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Adolescent female rats are more resistant than males to the effects of early stress on prefrontal cortex and impulsive behavior

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Abstract

We tested the hypothesis that adolescent Sprague-Dawley females may be more resistant than males to display impulsive behavior and lower prefrontal cortex thickness after mother–infant separation (MS). Starting at postnatal day 2 (P2), the MS group was separated 6 hours/day and the early handled (EH) group 15 min/day for 10 days, and another group was standard facility reared (SFR). Subjects were examined for novel open-field activity (P28), light–dark apparatus (P29), familiar open-field (P30) and frontal cortical thickness. This protocol resulted in impulsive behavior in MS rats relative to EH and SFR, but this effect was less pronounced in females than males. MS affected the two sexes differently in terms of decreased prefrontal cortex dorsoventral thickness, with this effect being significant in males but not females. Neuroanatomical and behavioral documentation that adolescent females are more resistant than males to ADHD-like effects of maternal separation have not been previously reported.

Keywords

Females; Sex differences; Maternal separation; Neonatal stress; Adolescence; Prefrontal cortex; ADHD

INTRODUCTION

Anatomical alterations have been found in the frontal cortex of children and adolescents with attention-deficit/hyperactivity disorder (ADHD), especially a reduced size of dorsal prefrontal cortex bilaterally (Sowell, Thompson, Welcome, Henkenius, Toga, & Peterson, 2003) that appears to be due to maturation delay in cortical thickness (Shaw, Eckstrand, Sharp, Blumenthal, Lerch, Greenstein, Clasen, Evans, Giedd, & Rapoport, 2007). As children mature to become adolescents, the effects of early life stressors may contribute to prefrontal cortical thickness and behavioral disorders such as ADHD, anxiety and depression (Heim & Nemeroff, 1999; Zappitelli, Pinto, & Grizenko, 2001; Teicher, Andersen, Polcari, Anderson, Navalta, & Kim, 2003).

One early life stressor that can be analyzed experimentally in rats is maternal separation (MS), which involves separating pups from their mothers for a prolonged period of time. MS is a well-established early stressor, based on the degree to which rat pups are dependent

on their mothers for stimulation and nutrition (Hofer, 1975; Janus, 1987; Fumagalli, Molteni, Racagni, & Riva, 2007; Sanchez, Ladd & Plotsky, 2001), and is clearly demonstrated to alter HPA function later in life (Plotsky & Meaney, 1993).

In the present study, we tested the hypothesis that adolescent Sprague-Dawley female rats may be more resistant than males to display ADHD-like effects of MS stress such as impulsive behavior and lower prefrontal cortex thickness. Renard, Rivarola & Suarez (2007) have proposed that female rats are more resistant than males to certain adverse effects of MS, which is supported by previous MS studies (Ruedi-Bettschen, Zhang, Russig, Ferger, Weston, Pedersen, Feldon, & Pryce, 2006; Spivey, Barrett, Padilla, & Gonzalez-Lima, 2008) and other stress-related manipulations such as restraint stress (Conrad, Jackson, Wiczorek, Baran, Harma, Wright, & Korol, 2004) and learned helplessness (Dalla, Edgecomb, C., Whetstone, A. S., & Shors, T. J., 2008).

Of particular interest to the current study is that certain MS protocols and rat strains have demonstrated sex-biased behavioral differences (Ruedi-Bettschen et al., 2006; Renard et al., 2007). For instance, adolescent Holtzman rats show no baseline sex differences in open field ambulation; however, baseline impulsive behavior is greater in males than females (Spivey et al., 2008a). For the present study we used this 6-hour per day MS protocol (Kaneko, Riley & Ehlers, 1994; Jimenez-Vasquez, Mathe, Thomas, Riley, & Ehlers, 2001) that produces a hyperactive/impulsive phenotype in adolescent Sprague-Dawley male rats (Colorado, Shumake, Conejo-Jimenez, Gonzalez-Pardo, & Gonzalez-Lima, 2006), similar to that found in animal models of ADHD (King, Barkley, Delville, & Ferris, 2000; Gonzalez-Lima, 2005).

Only a few studies have studied the behavioral effects of prolonged MS in adolescent rats (Marin & Planeta, 2004; Ryu, Lee, Yoo, Gu, Moon, & Jahng, 2008; Spivey et al., 2008a). During adolescence, rats are more exploratory and active, which may be an ideal time to test novelty seeking and ambulatory behaviors in the open field (Spear, 2000; Shumake, Barrett, & Gonzalez-Lima, 2005). Similarly, school-age in human children is also a time in which ADHD is identified, and in which sex differences can be observed relative to the behavioral aspects of ADHD (Carlson, Tamm, & Gaub, 1997).

Behavioral components of ADHD's hyperactive/impulsive phenotype include increased locomotion, deficits in attention, and increased impulsivity and risk-taking (Gonzalez-Lima, 2005). In rats, the open field (OF) and light-dark (LD) tests are ways to measure multiple behaviors related to ambulation (such as frequency and velocity), attention (such as rearing counts and duration), and risk-taking (such as increased exposed-zone time), behaviors which are similar to those found in ADHD. The velocity of movement, defined as bursts of fast ambulation, serves as an index of impulsivity analogous to what is found in hyperactive/impulsive models of ADHD (Gonzalez-Lima, 2005; Colorado et al., 2006). Rearing counts and average rearing duration are used to index orienting behavior because the duration of rearing is associated with orienting or non-selective attention, where longer rearing indicates more orienting behavior (Aspide, Gironi Carnevale, Sergeant, & Sadile, 1998; Gallo, Gonzalez-Lima, & Sadile, 2002). In the OF test, more time spent in the periphery (also called thigmotaxis) is related to anxiety-like behavior, while more time in the center places the rat at risk (Clement, Martin, Venault, & Chapouthier, 1995). In the LD test, more time spent in the light compartment can serve as another measure of risk-taking since adolescent rats prefer the covered dark compartment to the exposed lighted compartment (Colorado et al., 2006).

In adolescent rats, sex differences in the ADHD-like behavioral effects of MS may be related to reduced prefrontal cortical thickness. Adolescence is a time of major physiological

changes in sex hormones that are accompanied by marked maturational changes in the brain, especially changes in cortical synaptic remodeling, neurogenesis, and neurotransmitter receptors and transporters (Romeo, 2003; Sisk and Zehr, 2005). Animal models and human studies indicate that androgenized individuals show frontal cortex hypometabolism and reduced size and are more likely to display ADHD-like behaviors (King et al., 2000, 2003; Gonzalez-Lima, 2005; Dickstein, Bannon, Castellanos, & Milham, 2006; Martel, Gobrogge, Breedlove, & Nigg, 2008). In particular, juvenile Sprague-Dawley male rats display lower frontal cortex metabolic capacity than females (Spivey, Colorado, Conejo-Jimenez, Gonzalez-Pardo, Gonzalez-Lima, 2008). Sex differences may contribute to male-biased behavioral alterations related to the prefrontal cortex, such as ADHD, which is more prevalent in males than females, and especially in more masculinized males (Martel et al., 2008).

The present study fills a gap in the existing literature, by investigating whether an MS protocol (Kaneko et al., 1994; Jimenez-Vasquez et al., 2001) that may lead to ADHD-like behavior affects frontal cortical thickness and impulsive behavior of adolescent Sprague-Dawley rats in a sex-dependent fashion, compared to handled controls or facility reared animals. Female Sprague-Dawley rats have a higher baseline locomotor activity in the open field than males (Meisel, Dohanich & Ward, 1979; Slob, Bogers & Van Stolk, 1981; von Hoersten, Dimitrijevic, Markovic, & Jankovic, 1993). We hypothesize that these baseline sex differences in ambulatory behavior may not persevere as a result of the stressful MS manipulation, but rather, that females will be more resistant to MS-induced changes in ambulatory behavior and frontal cortex thickness, while males will be affected more strongly by the stressor. We predict that female adolescent rats may be more resistant to display the hyperactive/impulsive behavioral effects of MS that we previously found in Sprague-Dawley male rats (Colorado et al., 2006). This hypothesis is supported by a frontal cortex sex difference in adolescent rats in which male rats have lower prefrontal cortex metabolic capacity than females (Spivey et al., 2008b).

Additionally, MS is known to increase ambulatory activity in adolescent rats placed in a novel environment (Marin & Planeta, 2004; Caldji, Francis, Sharma, Plotsky, & Meaney, 2000), but few studies have continued this line of inquiry in order to determine whether the increases in activity are retained during repeated exposures to the once-novel environment (Colorado et al., 2006). The rationale for analyzing the effects of MS on novel, versus familiar, environments is that in human ADHD there is more hyper-reactivity to novel than to familiar environments (Aspide et al., 1998). Finding an effect in a novel, but not familiar, environment would support the MS paradigm as a model for human ADHD. Therefore, we also tested the hypothesis that MS-related increase in ambulatory behavior would be greater in novel vs. familiar environments.

Some behavioral data from the males used in this study have been reported (Colorado et al., 2006). Male data previously reported in Colorado et al. (2006) are velocity and rearing. New data from the males for the current study are ambulatory counts and percent exposed zone time in novel open field, light-dark test, and familiar open field. All the cortical anatomy data are also new.

MATERIALS AND METHODS

Subjects

Subjects were 60 Sprague-Dawley rats (30 male, 30 female) divided into three treatment groups: standard facility reared (SFR), early handled (EH) and prolonged maternal separation (MS), each group containing 10 male and 10 female rats. Forty (20 male, 20 female) newborn pups for EH and MS groups came from five pregnant females acquired

from a commercial supplier (Harlan, Houston, TX). Additionally, twenty (10 male, 10 female) standard facility reared (SFR) age-matched rats were obtained from different litters from the same commercial supplier. The pregnant females were singly housed and maintained on a 12-hour light/dark cycle in a temperature-controlled room at 22 centigrade and 40% humidity. Food and water were available *ad libitum*. When the dams gave birth (postnatal day 0), the newborns were culled so that each litter contained 5 males and 5 females. This study was conducted in accordance with the guidelines of the National Institutes of Health and the American Association for the Accreditation of Laboratory Animal Care and was approved by the Institutional Animal Care and Use Committee.

Treatments

The MS protocol used was based on previous studies from our lab and others that resulted in hyperactive/impulsive behavioral outcomes as compared to EH and SFR groups (Colorado et al., 2006; Jimenez-Vasquez et al., 2001). Early Handled group was included in order to rule out the effects of handling the rat pups; i.e., the pups from both groups were handled in an identical fashion, but any differences arising in the MS group would be attributed to the duration of separation from the dam, and not due to handling by the experimenters.

Beginning on Postnatal Day (P) 2, EH pups were handled and separated from the dams for 15 minutes; whereas MS pups were handled and separated from the dams for 6 hours. Maternal separation and early handling occurred daily from P2 through P6; the animals were left undisturbed from P7-P8, and separation occurred again daily from P9-P13. During the separation period, the pups were kept in an incubator at 30–34° C in order to maintain appropriate body temperature. SFR pups were not separated or handled postnatally at the Harlan facility except for standard once-weekly cage maintenance, and the cage environment at the supplier was similar to ours (Colorado et al., 2006).

Following P13, the subjects remained undisturbed until the day of weaning at P21, when they were separated from the mother and housed with the littermates. Food and water continued to be freely available and the 12-hour light/dark cycle was maintained. From P21 to P28, the subjects were handled daily for 5 minutes in order to habituate them to the experimenters.

Behavioral measures in open field and light-dark tests

All animals were tested for open field (OF) activity during the first day of behavioral testing (P28), followed by the light-dark (LD) test (P29). To evaluate the effects of novelty in the OF, subjects were tested a second time in the OF on P30, to measure any differences in activity between the novel (P28) and familiar (P30) OF tests. In the OF test, each animal was placed in the middle of an open-field activity chamber and ambulatory behavior was recorded for 10 minutes. In the LD test, the animals were initially placed in one of the corners of the illuminated compartment and ambulatory behavior was recorded for 10 minutes.

These tests were conducted in an OF activity chamber (43 × 43 × 30.5 cm) (Med Associates, St. Albans, VT). The four lateral sides of the chamber were made of clear plastic, with a fiberglass white bottom. Activity was detected by arrays of infrared light beam motion detectors (16 × 16, 2.54 cm apart) aligned at the base and middle of each side of the chamber, thus creating a detection grid. One array of detectors was located 1 cm above the floor to detect ambulatory (horizontal) movement. Another array located 6 cm above the floor, was in place to detect rearing (vertical) movement. The chambers were controlled by the Activity Monitor program, version 5.10 (Med Associates), which recorded various parameters related to the time course of the subject's behavior. The LD test box was a

modified setup of the OF activity chamber. A dark compartment that covered half of the total area of the chamber was inserted in the chamber, dividing its total area into two compartments: an illuminated side and a dark side. The center wall included a hole that allowed the animals to move between the dark and light compartments of the chamber. The chambers were washed and dried between each session.

The Activity Monitor program automatically recorded OF and LD parameters, including measures of locomotion (ambulatory counts), orienting behavior (rearing frequency and duration), movement velocity, and exposed-zone time (entering the center of the OF or the light compartment of the LD box). The parameters measured in the novel and familiar OF and LD tests were defined as follows:

1. *Ambulatory counts.* The number of horizontal beam breaks (counts) recorded while a subject is engaged in ambulatory (locomotion) movement.
2. *Velocity.* The mean ambulatory distance (cm) divided by time (seconds).
3. *Rearing counts.* Number of periods of continuous vertical beam breaks (counts) reported by the upper array of beams, when an animal rears on its hind legs. Rearing consist of stopping ambulation and standing on the hind limbs.
4. *Average rearing duration.* Total vertical time divided by total vertical counts.
5. *Exposed-zone time.* In the OF, this measure consisted of percent time in the center area of the OF, where the center/surround border is defined as 68%:32% of the total area. For the LD test, this measure was defined as percent time spent in the exposed light compartment rather than the covered dark zone. While these two measures are different for the OF and LD tests, they both involve leaving a defensive zone to enter a more exposed zone, and therefore they were both analyzed as risk-taking indices.

Each behavioral measure was analyzed separately using a 3 (session) $\times$ 2 (sex) $\times$ 3 (separation group) repeated-measures analysis of variance (ANOVA) using SPSS for Windows, version 11.5. Data from each 10-minute session were summed for the purposes of the analysis, except in the case of the velocity and exposed-zone measures, which were averaged across the 10-minute session. Planned comparisons to assess the effects of novelty and familiarity in the open field test were performed by separate 2 (session) $\times$ 2 (sex) $\times$ 3 (separation group) repeated-measures ANOVAs. Post-hoc simple effects were analyzed and Bonferroni-corrected in order to reduce error from multiple comparisons.

Frontal cortex thickness analysis

MS and EH brains were extracted after testing and processed as previously described (Spivey et al., 2008b). Frontal cortex thickness was measured at Bregma level 3.7 using an image-processing system and histochemical rat brain atlas (Gonzalez-Lima and Cada, 1998). The vertical (dorsoventral) distance was measured parallel to the midsagittal line, using the rhinal fissure endpoint to center the placement of the line. The horizontal (mediolateral) distance was measured perpendicular to the midsagittal line, crossing midway through the dorsoventral line. Nonparametric distribution data were analyzed with independent Mann-Whitney U-tests.

RESULTS

Sex-dependent behavioral effects of separation

MS effects were more manifest in males than females, especially the increase in velocity of movement and the decrease in rearing (Fig. 1B and C, respectively). For example, MS

differentially affected males and females in terms of ambulation velocity, as reflected by a significant Group $\times$ Sex interaction ($F(2,57) = 5.488$; $p = 0.007$). Simple effects tests showed that movement velocity was increased in MS males as compared to EH males ($p < 0.001$) and SFR males ($p = 0.009$), but was unaltered in MS females. MS resulted in similar numbers of ambulatory counts for males and females (Fig. 1A). Since females showed greater ambulation than males in both SFR and EH groups, MS elevated ambulation relatively more in males (increase of 188% compared to combined SFR and EH males) than it did in females (increase of only 34% compared to combined SFR and EH females). However, the Group $\times$ Sex interaction did not reach significance ($F(2,58) = 3.068$; $p = 0.054$). No other behavior showed a significant Group $\times$ Sex interaction.

Main effects of separation group and sex on behavior

Generally, statistically significant main effects of separation group were found, in which the MS group had more ambulatory counts ($F(2,58) = 11.477$; $p < 0.001$), higher average velocity ($F(2,57) = 6.059$; $p = 0.004$), higher exposed zone time ($F(2,57) = 3.340$; $p = 0.042$), and fewer rearing counts ($F(2,58) = 5.635$; $p = 0.006$) than both EH and SFR animals. Moreover, main effects of sex revealed that females had significantly greater ambulatory counts ($F(1,58) = 11.706$; $p = 0.001$), average velocity ($F(1,57) = 5.171$; $p = 0.027$), and rearing counts ($F(1,58) = 15.007$; $p < 0.001$) than males (Fig. 1), but no significant main effects of sex were found in average rearing duration or exposed-zone time.

Group effects evoked by novel environment

Generally, when comparing the novel to the familiar OF tests, significant Session $\times$ Separation Group interactions were found for average velocity ($F(2,58) = 4.247$; $p = 0.019$), rearing counts ($F(2,58) = 23.579$; $p < 0.001$), average rearing duration ($F(2,58) = 3.849$; $p = 0.027$), and exposed zone time ($F(2,57) = 4.570$; $p = 0.014$). No significant repeated-measures interactions were found for ambulatory counts.

MS animals reacted with faster ambulatory average velocities than both EH ($p = 0.001$) and SFR ($p = 0.003$) animals in the novel OF; However, there were no significant group differences in average velocity during the familiar OF (Fig. 2A). A similar novelty effect was seen for the exposed-zone time, with MS animals having much longer exposed zone times than EH animals ($p = 0.004$) in the novel but not the familiar OF (Fig. 2B).

In addition, MS animals showed the highest rearing counts relative to SFR group during the novel OF ($p = 0.002$), and the lowest relative to both EH and SFR during the familiar OF ($p < 0.001$ for both comparisons) (Fig. 2C). Average rearing duration was also affected by novelty, though in the opposite direction: the novel OF test showed no significant group differences, but in the familiar OF test, the MS animals had the shortest average rearing durations relative to both SFR and EH animals ($p < 0.001$ for both comparisons) (Fig. 2D).

Sex differences evoked by familiar environment

In assessing sex-dependent effects of novel vs. familiar OF tests, the general trend was that females' behaviors changed during the familiar OF where the males' behaviors did not. The measures that revealed significant Session $\times$ Sex interactions were rearing counts ($F(1,58) = 7.494$; $p = 0.008$), and exposed zone time ($F(1,58) = 4.074$; $p = 0.048$). For rearing counts (Fig. 3A), males and females behaved with similar rearing counts during the novel OF, but the females' rearing counts increased significantly during the familiar OF while the males' counts remained the same ($p < 0.001$). For exposed zone time (Fig. 3B), like the rearing counts, there were no sex differences in the novel OF, but in the familiar OF, females significantly increased their exposed zone time, while the males' exposed zone time remained the same ($p = 0.025$).

Frontal cortex thickness reduction in MS males

Dorsoventral cortical thickness (Fig. 4) was significantly reduced in MS as compared to EH males ($U = 19.5$; $p = 0.035$). No other significant effects were observed (Table 1). The cortex of SFR animals was not analyzed because they did not show any significant behavioral main effects or interactions as compared to EH animals.

Litter effects

To rule out the possibility that an aberrant litter could artificially drive a group difference, the individual means from each litter are also presented in Table 2. The means from each litter show that litters within groups were very similar in behavior.

DISCUSSION

Since adolescence is a time of major sexually dimorphic changes in the rat, we sought to determine sex-specific MS behavioral effects. Movement velocity was affected by MS in a sex-dependent fashion, increasing in MS males as compared to males of the comparison groups, but females were resistant to these increases in activity. A similar trend in which MS affected males but not females was evident in ambulatory counts. Dorsoventral cortical thickness was reduced in MS males, as compared to EH males, but was not different between groups in females. Adolescent females were more resistant to display the hyperactive/impulsive behavioral effects of MS found in male counterparts.

Reports of sex differences in the MS literature have described other separation-group dependent sex differences. One behavioral difference that stands out is that males exhibit increased anxiety-like behaviors compared to females (Barna, Balint, Baranyi, Bakos, Makara, & Haller, 2003; Wigger & Neumann, 1999; Kaneko et al., 1994). Behaviors related to locomotion and exploration such as OF activity, thigmotaxis and rearing all show sex differences; females show less thigmotaxis and rearing, while showing more ambulatory behavior in the OF (Kaneko et al., 1994). With regard to reward-seeking behaviors, MS females failed to acquire conditioned locomotor activity (Matthews, Wilkinson, & Robbins, 1996). In another study, MS females self-administered cocaine at higher levels than their male counterparts, suggesting a sex-dependent alteration in the dopaminergic system following MS (Matthews, Robbins, Everitt, & Caine, 1999).

Overall, sex affected nearly every behavior measured in adolescent Sprague-Dawley rats. Females had more ambulatory behavior, higher velocity during those ambulatory episodes, and more rearing counts than males. Particularly, SFR and EH females demonstrated greater activity than males in each measure. However, mean duration of each rearing episode was not different between the sexes. Females also appeared to show some behavioral effects of familiarity with the OF setting, in which rearing counts and exposed zone time were increased in the familiar OF, but no such changes occurred in the males' behavior.

While other rat strains, such as Holtzman, have been reported to show reduced or even reversed direction of sex differences in certain OF behaviors (Spivey et al., 2008a), the overall review of the literature provides a picture in which females are described to be more active and ambulatory than males (Blizard, Lippman & Chen, 1975; Slob, Bogers, & van Stolk, 1981; Slob, Huizer & van der Werff ten Bosch, 1986). It is therefore not surprising that in the current study, SFR and EH females had higher scores in measures of activity (both horizontal and vertical) than males.

Separation group behavioral effects were also strong, with MS increasing ambulatory counts, velocity, exposed zone time, and reducing rearing counts compared to the comparison groups. The effect of being in a novel environment appeared to affect MS

animals to a greater degree than the other two groups. Particularly, many of the measures of MS animals' ambulatory behavior were significantly higher than the other two groups in the novel OF, but this behavior remained high even when returned to the OF for a second, familiar, visit. However, regarding orienting behavior, the familiar OF is where MS animals were particularly lower compared to the other groups.

Our findings in Sprague-Dawley rats support existing literature which describes MS effects resulting in hyperactive/impulsive behavior. Various experimenters have reported increased ambulation in MS animals during a novel OF test (Hofer, 1975; Matthews, Wilkinson & Robbins, 1996; Brake, Zhang, Diorio, Meaney, & Gratton, 2004). Some MS protocols induce risk-taking behavior, such as increased venturing into exposed zones of an OF (Arnold & Sivi, 2002; Braun, Kremz, Wetz, Wagner, & Poeggel, 2003; Colorado et al., 2006; Kaneko et al., 1994; von Hoersten et al., 1993); however, others lead to anxious behavior, such as increased time in a dark compartment of an OF or the enclosed arms of an elevated plus maze (Spivey et al., 2008a; Janus, 1987; Matthews & Robbins, 2003; Daniels, Pietersen, Carstens, & Stein, 2004; Huot, Plotsky, Lenox, & McNamara, 2002). Potentially, some of the variation in phenotype resulting from MS may come from protocol differences (e.g., separation periods ranging from 3 to 6 hours). The common finding appears to be that MS can cause significant behavioral changes to the organism later in life.

Our measures of an ADHD-like behavioral profile in MS subjects rely on voluntary behaviors in OF and LD tests rather than learning tasks, while some measures of impulsivity are dependent on learning to delay gratification (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001). Our measures have the advantage of avoiding a possible confounding influence because learning deficits induced by MS (Matthews et al., 1996) could be interpreted as impulsivity (Colorado et al., 2006). The 6-hour/day MS protocol for 10 days (Kaneko et al., 1994; Jimenez-Vasquez et al., 2001) produced impulsive behavior in adolescent male Sprague-Dawley rats (Colorado et al., 2006) that was similar to that found in animal models of ADHD (King et al., 2000; Gonzalez-Lima & Sadile, 2000; Gonzalez-Lima, 2005).

The effect of being in a novel environment appeared to affect MS animals to a greater degree than the other two groups, which supports existing literature in which MS animals are more active and display more risk-taking behaviors in novel environments (Kaneko et al., 1994; Brake et al., 2004). Particularly, many of the measures of MS animals' ambulatory behavior were significantly higher than the other two groups in the novel OF, but this behavior remained high even when returned to the OF for a second, familiar, visit. However, regarding orienting behavior, the familiar OF is where MS animals were particularly lower compared to the other groups. This is consistent with the interpretation that orienting behavior as indexed by rearing is lower in a familiar environment in animal models of ADHD (Gonzalez-Lima, 2005).

Together the results of OF and LD test measures suggest an ADHD-like behavioral profile in MS subjects because the hyperactive/impulsive type of ADHD in humans and rat models is similarly characterized by hyper-reactivity to spatial novelty, increased frequency and faster velocity of locomotion, decreased frequency and shorter duration of orienting behavior, and more risk-taking behaviors (Gonzalez-Lima, 2005; Dickstein et al., 2006).

Our findings of sex differences in ADHD-like behaviors together with a reduction of frontal cortex thickness in MS males are consistent with the literature describing sex differences in prefrontal cortex anatomy and physiology as well as its involvement in impulsivity. Animal studies indicate that androgen exposure in males facilitates their development of a hyperactive/impulsive phenotype that is consistent with decreased frontal cortex

catecholamines and higher expression of ADHD-like behaviors (King et al., 2000; Li & Huang, 2006). A recent study by Spivey et al. (2008b) reported sex differences in cortical metabolic capacity in the Sprague-Dawley rats at 6 weeks of age. This neural finding in juvenile rats is relevant to the current MS findings because sex differences were found in cortical cytochrome oxidase, a rate limiting enzyme in cellular respiration. Cytochrome oxidase activity is an index of brain metabolic capacity that is correlated with behavioral predispositions (Sakata, Crews, & Gonzalez-Lima, 2005), and in particular with sex-related effects in various species including rats (Sakata, Coomber, Gonzalez-Lima, & Crews, 2000; Sakata, Gonzalez-Lima, Gupta, & Crews, 2002; Sakata, Gupta, Gonzalez-Lima, & Crews, 2002). Importantly, compared to females, males displayed reduced amounts of cytochrome oxidase in prefrontal cortical regions of the brain, regions implicated in the maturation of behavioral inhibition in infant rats (Nair, Berndt, Barrett, & Gonzalez-Lima, 2001), extinction of fear in rodents (Barrett, Shumake, Jones, & Gonzalez-Lima, 2003) and ADHD in humans (Dickstein et al., 2006).

The human literature indicates the dorsal prefrontal cortex of children and adolescents with ADHD is reduced bilaterally (Sowell et al., 2003), which is consistent with our dorsoventral cortical thickness findings. This anatomical alteration appears to be due to maturation delay in cortical thickness from children to adolescent patterns in individuals diagnosed with ADHD (Shaw et al., 2007). Animal models have also shown that prefrontal cortex hypometabolism is an important factor in ADHD-like behavior (Gonzalez-Lima, 2005). Since adolescent Sprague-Dawley female rats have higher frontal cortex metabolic capacity than males (Spivey et al., 2008b), females could be more resistant to display the MS-induced impulsive behavior and reduced cortical thickness that we found in male rats. This hypothesis was supported by our findings because MS affected adolescent male rats more than females in impulsivity (ambulatory velocity), orienting (rearing counts) and prefrontal cortex (dorsoventral thickness) measures. Baseline differences in prefrontal cortex metabolic capacity showing that males are hypometabolic relative to females may predispose males to the adverse effects of an early stressor such as mother-infant separation. Therefore, MS may have reduced cortical thickness preferentially in cortically hypometabolic males as compared to females. However, these differences in cortical thickness cannot account for the main effect of sex on behavior, but may be responsible for sex-dependent effects of MS on behavior. Nevertheless there are many other potential sex differences between adolescent rats that could account for the findings that female rats are more resistant than males to some adverse effects of MS. Further research is needed to understand whether cortical thickness is causally related to MS-induced impulsive behavior.

Overall, the findings are consistent with previous research demonstrating that this MS protocol results in hyperactive/impulsive behavior. However, this is the first study to our knowledge that finds sex-dependent effects of MS in adolescent Sprague-Dawley rats; particularly, that adolescent females are more resistant to the effects of MS on impulsive behavior and prefrontal cortex thickness. MS affected the two sexes differently, especially in terms of increased impulsivity as indexed by faster velocity of movement, and decreased prefrontal cortex maturation as indexed by reduced cortical thickness, with these effects reaching significant levels in males but not females. Findings of impulsivity and prefrontal cortex alterations from MS studies in rats may translate into an animal model for some aspects of ADHD in humans. Future directions for this line of research may involve observing the longitudinal effects of MS on hyperactive/impulsive behavior across the lifespan of rats, and further investigation of brain areas related to these behavioral effects.

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Figure 1.

Activity measures in adolescent rats averaged across three daily test sessions. Females show greater overall (A) ambulation, (B) velocity, and (C) rearing than males. Maternally separated (MS) rats show greater overall ambulation and velocity and less rearing than early handled (EH) and standard facility reared (SFR) rats. MS effects were generally more manifest in males than females, especially the increase in velocity of movement (B) and the decrease in rearing (C). a = female score significantly greater than male; b = MS group significantly different than EH and SFR groups.

Figure 2.

Effects of maternal separation as a function of novel vs. familiar environment. Data from both males and females were averaged and compared for the three groups, to identify group effects regardless of sex. (A) Maternally separated (MS) rats show high movement velocity in both a novel and familiar open field, whereas early handled (EH) and standard facility reared (SFR) rats show reduced velocity in the novel open field. (B) MS rats spend more time than EH and SFR rats exploring the exposed center zone of the novel open field. SFR rats match this level of risk taking in the familiar open field while EH rats continue to show less risk taking. (C) MS rats decrease rearing while EH and SFR rats increase rearing after repeated exposure to an open field. (D) SFR rats increase the duration of their rearing episodes while MS and EH rats decrease the duration of their rearing episodes after repeated exposure to an open field. MS rats show briefer rearing episodes than EH and SFR rats across both test sessions. * = MS group significantly different than EH and SFR groups.

Figure 3.

Sex differences evoked by familiar environment. Data from all groups were averaged to identify sex effects regardless of group. Females show significantly greater (A) rearing and (B) time in the center (exposed zone) of a familiar, but not novel, open field. * = female score significantly greater than male.

Figure 4.

A coronal hemisphere of a representative section of the rat prefrontal cortex (Bregma +3.7; figure adapted from Gonzalez-Lima & Cada, 1998). Cortical thickness was measured along the two white lines. DV = Dorsoventral; ML = Mediolateral, RF = rhinal fissure.

Table 1Means $\pm$ SEM for dorsoventral and mediolateral cortical thickness in adolescent rats.

SEX	GROUP	Dorsoventral (mm)	Mediolateral (mm)
Male	EH	3.97 $\pm$ 0.11	3.98 $\pm$ 0.55
	MS	3.77 $\pm$ 0.04	4.05 $\pm$ 0.04
	% Difference	5.3 *	1.8
Female	EH	3.82 $\pm$ 0.04	4.12 $\pm$ 0.01
	MS	3.8 $\pm$ 0.08	4.04 $\pm$ 0.07
	% Difference	0.05	1.9

*
 $p < 0.05$

Table 2

Behaviour measures with group differences (mean $\pm$ SEM) for individual MS and EH litters, and SFR group.

	EH - Litters 1		EH - Litters 2		MS - Litter 3		MS - Litter 4		MS - Litter 5		SFR	
	male	female	male	female	female	male	male	female	male	female	male	female
Total Ambulatory Counts												
OF Novel	407 $\pm$ 171	625 $\pm$ 153	341 $\pm$ 120	656 $\pm$ 98	936 $\pm$ 120	909 $\pm$ 157	1175 $\pm$ 223	1175 $\pm$ 171	545 $\pm$ 75	656 $\pm$ 50		
Light-Dark	711 $\pm$ 146	1000 $\pm$ 63	795 $\pm$ 117	1199 $\pm$ 88	1142 $\pm$ 100	992 $\pm$ 174	1120 $\pm$ 238	1054 $\pm$ 295	989 $\pm$ 76	1101 $\pm$ 73		
OF Familiar	518 $\pm$ 289	956 $\pm$ 51	752 $\pm$ 160	1357 $\pm$ 142	1369 $\pm$ 130	1063 $\pm$ 116	1139 $\pm$ 217	1062 $\pm$ 117	873 $\pm$ 80	1026 $\pm$ 82		
Mean Velocity (cm/s)												
OF Novel	30 $\pm$ 4	38 $\pm$ 9	28 $\pm$ 8	36 $\pm$ 2	43 $\pm$ 2	48 $\pm$ 4	49 $\pm$ 5	50 $\pm$ 3	31 $\pm$ 4	38 $\pm$ 3		
Light-Dark	66 $\pm$ 9	83 $\pm$ 2	69 $\pm$ 7	85 $\pm$ 2	76 $\pm$ 5	87 $\pm$ 5	91 $\pm$ 7	80 $\pm$ 2	77 $\pm$ 3	76 $\pm$ 4		
OF Familiar	29 $\pm$ 9	48 $\pm$ 4	43 $\pm$ 9	56 $\pm$ 5	52 $\pm$ 2	49 $\pm$ 6	47 $\pm$ 6	45 $\pm$ 4	46 $\pm$ 3	53 $\pm$ 3		
Total Vertical Counts												
OF Novel	65 $\pm$ 15	98 $\pm$ 25	60 $\pm$ 17	81 $\pm$ 16	107 $\pm$ 11	82 $\pm$ 19	96 $\pm$ 20	86 $\pm$ 14	54 $\pm$ 11	54 $\pm$ 6		
Light-Dark	129 $\pm$ 19	164 $\pm$ 5	136 $\pm$ 8	143 $\pm$ 12	136 $\pm$ 13	34 $\pm$ 11	33 $\pm$ 9	36 $\pm$ 12	93 $\pm$ 5	90 $\pm$ 8		
OF Familiar	72 $\pm$ 26	153 $\pm$ 14	115 $\pm$ 27	167 $\pm$ 13	145 $\pm$ 9	29 $\pm$ 7	31 $\pm$ 7	21 $\pm$ 6	100 $\pm$ 11	115 $\pm$ 5		
Exposed Zone Time (%)												
OF Novel	9 $\pm$ 4	15 $\pm$ 5	5 $\pm$ 2	7 $\pm$ 2	12 $\pm$ 2	22 $\pm$ 6	25 $\pm$ 6	20 $\pm$ 4	11 $\pm$ 2	13 $\pm$ 2		
Light-Dark	10 $\pm$ 6	35 $\pm$ 1	35 $\pm$ 1	35 $\pm$ 5	54 $\pm$ 12	29 $\pm$ 7	36 $\pm$ 6	26 $\pm$ 6	25 $\pm$ 4	25 $\pm$ 4		
OF Familiar	7 $\pm$ 2	10 $\pm$ 3	10 $\pm$ 3	14 $\pm$ 4	14 $\pm$ 5	17 $\pm$ 2	20 $\pm$ 4	14 $\pm$ 4	22 $\pm$ 2	24 $\pm$ 2		