior to parturition, with rat chow and water ad libitum and cage and bedding changed one a week. Pregnant animals were left undisturbed in cages until E15, allowing them a week of habituation prior to testing. Following weaning, offspring animals were maintained in similar circumstances regarding cage changes, food and water. Offspring were either double, quadruple or quintuple housed, based on treatment and sex, and handled and weighed bi-weekly by experimental staff, up until testing.
2.1.2. Apparatus

**Open Field:** A circular open field (203 cm diameter) with an opaque black plastic floor and 16 inch high, white, patterned walls.

**Prepulse Inhibition:** The prepulse inhibition apparatus, hardware and software were custom designed; see Waddell, Heldt, & Falls, 2003 for full description of the apparatus.

![Figure 2: (A) The placement of the novel object and nest within the home cage during the habituation phase. (B) The placement of the pups after 5 minutes of habituation, with half the pups within an established nest and the other half placed throughout the other side.](image)

2.1.3. Procedure

**Culling:** Following parturition on P1, pups were culled down to 12 per mother to create even groups.
**Pup Retrieval:** On P3, dams and pups were kept within their home cages and colony room, but moved to a more accessible shelf for this test. A novel, wooden object, cleaned with EToH, was placed within the middle of the cage with the nest on one side and left there for 5 minutes to allow the mother to habituate to its presence (Figure 2, A). Following habituation, half of the pups were moved to the other side of the novel object and scattered throughout the area (Figure 2, B). Mothers were then timed to their retrieval of the 1st pup and the last pup, allowing a maximum of 15 minutes to perform this behavior. Retrieval was defined as the mother picking up a pup and moving it back across the object to the side of the cage containing the intact nest.

**Gestational Stress:** This procedure and cross fostering were performed as reported in (Maccari et al., 1995). Through pregnancy days 15–20, 4 of 8 pregnant rats- randomly assigned by coin flip – were subjected to 45 minute restraint stress by being placed into a 8.5 inch long, 3 in diameter cylinder plexiglass restraint (Stoelting, USA) and positioned in a brightly lit room. Four other Non-stressed control mothers were not handled, except during cage changes, during this period, minimizing stress exposure.

**Weaning:** On P21, animals were weaned from their rearing dams and double housed with animals that were treated similarly during prenatal and early-life.

**Social Interaction:** Naïve Sprague-Dawley rats were acquired from Charles River age-matched to our experimental animals; they were allowed to habituate for a week to the colony conditions before being handled by experimenters.
Following P30 or P60, naïve animals were placed in the open field (described above) to allow them to habituate to the environment for 10 minutes. Following habituation, animals were returned to their home cage, following which each experimental animal was allowed to interact with a naïve animal for 10 minutes in the open field while behavior was recorded. The experimental animal was placed within the open field and allowed to behave within the field for approximately 30 seconds, at which time a gender-matched, non-experimental animal was placed in the field with it, and behavior was recorded for 10 minutes. Following recording, animals were removed from the open field and returned to their home cages with their cage mates, and the field was cleaned with 75% ETOH and allowed to dry before the next run began.

Two days following naïve interaction, treatment-paired animals whom had never encountered each other, yet had received the same PS, were placed in the open field in a manner similar to the naïve interaction described above. The first animal was taken from its home cage and allowed to spend approximately 30 seconds in the field before the second animal was placed within as well. Behavior was recorded for 10 minutes and hand scored for time spent interacting; social interaction was defined as time the animals spent with their main bodies within 1 inch of each other or less or as one rat following the other within 1 tail length.

**Open Field:** Animals were placed in the open field, to which they had never been exposed previously, near the wall and allowed 10 minutes of unimpeded
exploration, during which time their movements were filmed from above. Following exploration, animals were then removed, returned to home cage, and the field was cleaned with 75% ETOH and allowed to dry to prepare for the next animal. All videos of these behaviors were analyzed with Ethovision to determine number of entries to the center and velocity of the animals.

**Prepulse Inhibition:** The prepulse inhibition apparatus, hardware and software were custom produced, and consist of a plastic chamber 7.75 inches high, 6 across and 3.5 deep within a sound-attenuating cubical 16 inches by 15 by 23.5, containing a speaker and a load-cell platform for measuring startle amplitude. Animals were placed in the chamber and allowed to acclimate to the environment for 5 minutes with white noise of 55 dB produced by surrounding equipment. Following acclimation, the animals received 30 noise bursts (120 dB, 20 ms) over 30 minutes, with an average inter-pulse interval of 1 minute. 24 of these noise bursts were preceded by a prepulse noise of 70 dB. Degree of prepulse inhibition was calculated by subtracting the animal’s startle amplitude on trails in which the prepulse preceded the noise burst from the amplitude of trials in which there was no prepulse, and this difference score was then be divided by startle on no-prepulse trails. In this manner, a difference score of 1.0 means complete inhibition of startle due to prepulse, whereas 0 means no inhibition due to prepulse. Prepulse inhibition tests were used in offspring animals at 3 months of age.
**Statistical Analysis:** Pup retrieval data was analyzed using a one-way ANOVA between stressed and unstressed mothers. All other data was analyzed using SPSS 22 to perform a ANOVA for PS and determine descriptive statistics to evaluate potential interactions, with each sex analyzed separately. Graphs were generated using Prism 6, with error bars representing one standard deviation (SD).

2.2. Results

2.2.1. Pup Retrieval

While it was found that there was no significant difference between the time it took for stressed and unstressed mothers to retrieve their first pup ($F(1, 7)=0.55$, $p=0.49$), there was a significant difference in time to retrieval of the last pup dependent upon stress ($F(1, 7)=7.46$, $p=0.03$), where non-stressed dams retrieved later ($M=734.00$, $SD=191.68$) than stressed dams ($M=401.5$, $SD=150.18$).

2.2.2. Weight

At P30, females weighed 84.75 grams ($SD=7.08$) while males weighed around 92.54 grams ($SD=7.16$), while at P60 female animas were around 258.25 grams ($SD=25.69$), while males were 414.45 grams ($SD=36.96$). There was no distinction between animals based on PS treatment at any time point and across sexes. At P30, there was no significant effect of stress on animal weight in either females ($F(1, 19)=0.32$, $p=0.58$; Figure 3, A) or males ($F(1, 23)=2.09$, $p=0.16$;
Figure 3, B). Additionally, PS was not a significant predictor of animal weight at P60 in either females (F(1, 19)=1.40, p=0.25; Figure 4, A) or males (F(1, 21)=0.97, p=0.34; Figure 4, B).

Figure 3: (A) The comparative weight of PS treated (PS+) and untreated (PS-) females and (B) males at the P30 time point of Experiment 1.

Figure 4: (A) The comparative weight of PS treated (PS+) and untreated (PS-) females and (B) males in Experiment 1’s P60 time point.
2.2.3. Startle

There was no effect of PS treatment on startle amplitude in response to a noise burst unpaired with a lower-intensity audio prepulse in either females (F(1, 19)=0.26, p=0.61; Figure 5, A) or males (F(1, 23)=2.33, p=0.14; Figure 5, B) at the P30 time point. This same effect was seen at the P60 time point, in both females (F(1, 19)=1.01, p=0.33; Figure 6, A) and males (F(1, 21)=.62, p=0.44; Figure 6, B).

![Figure 5: (A) Startle amplitude of female and (B) male animals, comparing PS treated (PS+) animals with those not so-treated (PS-) at day P30 of age.](image-url)
At P30, there was no difference between animals that were treated with PS in tests of PPI in either females (F(1, 19)=0.22, p=0.64; Figure 7, A) or males (F(1, 23)=0.01, p=0.91; Figure 7, B). This lack of effect was conserved at day P60 in both females (F(1, 19)=2.39, p=0.14; Figure 8, A) and males (F(1, 21)=.073, p=0.40; Figure 8, B).

Figure 6: (A) Startle amplitude for female and (B) male animals at the P60 time point, comparing animals that received PS (PS+) to those that did not (PS-).
2.2.4. Open Field

In a novel open field at 30 days of age, females moved approximately as much if they were treated with PS as if they weren’t (F(1, 19)=0.15, p=0.70;
Figure 9, A). However, males moved significantly less distance during the 10 minutes in the open field if they were treated with PS than if they weren’t (F(1, 23)=13.99, p<0.001; Figure 9, B). Similarly, though the effect was less pronounced, females at P60 moved approximately the same amount regardless of treatment (F(1, 19)=1.27, p=0.27; Figure 10, A), while males moved significantly less if they were treated with PS than if they weren’t (F(1, 21)=4.35, p=0.05; Figure 10, B).

Figure 9: (A) Comparison of PS treated (PS+) to untreated (PS-) animals at P30 in females, whom were largely comparable, and (B) males, whom moved significantly more when untreated by PS during gestation than those so-treated, to a measured significance of less than 0.001 (***)
Frequency of center field entries differed by sex at P30, with female center field entries being not significantly different between animals treated with PS compared to those that were not (F(1, 19)=1.44, p=0.25; Figure 11, A), while males were so influenced (F(1, 23)=7.49, p=0.01; Figure 11, B). At P60 this distinction was abolished, with females entering the center field with similar frequency regardless of whether or not they were treated with PS (F(1, 19)=2.23, p=0.15; Figure 12, A), as did males (F(1, 21)=0.99, p=0.33; See Figure 12, B).

Figure 10: (A) Comparison of PS treated (PS+) to untreated animals (PS-), with females moving about as much regardless of treatment at P60, while (B) similarly-aged males traveled significantly more if they were PS- than if they'd received the prenatal stressor (* = p of 0.05).
2.2.5. Social Interaction

At the P30 time point, treatment-paired animals didn’t interact with each other any more or less dependent upon PS treatment in either females (F(1,
9)=0.47, p=0.51; Figure 12, A) or males (F(1, 12)=0.67, p=0.43; Figure 12, B).

This effect was repeated at the P60 time point, with female movement clearly independent of PS treatment (F(1, 11)=0.17, p=0.69; Figure 13, A), while males also didn't have a significant distinction, but with a noteworthy trend towards significance that a greater number of comparisons might discover (F(1, 11)=3.80, p=0.08; Figure 13, B).

Figure 13: (A) PS-treated (PS+) females and (B) males statistically moved as much in a novel, open field as did their un-treated (PS-) cohort at age P30.
2.2.6. Discussion

Though experiment 1 shows little support for the hypothesis that PS results in behaviors similar to what might be seen in an animal model for SCZ or ASD, it does replicate previous literature’s observations regarding the effect of PS upon animal locomotion in an open field. Alonso et al., 1991, observed that male animals exposed to stress prenatally locomoted less in an open field than their female or control comparisons, as noted in the introduction, though those animals were significantly older. The frequency of center field entries at P30 in males is very interesting, suggesting that animals exposed to gestational stress have greater anxiety than those that don’t and corresponding well to reported literature (Whimbey & Denenberg, 1967), but is not relevant to our hypotheses regarding SCZ and ASD. The negligible non-significance of male social interaction at P60 is in the direction that would support this PS treatment being
associated with ASD-like behavior, but, again, does not reach significance.

Increased power, perhaps through utilization of a different method of testing social interaction that allowed for each animal to contribute individually to the statistics, rather than pairing them by treatment and halving our power, might serve to disambiguate this finding. However, in lieu of additional data to that effect, we must conclude that this PS manipulation doesn't significantly affect rates of social interaction.

Our manipulation check regarding animal weight failed to distinguish between animals that received PS versus those that didn't. However, this methodology has been used previously to great effect to produce long lasting changes in an animals stress reactivity and behavior, as noted in numerous papers such as Maccari et al., 1995, though that study in particular used Wistar animals. Other studies in this vein, such as Vallée et al., 1999, have validated and expanded upon the earlier study's findings in Sprague-Dawley animals. This makes it unlikely that the specific protocol is faulty, and it's worth noting that these classic experiments didn't report weight as a manipulation check, so it's conceivable that the long-term effects of PS due to this manipulation are expressed without effecting animal weight directly. In the pup retrieval task, there was a difference in the time it took mothers to retrieve their last, but not first, pup of six; however, the non-stressed mothers presented a deficit in that regard, against expectation. This is due to 2 of the mothers within the non-stress treated group failing to retrieve all of their pups during the 15 minute testing
period. Deficits in pup retrieval are associated with reduced functionality in the medial prefrontal cortex (Febo, Felix-Ortiz, & Johnson, 2010) and the medial preoptic area (Terkel, Bridges, & Sawyer, 1979), so it’s surprising that essentially untreated, maternal animals would display this deficit. It’s possible, given the low power of this behavioral test on the mothers (N=8), that these animals may simply be behavioral outliers that contribute to the overall significant difference between these two groups.

CHAPTER 3: EXPERIMENT 2

To differentiate the effects of PS from those of the environment created by the rearing dam due to the influence of the stress placed upon it during pregnancy, which we chose to term MS for the purposes of this report, we cross fostered the pups birthed by half of the mothers stressed during gestation with the pups of mothers were not stressed at P1. The classic study Maccari et al., 1995, showed that cross fostering effectively reversed the influence of a PS treatment similar to those used here on stress reactivity, as measured by corticosterone. We anticipated that cross fostering would alleviate the effects of PS on the male sex, as regards movement in the open field. The P30 time point was not included in this experiment.

3.1. Methods

Methods are as in experiment 1 except where noted.
**Open Field:** In experiment 2, due to co-occurring experiments, 4 black-painted rectangular columns, 8 inches high and 3 1/4\(^{th}\) inches across, were positioned throughout the field, 24 inches from each other and 17 inches from the wall. Exploration time was limited to 3 minutes, due to time constraints.

**Pup Retrieval:** Animals were assigned by experimenter selection, rather than through randomized coin flip.

**Culling and Cross Fostering:** On P1, pups from half of the stressed mothers were exchanged with the pups from half of the non-stressed mothers, and vice versa. If pup attenuation occurred prior to or post cross-fostering, bringing the litter to below 12, their number were subtracted from the P30 group, rather than the P90.

**Social Interaction:** These tests were not performed in experiment 2.

**Prepulse Inhibition:** Animals were placed in the chamber and immediately presented with 10, 120 dB, 20 ms noise bursts over 5 minutes. Following initial presentation, the animals received 30 additional noise bursts over the next 15 minutes, with an average inter-pulse interval of 30s. 15 of these noise bursts were preceded 100 ms before by a prepulse noise of 60 dB.

**Statistical Analysis:** All other data was analyzed using SPSS 22 to perform a 2 x 2 multivariate ANOVA for PS and MS and determine descriptive statistics to evaluate potential interactions, with each sex analyzed separately. Graphs were generated using Prism 6.
3.2. Results

3.2.1. Pup Retrieval

Here there was found to be no distinction in pup retrieval rates between stressed and unstressed mothers in either the first (F(1, 7)=1.69, p=0.24) or the last pup retrieved (F(1, 7)=0.14, p=0.72).

3.2.2. Weight

The stress rearing dams experienced during pregnancy did not significantly impact weight in females (F(1, 23)=1.98, p=0.17; Figure 14, A), but did in males (F(1, 23)=14.29, p=0.001; Figure 14, B), where males reared by stressed mothers were lighter (M=367.75, SD=37.23) than those that were reared by mothers unstressed during pregnancy (M=415.25, SD=23.30). Conversely, while there was no influence of PS on male weight (F(1, 23)=1.18, p=0.29), in females it could be argued there was a negligible, but not significant, effect of PS (F(1, 23)=3.46, p=0.08). PS and MS did not interact to produce effects above and beyond the main in either females (F(1, 23)=0.03, p=0.85) or males (F(1, 23)=1.21, p=0.28).
3.2.3. Startle

PS was not a significant predictor of startle amplitude in response to an acoustic burst in females (F(1, 23)=0.17, p=0.68; Figure 15, A) or males (F(1, 23)=2.28, p=0.15; Figure 15, B). Similarly, MS didn’t significantly influence startle in either females (F(1, 23)=0.39, p=0.54) or males (F(1, 23)=0.16, p=0.69). Finally, there was no interaction between PS and MS in females (F(1, 23)=0.87, p=0.36), though males showed a negligible trend towards significance (F(1, 23)=3.38, p=0.08).
In females, neither PS (F(1, 23)=0.10, p=0.76; Figure 16, A) nor MS (F(1, 23)=0.01, p=0.97) were significant predictors of %PPI when a noise burst was preceded by a non-startling, lower dB prepulse, and nor was there a significant interaction between the two treatment conditions (F(1, 23)=0.06, p=0.80).

Similarly, in males, PS (F(1, 23)=0.05, p=0.82; Figure 16, B) and MS (F(1, 23)=0.74, p=0.40) were not significant indicators of %PPI, and an interaction between these factors was not detected (F(1, 23)=1.42, p=0.25).

Figure 16: (A) In females, there was no significant distinction between animals that were treated with PS (PS+) or not (PS-), nor was there a distinction between those that experienced stress due to maternal behavior (MS+) and those that did not (MS-). (B) A similar lack of effect was found among males, though an interaction between PS and MS was discovered and decomposed within the text.
3.2.4. Open Field

In regards to distance traveled in a novel open field, PS ($F(1, 23)=6.85$, $p=0.02$; Figure 17, A), but not MS ($F(1, 23)=0.11$, $p=0.17$), was a significant predictor of locomotion in females, with prenatally stressed animals moving more ($M=2159.64$, $SD=995.71$) than those that weren’t ($M=1025.28$, $SD=1037.46$). Among male animals, PS did not significantly predict rate of moment in the field ($F(1, 23)=0.01$; Figure 17, B), $p=0.93$), but MS did ($F(1, 23)=5.62$, $p=0.03$). There was no interaction between PS and MS in females ($F(1, 23)=0.08$, $p=0.78$), but in males there was a differential effect of PS dependent upon whether the rearing dam was stressed during pregnancy ($F(1, 23)=6.67$, $p=0.02$).
In females, frequency of center field entries was not significantly associated with PS (F(1, 23)=1.73, p=0.20; Figure 18, A) or MS (F(1, 23)=0.29, p=0.59), and nor was there an interaction between these two treatments (F(1, 23)=0.73, p=0.40). In males, this pattern was repeated, with neither PS (F(1, 23)=0.66, p=0.43; Figure 18, B) nor MS (F(1, 23)=0.66, p=0.43), again with no interaction (F(1, 23)=0.24, p=0.63).

Figure 18: (A) PS was a significant predictor of distance traveled in a novel open field in females, with animals that received PS (PS+) moving more than those that didn’t (PS-). (B) In males, MS better predicted locomotion distance, with animals reared by a dam that suffered stress during pregnancy (MS+) traveling more than those which didn’t (MS-; * = p < 0.05).
3.2.5. Discussion

In experiment 2, we've found some interesting effects in our open field data that deserve consideration. Females seemed more influenced by PS in terms of open field locomotion, with stressed animals moving more, while male animals that were reared by dams stressed during pregnancy moved more than those reared by unstressed mothers. This is in contradiction of both the literature cited earlier in this study, and of experiment 1 presented here. That PS would result in increased locomotion is consistent with an animal model of SCZ, but that this effect occurs only in females is against the model, since the age of onset of SCZ in human females is later than that of males; so, if we were to see such an effect, we'd anticipate preferentially seeing it in male animals, rather

Figure 19: (A) There was no significant effects of animals treated prenatally with restraint stress (PS+) compared to those that weren't (PS-), or whom received early life stress due to maternal stress during pregnancy (MS+) versus those that didn’t (MS-) in either females or (B) males.
than female. The influence of MS on the males, however, is somewhat surprising. If the effects of experiment 1 were preserved and the canonical reversal of effect of PS from Maccari et al., 1995, were in effect, then we would anticipate that animals that received PS in utero and were reared by a gestationally-stressed dam would have reduced locomotion and all other groups would have normal behavior in the open field. Instead, this group (PS+, MS+) has the greatest amount of locomotion. Perhaps, the double-hit of stress during the prenatal period and stress in early life was sufficient to reverse the normal influence of PS on locomotion in a novel open field, and instead create a phenotype typical of SCZ, of which hyperlocomotion is a feature.

It’s also worthy of commentary that in experiment 2 we see a difference in our male animals due to treatment, specifically MS, with animals reared by stressed dams being significantly lighter than those reared by unstressed dams. Given that this effect is present, and that an effect of PS is seen neither here nor in experiment 1, it’s worthy of consideration that MS may play a more central role in development, as regards weight, than PS.

CHAPTER 4: EXPERIMENT 3

In the third experiment, we continued cross fostering pups, but we also extended the second time point from P60 to P90, to determine if effects seen at the P60 time point would persist into near adulthood.
4.1. Methods

To examine the influence of PS and MS on a later time point, offspring animals were allowed to develop out to P90, rather than P60 as in experiments 1 and 2. Methods are as in experiment 2, except as described.

**Open Field:** Exploration time was allowed out to 10 minutes. At the P90 time point, the towers described in experiment 2 were removed, due to no longer co-utilizing the field with another experiment.

**Prepulse Inhibition:** Animals were placed in the chamber and immediately presented with 10, 120 dB, 20 ms noise bursts over 5 minutes. Following initial presentation, the animals received 30 additional noise bursts over the next 15 minutes, with an average inter-pulse interval of 30s. 7 of these noise bursts were preceded 100 ms before by a prepulse noise of 60 dB, 6 presentations of 70 dB and 7 presentations of 80 dB.

4.2. Results

4.2.1. Pup Retrieval

In experiment 3, we found no distinction in stressed and unstressed mothers in time to retrieval of either the first \((F(1, 7)=1.57, p=0.26)\) or last pup \((F(1, 7)=0.06, p=0.81)\)
4.2.2. Weight

At P30, among females there was no significant effect of PS (F(1, 16)=0.01, p=0.95; Figure 19, A) or MS (F(1, 16)=0.33, p=0.57), and there was no interaction between the two factors on animal weight (F(1, 16)=0.12, p=0.74). This is also true for males at the P30 time point, as PS (F(1, 20)=0.75, p=0.40; Figure 19, B), MS (F(1, 20)=0.05, p=0.83) and the interaction between the two (F(1, 20)=3.09, p=0.10) were not significant predictors of animal weight.

At the P90 time point, there wasn’t a considerable effect of either PS (F(1, 23)=0.18, p=0.67; Figure 20, A) or MS (F(1, 23)=0.34, p=0.56) in females, but while there was no statistically significant interaction between PS and MS (F(1, 23)=3.95, p=0.06), there could be considered a trend toward significance.

Among males at P90, PS was shown to be a significant predictor of animal weight (F(1, 23)=6.18, p=0.02; Figure 20, B), while MS was not (F(1, 23)=3.13,
p=0.09). There was additionally found to be no differential effect of PS dependent upon stress due to maternal stress (F(1, 23)=16.9, p=0.21).

4.2.3. Startle

At P30, among females, neither PS (F(1, 16)=0.51, p=0.49; Figure 21, A) nor MS (F(1, 16)=0.02, p=0.89) were significant predictors of startle due to an unexpected 120 dB noise burst, and nor did these treatments interact such that startle amplitude due to PS treatment changed dependent upon MS treatment (F(1, 16)=0.09, p=0.74). Similarly, in males of this age, PS (F(1, 20)=1.49, p=0.24; Figure 21, B) and MS (F(1, 20)=0.79, p=0.38) did not considerably influence startle amplitude, and there was not interaction between PS and MS (F(1, 20)=0.32, p=0.58)

Figure 21: (A) At P90, there was no significant influence of PS or MS on animals weight. (B) Among male animals, treatment with stress during gestation (PS+) was associated with greater weight compared to those unstressed in that period (PS-), but with no main effect for being reared by a stressed mother (MS+) as opposed to being reared by an unstressed mother (MS-).
At the P90 time point, there was no significant influence of PS (F(1, 23)=0.42, p=0.52; Figure 22, A) or MS (F(1, 23)=1.43, p=0.24) on amplitude of startle in females, and nor was there an interaction between these factors (F(1, 23)=0.42, p=0.52). Among males at the same time, PS wasn’t a significant predictor of startle amplitude (F(1, 23)=0.54, p=0.47; Figure 22, B), but MS was (F(1, 23)=6.50, p=0.02). There was no effect of MS or PS above and beyond their main effects in males (F(1, 23)=0.10, p=0.76).
PPI at time point P30, in females didn’t vary due to PS at any dB of prepluse preceding the noise burst (80 dB $F(1, 16)=0.19, p=0.67$; 70 dB $F(1, 16)=2.83, p=0.12$; 60 dB $F(1, 16)=0.33, p=0.58$; Figure 23, A), nor due to MS (80 dB $F(1, 16)=0.13, p=0.72$; 70 dB $F(1, 16)=0.13, p=0.72$; 60 dB $F(1, 16)=0.04, p=0.84$). Additionally, there was no differential effect of PS dependent upon MS treatment at any prepulse intensity (80 dB $F(1, 16)=0.001, p=0.97$; 70 dB $F(1, 16)=1.26, p=0.28$; 60 dB $F(1, 16)=0.19, p=0.67$). Among males of this age, there was no distinction in groups due to PS (80 dB $F(1, 20)=0.35, p=0.56$; 70 dB $F(1, 20)=0.59, p=0.45$; 60 dB $F(1, 20)=0.20, p=0.66$; Figure 23, B) or MS (80 dB $F(1, 20)=0.01, p=0.93$; 70 dB $F(1, 20)=0.30, p=0.59$; 60 dB $F(1, 20)=0.10, p=0.76$), and there was no interaction between these treatments at any prepulse intensity (80 dB $F(1, 20)=0.91, p=0.35$). Figure 23: (A) While females didn’t differ significantly as a result of any treatment at P90, (B) males didn’t differ due to either receiving restraint stress while in utero (PS+) or not (PS-), but were distinguished in startle amplitude based on whether their rearing dam was stressed during pregnancy (MS+) or not (MS-).
At the P90 time point, females didn’t vary significantly in PPI due to treatment by PS ($80 \text{ dB } F(1, 23)=0.52, p=0.48; 70 \text{ dB } F(1, 23)=0.17, p=0.69; 60 \text{ dB } F(1, 23)=1.98, p=0.17$; Figure 24, A) or MS ($80 \text{ dB } F(1, 23)=2.68, p=0.12; 70 \text{ dB } F(1, 23)=0.05, p=0.82; 60 \text{ dB } F(1, 23)=0.001, p=0.97$), and nor was there an interaction between these treatments, regardless of prepulse dB ($80 \text{ dB } F(1, 23)=0.29, p=0.60; 70 \text{ dB } F(1, 23)=1.66, p=0.21; 60 \text{ dB } F(1, 23)=0.00, p=0.99$).

Among males of this age, PS did not significantly contribute to PPI at any intensity of prepulse ($80 \text{ dB } F(1, 23)=0.54, p=0.47; 70 \text{ dB } F(1, 23)=0.08, p=0.78; 60 \text{ dB } F(1, 23)=0.10, p=0.75$; Figure 24, B), and neither did MS, though there might be interpreted as a trend towards significance in the 80 dB prepulse only ($80 \text{ dB } F(1, 23)=3.11, p=0.09; 70 \text{ dB } F(1, 23)=2.40, p=0.14; 60 \text{ dB } F(1, 23)=0.88, p=0.36$). Additionally, there was no interaction between PS and MS above and
beyond the main effects of both \(80\ dB\ F(1, 23)=0.005, p=0.94; 70\ dB\ F(1, 23)=0.002, p=0.96; 60\ dB\ F(1, 23)=0.007, p=0.93\).

4.2.4. Open Field

At P30, female animals didn’t move more due to PS \(F(1, 16)=3.06, p=0.10\); Figure 25, A) or MS treatment \(F(1, 16)=0.19, p=0.67\) in a novel open field; in addition, there was no interaction between PS and MS in determining distance moved \(F(1, 16)=0.08, p=0.77\). A similar effect was seen in males of this age, with neither PS \(F(1, 16)=0.06, p=0.81\); Figure 25, B) nor MS \(F(1, 16)=0.002, p=0.97\) predicting movement distance, and nor was there an effect of PS dependent upon MS treatment \(F(1, 16)=0.55, p=0.47\).
At the P90 time point, female animals didn’t move statistically differently dependent upon treatment by PS (F(1, 23)=0.71, p=0.41; Figure 26, A) or MS (F(1, 23)=0.02. p=0.89), and nor was there an interaction between these treatments (F(1, 23)=1.49, p=0.24). Similarly, males weren’t influenced in their movement distance by PS (F(1, 23)=0.38, p=0.54; Figure 26, B), though there seemed to be a trend towards MS influencing movement in novel open field (F(1, 23)=3.98, p=0.06. There was no effect PS dependent upon treatments of MS (F(1, 23)=0.25, p=0.62).

Figure 26: (A) At P30, there was no influence of either gestational stress (PS+) or lack there of (PS-) or stress due to maternal rearing (MS+) or its absence (MS-) in either females or in (B) males.

At the P90 time point, female animals didn’t move statistically differently dependent upon treatment by PS (F(1, 23)=0.71, p=0.41; Figure 26, A) or MS (F(1, 23)=0.02. p=0.89), and nor was there an interaction between these treatments (F(1, 23)=1.49, p=0.24). Similarly, males weren’t influenced in their movement distance by PS (F(1, 23)=0.38, p=0.54; Figure 26, B), though there seemed to be a trend towards MS influencing movement in novel open field (F(1, 23)=3.98, p=0.06. There was no effect PS dependent upon treatments of MS (F(1, 23)=0.25, p=0.62).
Frequency of entry into the center of the novel, open field was measured at P30 in females, on which neither PS (F(1, 16)=0.000, p=1.00; Figure 27, A) nor MS (F(1, 16)=1.89, p=0.19) had a significant impact, and nor was there an additional effect of PS dependent upon varying treatments of MS (F(1, 16)=0.000, p=1.00). Among males of this age, neither PS (F(1, 20)=0.19, p=0.67; Figure 27, B), nor MS (F(1, 20)=0.19, p=0.67), and additionally there was no significant interaction between PS or MS (F(1, 20)=2.36, p=0.14).

Figure 27: (A) Female animals didn’t express differences in locomotion in a novel open field due to either gestational stress (PS+) or a normal gestational period (PS-), or being reared by dams the were themselves either exposed to stress during pregnancy (MS+) or not (MS-). (B) Males similarly have no significance between groups.
At an age P90, the frequency of entry into the center of the open field was not significantly controlled by either PS (F(1, 23)=1.23, p=0.28; Figure 28, A) or MS (F(1, 23)=2.65, p=0.12), and neither was their an interaction between these two treatments (F(1, 23)=0.44, p=0.51). Among males, again, neither PS (F(1, 23)=0.35, p=0.56; Figure 28, B) nor MS (F(1, 23)=1.72, p=0.20) significantly predicted frequency of center field entries, and nor was there an interaction between PS and MS treatments (F(1, 23)=0.52, p=0.48).

Figure 28: (A) At P30, neither female nor (B) male frequency of center field entries was dependent upon either receiving stress prenatally (PS+) or not (PS-), and neither was a rearing dam’s experience of stress during pregnancy (MS+) or not (MS-) a significant predictor.
4.2.5. Discussion

Here we do not find the influence of PS or MS on open field movement dependent on animal sex that we saw differentially in experiment 1 and 2. Our manipulation check of weight reveals an unexpected result, where male animals subjected to PS had increased weight compared to unstressed males. We would normally anticipate that PS would result in a significant reduction in animal weight (Drago et al., 1999). Additionally, we find that male animals reared by dams stressed during pregnancy startled more in response to a 120 dB noise burst than those raised by unstressed mothers. Long-Evans rats subjected to maternal separation for 3 hours per day during early life showed considerably enhanced startle response at P120, an effect that was associated with increased corticosterone levels compared to controls (Kalinichev et al., 2002). The
inability of this analysis to detect any effect of PS or MS on movement in the open field in males, when previous experiments have found influences of PS and MS interacting with PS, is surprising given that the treatment would have been the same as in experiment 2, and as the previous study from Kalinichev et al., 2002, showed the later time point shouldn’t have the effect of rescuing the deficit due to MS. Perhaps this is a low probability event, or perhaps the animals used in experiment 3 were dissimilar in some way to the animals used in experiment 1 and 2 due to individual factors.

CHAPTER 5: GENERAL DISCUSSION

These experiments intended to determine the association between PS on behaviors associated with SCZ and ASD when accounting for the influence of the mother in early life in Sprague-Dawley rats. We hypothesized, based on previous literature, that PS would produce reductions in locomotion in a novel open field, reduce %PPI in response to an intense noise burst preceded by a non-startling prepulse, and reduce social interaction between age, sex and treatment matched animals. In experiment 1, we saw that animals subjected to PS moved less in a novel open field at both 30 and 60 days of age; a canonical behavior that replicates previous findings in the literature. When, in experiments 2, we added cross fostering to our protocol, we saw an effect of MS that eclipsed that of PS, combined with an interaction that showed that animals both prenatally stressed and reared by a stressed dam moved more in an open field than other males, a finding consistent with an animal having face validity for SCZ. However, in
experiment 3- which methodologically didn’t vary from experiment 2 on either manipulation or this measure, excepting time point of testing (P90 rather than P60)- we do not see this effect. Similarly, while experiment 1 and 2 don’t show any significance in startle amplitude in males or females, experiment 3 showed a significant effect of MS on amplitude of startle in male animals. This effect is unsurprising given the literature, but it is confusing why similarly-treated animals in previous experiments failed to elicit this same significance in their behaviors. The distinctions, as well as the observation that in experiment 3 PS-treated males weighed more than their unstressed counterparts- a very non-canonical finding- gives rise to the possibility that the animals tested in experiment 3 were of a different population than those tested in experiments 1 and 2.

Pregnant animals were all ordered time pregnant to E7 from Charles River Canada, and reportedly come from the same Sprague-Dawley breeding stock. However, individual differences have always been considered to play an important role in these experiments, as both SCZ and ASD are strongly dependent upon genetic factors (Sullivan, Kendler, & Neale, 2003; Szatmari, Jones, Zwaigenbaum, & MacLean, 1998). Because each experiment presented here examined the offspring animals descending from 8 dams, there’s considerable concern that a litter effect could significantly influence our findings. Given experiment 3’s considerable departure from the findings in experiment 1 and 2- differences between which can be explained by methodological
distinction, it’s reasonable to hypothesize that variation in a single or multiple dams could have influenced behavioral outcomes in a significant manner, leading to the apparent lack of replication of earlier discoveries. If this were the case, then a greater number of dams, coupled with fewer animals preserved from each birth cohort and utilized in the study, would weaken the influence of the litter upon the findings and give greater distinction to the influences of PS and MS.

In several instances in experiments 2 and 3, we saw an interesting effect of MS that was not anticipated based either on the prevailing literature, or upon the hypotheses relating to SCZ. We had hypothesized that PS would result in an increase in locomotion, as associated with increased locomotion in individuals suffering from SCZ, but not that PS and MS would interact to increase locomotion above and beyond the effects of PS or MS alone. This suggests the efficacy of a “two-hit” model of the environmental factors of SCZ, a hypothesis that has gained some popularity in recent years, in producing animal behaviors with good face validity to the disease. Though frequently examined utilizing a neonatal stressor such as maternal separation and an adult stressor in much later life (Choy et al., 2009), it’s not unreasonable to hypothesize that PS could also act as a ‘hit’ for inducing behavioral phenotypes with good face validity for SCZ. This finding, in particular, is illuminating in regards to our hypotheses regarding modeling SCZ in animals, though due to concerns regarding litter effects requires further replication to see if the effect is consistent.
It’s also interesting to observe that, in experiment 2, while males had an enhancement of locomotion distance based on MS, female movement was instead enhanced by PS. A testable hypothesis that arises from this observation is whether there’s a differential effect of PS and MS dependent upon sex, whereby females are more influenced by PS and males more susceptible to early life stressors. There are certainly numerous studies finding a differential effect of PS due to sex, such as a study that examined the offspring of pregnant rats exposed to unpredictable lights and noises throughout pregnancy, 3 times a week, in which PS-treated females, but not males, showed enhanced corticosterone secretion, though they did not move significantly more in the open field than did the males (Weinstock et al., 1992). Indeed, in a study already presented in our introduction that used a PS procedure similar to ours and manipulated MS via maternal separation for 6 hours a day, it was found that MS had more of an effect on the weight of males than females (Lehmann et al., 2000). This study found that there was an interaction between PS and sex, whereby females moved less due to PS, but males did not; however, there was no significant effect of maternal separation on locomotion in either males or females. To further disambiguate the differential influence of PS and MS on the sexes would require further experimentation to parse apart these effects.

In regards to the experiment-dependent effect of PS or MS upon weight in males, we would typically anticipate that PS itself would result in a reduction of animal weight. In a study that used 15 minutes of forced swim from E5 till
parturition - a manipulation that might be considered more severe than that used in our study, due to the duration of the stressor, or less because the stressor became familiar by the time the dam entered the sensitive period of pregnancy-differences in animal weight were evident at P1 and extended out to P60 (Drago et al., 1999). The finding, in experiment 2, that MS significantly reduced male weight is meaningful and consistent with the belief that MS may have a greater influence upon the development of males than PS. Experiment 3’s result, as reflects weight, is entirely unanticipated, with PS-treated males at P90 weighing more than non-prenatally-stressed males. It has been proposed that increased consumption of food may be a compensatory response to stressors, however, examination of the literature suggests that an acute stressor results in reduced food consumption and weight loss over time (Rybkin et al., 1997).

It’s worthy of consideration that the historical influence of PS on animal weight may be incorrect, and in fact application of PS, as utilized in this study, does not directly result in reduced weight gain. Expounded on in the discussions of experiment 1, early studies using this stress protocol did not report on animal weight, and thus cannot reliably guide us as to what should be expected. As was discussed in the introduction, DEX administration has been associated with reduction of weight, locomotion in an open field and mineralocorticoid receptor (MR) and glucocorticoid receptors (GR) in the hippocampus in the offspring animals of rats treated with the drug (Welberg, Seckl, & Holmes, 2001). This suggests some of the results in this study may be related to dysfunction of the
corticosterone stress-reactivity system, which would serve to explain experiment 1’s finding related to animals untreated by PS entering the center field with greater frequency, as those animals may be less anxious than animals treated with PS (Whimbey & Denenberg, 1967).

In this series of experiments, we manipulated the stress placed upon the offspring animal through the rearing dam, relying on the stress the dam received during pregnancy to indirectly affect the neonates in early life; we termed this effect MS. The early life period has been extensively studied, but often using an alternate paradigm that separates the mother from her pups for a differential period of time. Pups separated from the mother for approximately 15 minutes throughout the neonatal period, a manipulation called handling, has been shown to increase maternal care through licking and grooming (L&G), which has been associated with reduced anxiety, while pup separated from their mothers for an hour or more had decreased weight at weaning, typically termed maternal separation (McIntosh, Anisman, & Merali, 1999). These manipulations have been well established as means of experimentally controlling stress during the early life period, especially in relation to the rearing dam. As relates to our experiments, a study that used a gestational stressor very similar to ours found that postnatal handling of exactly 15 minutes by experimenters rescued the effects of PS on measures of anxiety and locomotion in response to a novel arena (Vallée et al., 1997). This finding may imply that the influence of the early life
period is more important than the prenatal period for the development of behavioral deficiencies as were measured in this study.

As described above, maternal L&G and other maternal behaviors such as arched back nursing can be effectively controlled by pup separation from the rearing dam for either 15 minutes or more and may influence the stress response of reared animals (Caldji et al., 2000). Lactating Long-Evans rats that express high levels of L&G showed greater levels of OT receptors in the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST), central amygdala (CeA) and the ventral region of the lateral septum when compared to lactating rats with low L&G and those that aren’t in lactation (Francis, Champagne, & Meaney, 2001). Infusion of an OT antagonist into the left lateral ventrical reduced frequency of L&G in normally high L&G mothers, but not low L&G dams (Champagne et al., 2001). This same study found that ovariectomized (OVX) virgin rats of high, but not low, L&H dams had increased levels of OT receptor binding at greater levels of estrogen replacement. As relates to our study, transgenic mice with deficits in the OT gene (OT-/-) did not differ from genetically normal animals in startle or %PPI, but genotype and drug administration interacted in such a way that OT-/- mice treated with phencyclidine (PCP) had reduced %PPI, an influence not accounted for by either genotype or drug main effects (Caldwell, Stephens, & Young, 2008). In male Sprague-Dawley rats, subcutaneous OT administration rescued the PPI deficit in animals that were injected with a DA agonist or a non-competitive NMDA
antagonist (Feifel & Reza, 1999). As shown above, OT receptors are increased by an enhanced maternal environment in early life, and activation of OT receptor serves to rescue animals that would otherwise express reduced %PPI. This may have significance for our finding in experiment 3’s P90 time point, where there was a trend towards reduced %PPI in male animals exposed to an 80 dB prepulse and reared by mothers stressed during pregnancy; such mothers may have reduced L&G compared to dams that were not stressed during pregnancy (Smith et al., 2004), which may result in low OT in the offspring animals, and thus increased vulnerability to events that could result in reduced %PPI.

In experiment 1, we found that at P60, but not in early juveniles, there was a non-significant effect of PS, whereby gestationally stressed males interacted less with a treatment- and sex-paired, but unfamiliar, animal than those that weren’t stressed. These results are somewhat inconsistent with the literature, as an experiment that tested the effects of enhanced maternal L&G through brief separation from pups found that male rats that received the improved rearing had reduced duration of investigative social interaction with treatment-paired experimental companion (Todeschin et al., 2009). In regards to the system underlying social interaction, in ASD-diagnosed children from 6 to 11 there was less detectable serum OT than in healthy children and OT levels didn’t increase with age as in controls (Modahl et al., 1998). This same study observed that greater levels of OT were associated with reduced levels of social functioning in ASD-diagnosed children, which was an effect opposite of that seen
in healthy controls; this suggests that there may be a differential effect of OT in ASD sufferers and in healthy controls. Intravenous administration of synthetic OT to adults diagnosed with ASD improved their comprehension of emotional content in speech (Hollander et al., 2007). Given the earlier reported finding that OT reception rescues %PPI response in animals that would otherwise have reduced %PPI, and in consideration of the observation that subjects suffering from ASD have reduced OT levels, it is possible that reduced levels of OT may be associated with both ASD and SCZ, in which reduced %PPI is a well-established symptom. The adult female offspring of low L&G dams had reduced OT receptor binding when compared to high L&G females in the BNST and CeA, with no differences found within male offspring (Francis, Young, Meaney, & Insel, 2002); this suggests that animals exposed to low L&G during rearing may have reduced PPI after exposure to drugs such as PCP, effects unmitigated by OT neurotransmission in these animals, and social interaction deficits similar to those seen in ASD. Clearly, our manipulation using MS treatment was insufficient to differentiate animals consistently based on %PPI, but it’s conceivable that MS+ animals may be analogous to the offspring reared by low L&G mothers, which means differentiations between our treatment groups might have been more discernable following administration of PCP or other NMDA antagonist, a finding which would have relevance for SCZ.

Having some relevance to our social interaction measurement, a human study that utilized a simulated social partner with a computer and measured
blood oxygenation via functional magnetic resonance imaging (fMRI) showed that virtual interaction with the simulated other resulted in increased signal from the ventral MPFC (Schilbach et al., 2006). Human case studies of individuals whom have suffered frontal lobe brain damage show that individuals with injuries to the ventral frontal lobe, as opposed to other areas, showed deficit in recognizing expressions and vocal tone, possibly implying decreased capacity in tasks analogous to social interaction in rodents (Hornak, Rolls, & Wade, 1996). A structural MRI study in human males highly correlated performance on Cloninger’s temperament and character inventory, which purports to be able to determine if the reporting individual is sensitive to social reward for behavior, with grey matter density in the orbitofrontal cortex and ventral striatum (Lebreton et al., 2009). Frontal lobe deficit has been implicated in SCZ for several decades now (Ingvar & Franzén, 1974), and early imaging showed decreased volume within the frontal area specifically (Andreasen et al., 1986). This “hypofrontality” is typically meant to describe reduced PET signal due to attenuated blood flood (Volkow et al., 1987), especially in the dorsolateral prefrontal cortex (DLPFC; Glahn et al., 2005), and it’s important to observe that similar effects may be associated with ASD as well (Ring et al., 1999; Baron-Cohen et al., 1999). One of the classic symptoms associated with a diagnosis of schizophrenia is executive function impairment, traditionally assessed by the Wisconsin Card Sorting Test; people diagnosed with SCZ have difficulty altering the schema they use to determine the ‘correct’ card after previously establishing
an alternate schema (Goldberg et al., 1987; Everett, Lavoie, Gagnon, & Gosselin, 2001). There is some evidence that individuals suffering from ASD may also possess deficit at executive function tasks (Hughes, Russell, & Robbins, 1994), which may be related to reduced blood flow to the frontal lobes compared to age matched controls (Zilbovicius et al., 1995). Considering these reports, social interaction appears partially dependent upon the frontal lobe, an area where individuals suffering from SCZ and ASD possess reported deficits. This implies that observed deficit in experimental tests of social interaction may well be associated with frontal lobe dysfunction, and related back to the observed symptoms in both these disabilities.

In general, the findings of this study present a complex and at times contradictory picture of the effects of both stress during gestation, as mediated by the mother, and stress to the offspring due to the environment of the mother in the offspring’s early life. We have termed these factors PS and MS, and have found differing effects of them dependent upon behavior test, animal sex and experiment, as has been exhaustively considered throughout this discussion. While it may be tempting to suggest that our manipulation was not effective at instilling the influences of PS or MS into our experimental, offspring animals, that hypothesis is belied by the historical effectiveness of the treatment and the significant findings found in the experiments presented here. It’s worthy of consideration that the specific Sprague-Dawley rat strain used in this experiment may be unexpectedly resistant to this form manipulation; while
papers cited above suggest that Sprague-Dawley rats should be susceptible to this treatment, anecdotal evidence (Personal communications: Dr. William Falls), suggests that individual colonies of the same strain may have differential effects in how they respond to a given treatment.

Future experiments along these lines may benefit from giving greater consideration the litter effects and limiting the number of offspring utilized from each maternal animal. Postnatal manipulations, such as through maternal separation paradigms, could have a considerable influence on animal behavior, perhaps to a greater extent than PS manipulations, based on both studies reported above and our own observations here. Other rat strains may also be considered, such as those that provide for more genetic diversity through interbreeding with wild-type animals, to increase the likelihood that animals with the genetic predisposition towards behaviors associated with SCZ and ASD are contained within our sample. Further, examination of these behaviors associated with the diagnoses of SCZ and ASD in humans suggests molecular routes of inquiry that can be easily incorporated into the current experimental series. It may be worthwhile to examine OT levels in the preserved plasma of the animals described here for potential variations due to either PS or MS.
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