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# Early Adversity Alters Attention and Locomotion in Adult Sprague–Dawley Rats

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This study investigated the effects of prenatal stress and its interaction with artificial rearing (AR) on adult rat behavior. Pregnant dams underwent restraint stress from Gestational Day 10 to 21. After parturition, pups were raised by their mothers or in the AR paradigm, with or without stroking stimulation. In adulthood, rats were tested on prepulse inhibition (PPI), locomotor activity, elevated plus-maze, and spatial working memory. There were main effects and interactions of both prenatal stress and AR on activity. Additional stimulation reduced activity in nonstressed AR rats but increased activity in prenatally stressed AR rats. AR altered PPI and plus-maze behavior whereas additional stimulation partially reversed these effects. This study demonstrates that prenatal experiences can modulate the effects of postnatal treatments.

*Keywords:* prenatal stress, rat artificial rearing, attention, locomotion, emotionality

The central nervous system (CNS) is highly plastic and susceptible to various factors during the perinatal period. Environmental factors are especially salient during this period, and the effects of early experiences can continue into adulthood (Reisen, 1947). The mother–offspring relationship is often the central mediator of these environmental effects, and thus mothers exert a long-lasting influence on the physiology and behavior of their offspring (Lephart, Watson, Jacobson, Rhees, & Ladle, 1997; Patin et al., 2002; Zhang, Parent, Weaver, & Meaney, 2004). This can be accomplished through the direct effects of the mother's behavior as well as indirectly through other factors, such as the mother's prenatal hormones and stress, which can alter the developing CNS (see Knackstedt, Hammelmann, & Arck, 2005, for a review). Prenatal experience is almost exclusively mediated by the mother and provides the background for subsequent postnatal environmental factors. The primary focus of this study was to investigate the effects of prenatal stress and its interaction with postnatal experiences on the development of attention, locomotor activity, emotionality, and cognition in rats. These behavioral outcomes are known to be affected in human populations suffering from attention-deficit hyperactivity disorder (ADHD; Robbins, 2000) as well as in those raised under adverse environmental conditions, such as orphanages and institutions (Kreppner, O'Connor, Rutter, & The English and Romanian Adoptees Study Team, 2001).

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Prenatal stress in animals produces a wide range of behavioral and physiological changes in both the mother and her offspring. Behavioral changes in the offspring include reductions in sexual behavior, conditioned avoidance, and social behavior, as well as increases in aggressive behavior (see Chapillon, Patin, Roy, Vincent, & Caston, 2002, for a review). A number of learning and memory changes have also been observed. The most consistently reported are reduced rates of maze and reversal learning (Aleksandrov, Polyakova, & Batuev, 2001; Lemaire, Koehl, Le Moal, & Abrous, 2000; Weller, Glaubman, Yehuda, Caspy, & Ben-Uria, 1988).

Compared with controls, prenatally stressed offspring also demonstrate higher rates of emotionality in the open field, elevated plus-maze, and forced swim test along with a higher propensity to self-administer drugs (Alonso, Arevalo, Afonso, & Rodriguez, 1997; Deminière et al., 1992; Wakshlak & Weinstock, 1990). Performance on tests of attention, as measured through the sensorimotor gating task, prepulse inhibition (PPI; Barbazanges, Piazza, Le Moal, & Maccari, 1996; Lehmann, Stöhr, & Feldon, 2000), and locomotor activity (Deminière et al., 1992; Ordyan & Pivina, 2003; Weller et al., 1988) are also altered in these animals. In general, studies have suggested that prenatal stress has a greater effect on male rats. However, research conducted with female rats has not been as extensive (Kofman, 2002; Nishio, Kasuga, Ushijima, & Harada, 2001; Szuran, Pliška, Pokorny, & Welzl, 2000).

In accordance with changes in behavior systems, prenatal stress produces various effects on the CNS and endocrine systems, which may mediate these stress effects on behavior. Such changes include alterations in the activity of the prenatal gonads (Ward, 1972), decreased neurogenesis (Lemaire, Koehl, Le Moal, & Abrous, 2000), as well as hyperactivity and chronic up-regulation of the hypothalamic–pituitary–adrenal (HPA) axis (Fride, Dan, Feldon, Halevy, & Weinstock, 1986; Lemaire, Koehl, Le Moal, & Abrous, 2000; Maccari et al., 1995; Vallée et al., 1999; Vallée, Mayo, Maccari, Le Moal, & Simon, 1996).

The postnatal environment can also have a considerable impact on the development of an organism. To assess the effects of the postnatal environment on behavior and physiology, numerous studies have explored the effects of early separation from the mother on development of the offspring. Separation periods can range from a single separation during the preweaning period (Ellenbroek, van der Kroonenberg, & Cools, 1998) to repeated daily separations (15 min to 5 hr; Brake, Zhang, Diorio, Meaney, & Gratton, 2004; Meaney et al., 1991). In addition, separations can encompass almost the entire preweaning period (artificial rearing; see below; Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001; Lovic & Fleming, 2004). On the basis of these temporal parameters, maternal separation studies have found multiple and opposing effects on behavior and physiology. Short periods of separation or handling regimens appear to be beneficial to pups (Lehmann et al., 2000; Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; Meaney et al., 1985). In contrast, long periods of daily separations appear to be detrimental (Ellenbroek et al., 1998; Francis, Diorio, Plotsky, & Meaney, 2002).

In addition, separation of young from the mother and siblings throughout the preweaning period (artificial rearing) produces still greater behavioral and physiological effects (Gonzalez et al., 2001; Lovic & Fleming, 2004). Complete separations or isolations can be accomplished through an artificial rearing procedure, where artificially reared (AR) rats are removed from the mother on Postnatal Day (PND) 2 and are reared in a "pup-in-a-cup" regimen (Hall, 1975). This maternal separation paradigm allows for the manipulation of various maternal care and nest factors, including temperature, food type, and schedule, as well as exposure to tactile stimulations normally supplied by the mother and littermates (Gonzalez et al., 2001; Levy, Melo, Galef, Madden, & Fleming, 2003; Lovic & Fleming, 2004; Melo et al., 2006). The amount of tactile stimulation received by pups is a particularly important factor. This type of stimulation has been found to reverse many of the consequences associated with AR (Gonzalez et al., 2001; Lovic & Fleming, 2004). In addition, in a natural (nest) environment, tactile stimulation provided by the mother, has been found to be a moderator of HPA activity in the offspring (Champagne & Meaney, 2001; Fish et al., 2004).

In a series of studies we have found that rats reared without their mothers, in the AR pup-in-a-cup paradigm, show increased activity in the open field, reduced maternal care toward their own offspring, and reduced attention and sensorimotor gating (Gonzalez et al., 2001; Lovic & Fleming, 2004; Novakov & Fleming, 2005). However, providing AR rats with additional somatosensory, tactile licking-like stimulation reverses these effects (Gonzalez et al., 2001; Levy et al., 2003; Lovic & Fleming, 2004; Novakov & Fleming, 2005).

Although it is clear that prenatal stress and postnatal environmental manipulations affect various behavioral systems, few studies have explored how the effects of manipulations during these two periods in developmental life combine or interact with one another. The purpose of this study was to examine the effects of these pre- and postnatal manipulations on behavior within the same group of rats. We focused on a wide range of behavioral measures such as attention, hyperactivity, emotionality, and cognition.

In this study, attention was measured with the prepulse inhibition (PPI) of the acoustic startle response paradigm. The acoustic startle response is a rapid contraction of facial and skeletal muscles in response to an unexpected and intense stimulus (Koch & Schnitzler, 1997). PPI refers to a reduction of the startle response to a powerful pulse stimulus when immediately preceded by a weaker, nonstartling prepulse stimulus (Borrell, Vela, Arévalo-Martin, Molina-Holgado, & Guaza, 2002). PPI is a measure of sensorimotor gating, a nonvolitional aspect of attention, that has been used in both human and animal populations (Braff, Geyer, & Swerdlow, 2001; Koch & Schnitzler, 1997; Varty, Braff, & Geyer, 1999).

Both prenatal and postnatal manipulations can alter PPI. Prenatal stress has been shown to reduce PPI (Koenig et al., 2005; Mintz, Yovel, Gigi, & Myslobodsky, 1998). However, these finding have not been universal (Lehmann et al., 2000). In addition, isolation rearing during the juvenile period (Bakshi, Swerdlow, Braff & Geyer, 1998) and artificial rearing have also produced a reduction in PPI (Lovic & Fleming, 2004). Moreover, handling and artificial rearing with tactile stimulation partially reverse these effects (Lehmann et al., 2000; Lovic & Fleming, 2004).

The effects of prenatal stress on emotionality have been extensively investigated (Alonso, Arevalo, Afonso, & Rodriguez, 1997; Deminière et al., 1992; Lehmann et al., 2000). However, studies on the effects of maternal separation on emotionality have yielded variable results (Kalinichev, Easterling, Plotsky, & Holtzman, 2002; McIntosh, Anisman, & Merali, 1999). The effects seem to depend on the separation procedure, testing conditions, strain, and sex. Furthermore, the anxiogenic effects of prenatal stress have recently been demonstrated to be more robust than those of maternal separation (Estanislau & Morato, 2005).

Finally, although many studies have investigated the impact of early adversity on cognition, few have examined working memory. In terms of spatial memory, prenatal stress has been shown to impair long-term and working memory in the Morris water maze (Aleksandrov, Polyakova, & Batuev, 2001; Gue et al., 2004; Lemaire, Koehl, Le Moal, & Abrous, 2000). In contrast, periodic and complete maternal separation have not produced significant impairment in water maze learning and memory (Lehmann, Pryce, Bettschen, & Feldon, 1999; Levy et al., 2003). Although the effects of early adversity on long-term spatial memory have been the focus of a number of studies, the effects on spatial working memory have not been fully examined.

The purpose of this study was to examine the combined effects of prenatal stress and motherless rearing, with the use of the AR paradigm, on the development of attention, locomotor activity, emotionality, and spatial memory in adult rats. Additionally, because sex differences are either apparent or inconclusive for all of the aforementioned behaviors, this study assessed the effects on both male and female rats. In accordance with the majority of studies examining the effects of prenatal stress, this study used the restraint stress paradigm. This method has been shown to increase corticosterone and adrenocorticotropic (ACTH) levels in both the dam and the fetus (Williams, Davis, McCrea, Long, & Hennessy, 1999). Because both prenatal stress and maternal separation increase emotionality and locomotor activity, and decreases attention, we expected that combining both stressors would potentiate these effects and that tactile stimulation would partially reverse them. Further, we expected prenatal stress to impair spatial working memory in the water maze task but AR to have no effects.

Moreover, because males appear to have an increased sensitivity to prenatal factors, we expected their deficits to be more severe.

## Method

### Subjects

A total of 137 Sprague–Dawley rats (79 females and 58 males) were used in this study. The rats were born and raised at the University of Toronto at Mississauga from stock originally obtained from Charles River Farms (St. Constant, Quebec, Canada). The colony was maintained on a 12-hr light–dark cycle, with lights at 0800 in a room maintained at approximately 22 °C, humidity 50–60%. Beginning on PND 21, rats were housed two per cage (clear Plexiglas, 20 cm × 43 cm × 22 cm), with food (Purina Rat Chow) and water available ad libitum.[1]

### Apparatus and Procedure

*Mating.* Vaginal smears were taken from 19 virgin females over several days at approximately 1400. Once the females were in proestrus, they were placed with a sexually experienced male for 24 hours. Presence of spermatozoa in a vaginal smear after mating was considered Gestational Day (GD) 0.

*Prenatal stress.* Dams were left undisturbed until GD 10, at which point they were randomly assigned to the prenatally stressed (PS) or the prenatally nonstressed (NS) group. From GD 10 to 21, PS dams were weighed and then placed in a Plexiglas restrainer (8 cm diameter × 20 cm length) for 4 hr per day at random times between 900 and 1800. Restrainers were designed to limit, but not prohibit, movement of the pregnant female and to avoid constriction of her abdomen. The length of the chamber was flexible (15 to 18 cm) to accommodate the range of dam sizes. After each session, the restrainers were cleaned thoroughly with 30% alcohol. This type of stressor has been used previously (Lehmann et al., 2000; Lemaire, Koehl, Le Moal, & Abrous, 2000; Vallée et al., 1997). However, instead of beginning on the last week of pregnancy as in previous studies (Lehmann et al., 2000; Lemaire, Koehl, Le Moal, & Abrous, 2000; Vallée et al., 1996), our stressor began on GD 10 to encompass the entire development of the CNS. Further, we increased the daily length of the stressor from three 45-min sessions to a single 4-hr session in an attempt to heighten the stress experience (Fujioka et al., 1999; Hashimoto et al., 2001). Rats were monitored to ensure appropriate weight gain and any signs of poor health. NS dams were left undisturbed.

*General procedures.* Dams were allowed to give birth undisturbed. On the day of parturition (PND 0), litters were culled to 14 rats (7 males, 7 females). On PND 4, 3 male and 3 female pups were removed from the nest and implanted with a cheek cannula (see *Surgery and Artificial Rearing* below). The remaining two pups stayed with the dam (mother reared, control [MR–con]). One male and one female each received a sham surgery and were returned to the nest (mother reared, sham surgery [MR–sham]), and the remaining pups were artificially reared and randomly assigned to two groups (see *Treatment and Groups* below, which includes a description of the minimal [min] and maximal [max] stimulation conditions). This resulted in the following group compositions for males: PS AR–min = 9; PS AR–max = 6; PS MR–sham = 9; PS MR–con = 9; NS AR–min = 4; NS AR–max = 7; NS MR–sham = 7; and NS MR–con = 7. The group composition for females was as follows: PS AR–min = 7; PS AR–max = 7; PS MR–sham = 9; PS MR–con = 9; NS AR–min = 8; NS AR–max = 4; NS MR–sham = 8; NS MR–con = 8. These rats were derived from 19 litters, with only one rat from each litter per group.

*Surgery and artificial rearing.* Prior to surgery, pups were weighed, and a topical anesthetic (Eutectic Mixture of Local Anesthetics [EMLA], containing 2.5% lidocaine and 2.5% prilocaine) was applied to their right cheek. A leader wire (stainless steel, 0.25 mm in diameter), sheathed in lubricated (mineral oil), silastic tubing, and polyethylene (PE) 10 tubing was used to pierce the cheek. Once the flared end of the tubing contacted the inside of the cheek, the leader wire and silastic tubing were removed, and Polysporin antibiotic ointment was applied topically to the site of penetration. Another leader wire was then used to insert a t-washer, which was secured in place with Superglue. MR–sham pups had their cheeks pierced, but the PE 10 tubing was removed. Polysporin was applied to the site of penetration, and nontoxic permanent black marker was applied to the pups' ears prior to placing them back with the litter for later identification.

After surgery, AR pups were placed individually in plastic cups (11 cm diameter × 15 cm deep) containing corn cob bedding (Bed o'cobs, The Andersons, Maumee, OH), each of which was placed inside another weighted cup. The cups floated in temperature controlled (34–37 °C) water directly below time-controlled infusion pumps (PHD 22/2000 syringe, Harvard Apparatus, Holliston, MA) to which they were connected with the cheek cannula tubing. The pumps delivered milk (Messer diet) for 10 min every hour, 24 hr a day. The amount infused was calculated on the basis of mean pup body weight. Beginning on PND 4, pups received a volume of milk equal to 33% of the mean body weight, and this amount increased by 1% daily. Each morning, the pups were disconnected from the pumps, their weight was recorded, and all tubing was flushed with double distilled water. New syringes with fresh formula were prepared, and the new infusion rates were programmed on the basis of the new weights.

*Treatment and groups.* AR rats were randomly assigned to either AR–min or AR–max groups. Each day, AR–min pups were stimulated twice (morning and night) for 30 s each with a wet camel hair paintbrush in the anogenital region to stimulate urination and defecation. AR–max pups received the same anogenital stimulation twice daily, as well as 2 min of dorsal stimulation with a dry camel hair paintbrush eight times a day. Stimulations for both groups occurred daily from PND 4 to 16. On PND 17–18, pups were removed from the pumps and given milk formula, rat chow, and a mixture of the two.

On PND 21, all rats were weighed and paired with a social partner of the same sex from another litter that was not tested. Rats were left undisturbed until Day 60 when they were tested on PPI, locomotor activity, elevated plus-maze, and spatial working memory (in this order). Rats were left undisturbed for at least a week between tests. Procedures for PPI are based on Lovic and Fleming (2004). All behavioral testing occurred during the light cycle.

*Acoustic startle response and prepulse inhibition test.* Rats were tested in one of four acoustic startle cubicles (55.9 cm × 38.1 cm × 35.6 cm internal dimensions; Med Associates, St. Albans, VT), lit with a red light, and fan ventilated. A grid floor rat holder (16.5 cm × 7.6 cm × 8.9 cm) was mounted on top of a startle platform, which detected and transduced the movement of the rat. All acoustic stimuli were delivered through two supertweeter speakers (Model No. 40-1310, Radio Shack, Fort Worth, TX) located along the back wall, which were controlled by a personal computer equipped with Windows-based Startle Reflex Software (Version 3.35; Med Associates Inc., St. Albans, VT).

PPI was measured during a single testing session after PND 60. Prior to testing, rats were brought into an adjacent suite and allowed to acclimatize for 30 min. Rats were weighed and placed into a metal grid floor rat holder, which attached to the startle platform. The test lasted 25.5 min, beginning with a 7-min acclimatization period with a 70-dB background noise, which was presented throughout the test. In Block 1, there were four pulse/startle trials (120 dB, 10 kHz, 30 ms). In Block 2, there were 60 trials presented in random fashion but consistent between rats. These trials consisted of 8

[1] All the procedures described in this report conform to the guidelines set by the Canadian Council on Animal Care and are in accordance with Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. The procedures were approved by the University of Toronto at Mississauga Animal Care Committee.

startle trials (120 dB, 10 kHz, 30 ms long), 16 prepulse-alone trials (10 kHz, 20-ms long, 4 for each intensity: 72, 74, 80, 84 dB), four no-stimulus (null) trials, and 32 prepulse and startle trials (8 of each prepulse intensity, with a 120-dB startle pulse, 100 ms between prepulse and pulse). To calculate the percentage of PPI, we used the following formula: [1 − (prepulse and startle response/startle response)] × 100. Averaged responses during the 100 ms following the termination of sound stimulus were the dependent measures. The metal grid animal holder was cleaned thoroughly with 70% alcohol between each test.

*Locomotor activity.* Locomotor activity was assessed in one of eight activity boxes (clear Plexiglas cages, 22 cm × 44 cm × 30 cm; custom made by the Centre for Addiction and Mental Health, Toronto, Ontario, Canada). Two arrays of 16 infrared photocells, mounted immediately outside, extended lengthwise along the cages. The photocells were spaced 2.5 cm apart and 2 cm and 12 cm above the floor of the cage. Rats were tested individually in activity boxes for 1 hr in a dimly lit room. The test was repeated 24 and 48 hr after the initial test. Amount of ambulation was measured in 5-min intervals with photocells. Between rats, the cage was cleaned thoroughly with 70% alcohol.

*Elevated plus-maze.* The plus-maze apparatus was elevated 50 cm above the floor, and all arms of the maze were 10 cm wide. The enclosures of the closed arms were 40 cm high. Rats were placed in the middle of the maze and allowed to explore it for 10 min. The number of crossings into and the time spent in the open and closed arms were recorded. A crossing was counted when all four feet had passed into a particular arm. Time spent in the middle of the maze that intersected all four arms was considered part of the time spent in closed arms.

*Spatial working memory task.* Spatial working memory was assessed with a blue Nalgene cylindrical tank (50 cm height × 170 cm diameter; Nalge Nunc International, Rochester, NY) filled with water. The water was made white with a nontoxic tempera paint. The white platform was 20 cm tall × 10 cm in diameter and was submerged under 3 cm of water. The four cardinal points in the room (north, south, west, east) were demarcated with distinctive visual cues.

Spatial working memory was assessed over 7 consecutive days. The first 2 days were considered the training period. Every day the rats were administered four trials, with a 15-s intertrial interval, to learn the position of the platform. Trials were a maximum of 2 min; if the rat had not found the platform within this time frame, the rat was guided to the location. After each trial, rats were left on the platform for 20 s. Rats were released into the pool from a different cardinal location in a pseudorandom order on each trial. Each day the order of the release location and the position of the platform were changed. The position of the platform changed to one of seven new locations, which were situated in one of the quadrants of the pool. Therefore, the rats did not have to form a long-term memory, as the platform location would change every day. The rats had to use their working memory; they had to hold the location of the platform "online" only for the duration of a particular test. The latency to reach the platform in seconds was used as a measure of spatial working memory. The final test of the series, the spatial working memory task, was administered to male rats only, as females had to be mated for another study (data not presented here).

*Statistical analysis.* PPI data were examined with a 2 (stress: prenatally stressed vs. prenatally nonstressed) × 2 (sex: male vs. female) by 3 (rearing: AR–min vs. AR–max vs. MR) by 4 (decibel interval: 72 vs. 76 vs. 80 vs. 84 dB) repeated measures analysis of variance (ANOVA). Locomotor activity and elevated plus-maze data were examined with a 2 (stress) × 2 (sex) × 3 (rearing) multivariate ANOVA. Additionally, for locomotor activity, we used a 2 (stress) × 3 (rearing) × 3 (days) repeated measures ANOVA to analyze level of habituation over the 3 testing days. Water maze data were analyzed with a 2 (stress) × 3 (rearing condition) × 4 (trials) repeated measures ANOVA. We used Tukey's post hoc analysis to examine group differences. Finally, partial correlations controlling for sex and prenatal and postnatal conditions were conducted for measures of acoustic startle, PPI, locomotor activity, emotionality, and spatial working memory. The level of statistical significance was $p < .05$.

## Results

For all behavioral tests, no significant differences were found between the MR–sham and MR–con groups. Therefore, these groups were combined and are referred to as MR in the following discussion.

### *Acoustic Startle Response*

For analysis of the acoustic startle response, body weight was used as a covariate, as body weight can affect the extent of the startle response. No significant main effect of prenatal stress was found for acoustic startle responses. However, a significant main effect of rearing was observed, $F(2, 105) = 3.69$, $p = .028$. AR–min rats demonstrated a significantly greater startle response compared with MR rats ($p = .010$; Figure 1). Also, a significant main effect of sex was observed, $F(1, 105) = 6.83$, $p = .010$. Males exhibited a significantly higher startle response than did females. No significant interactions were found.

### *Prepulse Inhibition of the Acoustic Startle Response*

No significant main effects of prenatal condition, sex, or decibel level were found for PPI. However, there was a significant main effect of postnatal rearing conditions, $F(1, 105) = 4.78$, $p = .010$. As shown in Figure 2, AR–min rats had significantly lower levels of PPI than did MR rats ($p = .007$). AR–max rats did not differ from either group. No significant interactions were observed.

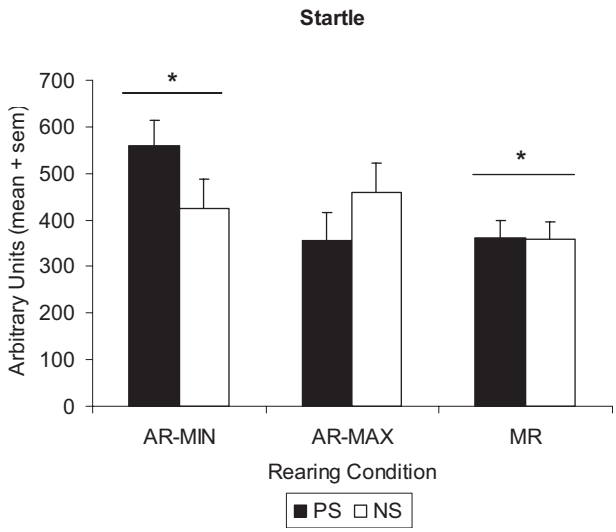


*Figure 1.* Artificial rearing with minimal stimulation increased acoustic startle response, whereas artificially reared rats with maximal stimulation performed similarly to mother reared rats. PS = prenatally stressed; NS = prenatally nonstressed; AR–MIN = artificially reared with minimal stimulation, AR–MAX = artificially reared with maximal stimulation; MR = mother reared; sem = standard error of the mean. PS AR–MIN = 16; PS AR–MAX = 13; PS MR = 34; NS AR–MIN = 11; NS AR–MAX = 11; NS MR = 32. *$p < .05$.

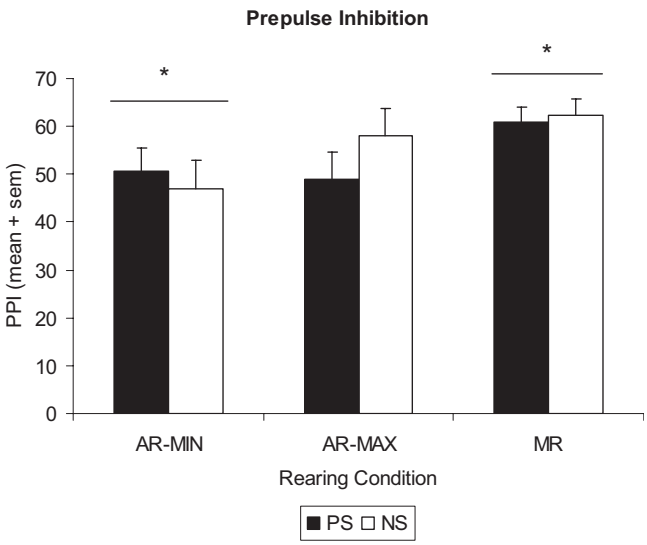


*Figure 2.* Maximal stimulation partially reversed the reduction in prepulse inhibition (PPI) produced by artificial rearing. PS = prenatally stressed; NS = prenatally nonstressed; AR–MIN = artificially reared with minimal stimulation; AR–MAX = artificially reared with maximal stimulation; MR = mother reared; sem = standard error of the mean. PS AR–MIN = 16; PS AR–MAX = 13; PS MR = 34; NS AR–MIN = 11; NS AR–MAX = 11; NS MR = 32. *$p < .05$.

### *Locomotor Activity*

There were significant main effects of prenatal stress, $F(1, 107) = 6.41$, $p = .013$; postnatal rearing, $F(2, 107) = 42.11$, $p < .0001$; and sex, $F(1, 107) = 13.90$, $p < .0001$, for general activity levels. Prenatally stressed rats were significantly more active than nonstressed rats; both AR–min and AR–max rats were significantly more active than MR controls ($p = .0001$ for both AR groups), and females were significantly more active than males.

Also, there was a significant interaction between prenatal and rearing conditions, $F(2, 107) = 4.24$, $p = .017$. AR–max rats had lower activity, more similar to MR animals, if nonstressed. If prenatally stressed, AR–max rats had the highest level of locomotor activity. Other groups did not show the same pattern. Separate repeated measures analyses revealed that this effect was a result of differences in habituation. MR rats in both prenatal conditions displayed a typical locomotor habituation over the 3 days. AR–min rats in both prenatal conditions failed to habituate over the testing sessions. However, AR–max rats exhibited significantly different responses depending on their prenatal condition, $F(1, 21) = 8.42$, $p = .009$. Nonstressed AR–max rats displayed locomotor habituation comparable to MR rats, but prenatally stressed AR–MAX rats did not show locomotor habituation over the course of testing (Figures 3a, 3b, 3c, and 3d).

### *Elevated Plus-Maze*

Prenatal stress did not significantly affect any plus-maze measure. However, a significant main effect of rearing condition was observed for time spent in the open arms, $F(2, 105) = 4.42$, $p = .014$. Specifically, AR–min rats spent significantly more time in the open arms then did MR rats ($p = .013$). AR–max rats did not significantly differ from MR rats or AR–min rats (Figure 4). None of the groups differed on the number of crossings or on ratio of open to total crossings. There were no significant interactions.

### *Spatial Working Memory Task*

Neither prenatal stress nor rearing condition significantly affected latency to reach the platform during the training or testing periods. Furthermore, there were no significant interactions (see Figures 5a, 5b, 5c, and 5d).

### *Correlations*

Correlations between acoustic startle, PPI, locomotor activity, and plus-maze performance were analyzed to establish whether these behavioral systems were related to one another. Analyses conducted with partial correlations revealed that startle was significantly positively correlated with average beam cuts ($r = .247$, $p = .009$). However, PPI and activity were not significantly correlated. Conversely, average beam cuts were significantly positively correlated with time spent in the open arms ($r = .20$, $p = .038$) and with crossings into open arms ($r = .289$, $p = .002$).

## Discussion

This study was conducted to evaluate the relation between prenatal stress and artificial rearing in terms of their effect on attention, locomotor activity, emotionality, and cognition in adult rats. These endpoints have been associated with a variety of human clinical conditions, including ADHD, schizophrenia, and the inattentive/overactivity syndrome of institutionalized children (Kreppner et al., 2001; Robbins, 2000; Weike, Bauer, & Hamm, 2000). Consistent with prior work, this study showed that prenatal stress and postnatal adversity, in the form of artificial rearing, produced alterations in a number of behaviors. In general, the effects of these manipulations were not additive. However, there also occurred a clear interaction between prenatal and postnatal adversity on locomotor activity. Providing AR rats with additional licking-like stimulation (AR–max) decreased locomotion in prenatally nonstressed rats such that they looked more like their mother-reared counterparts. In contrast, providing the same stimulation to prenatally stressed AR rats produced opposite effects. Prenatal stress increased their locomotor activity to levels that were higher than the already high levels shown by AR minimally stimulated animals. These results indicate that the effects of postnatal experience on certain behaviors can depend on the prior background of the animal being stimulated. Below we discuss the effects of prenatal and postnatal manipulations on each of the separate endpoints, finishing with a discussion of the potential mechanisms behind the interaction.

### *Effects of Prenatal and Postnatal Manipulations*

*Acoustic startle.* The acoustic startle response was disrupted by postnatal rearing conditions. AR–min rats, who received low levels of somatosensory stimulation, had a significantly higher startle response than did MR rats. These results are consistent with those of studies involving high- versus low-licking mothers (Zhang, Chretien, Meaney, & Gratton, 2005), although they are not consistent with previous findings obtained with the AR paradigm

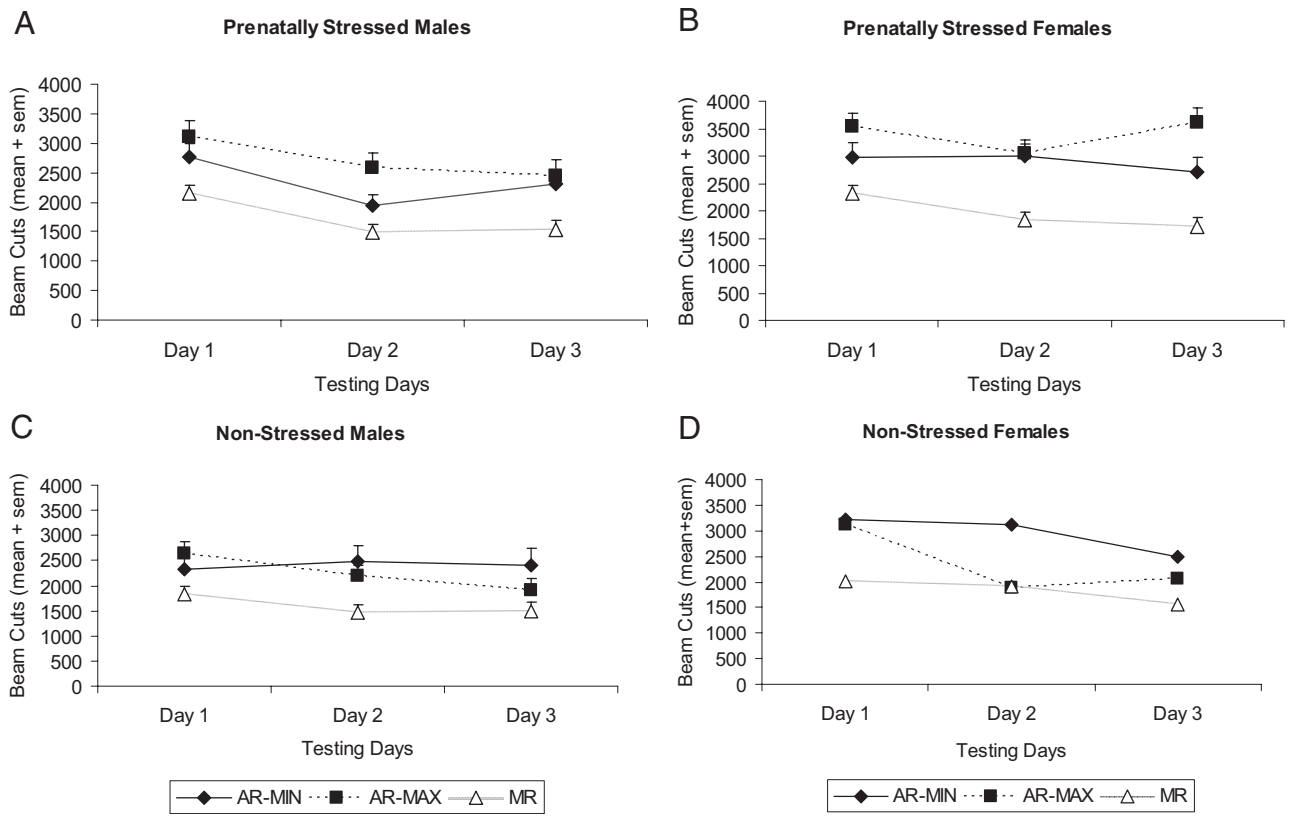


*Figure 3.* Locomotor activity. (A) Prenatally stressed (PS) and artificially reared (AR) male rats were significantly more active than prenatally stressed mother-reared male rats. PS AR–MIN = 9; PS AR–MAX = 6; PS MR = 18. (B) Prenatally stressed and artificially reared female rats were significantly more active than prenatally stressed mother-reared female rats. PS AR–MIN = 7; PS AR–MAX = 6; PS MR = 20. (C) Nonstressed and artificially reared male rats were more active than nonstressed mother-reared male rats. NS AR–MIN = 4; NS AR–MAX = 7; NS MR = 15. (D) Nonstressed and artificially reared female rats were more active than nonstressed mother-reared female rats. NS AR–MIN = 6; NS AR–MAX = 4; NS MR = 17. AR–MIN = artifiically reared with minimal stimulation; AR–MAX = artificially reared with maximal stimulation; MR = mother reared; sem = standard error of the mean.

among female rats (Lovic & Fleming, 2004). Hence, the difference in the results of our study and previous AR studies may be due to sex differences in the effects of AR on acoustic startle, as males in our study showed a higher startle response than did females. Further, although we did not find a significant interaction between prenatal condition and artificial rearing, it appears that the AR–min versus MR differences were largely driven by the prenatally stressed AR–min group. As can be seen in Figure 1, prenatally stressed AR–min rats have a larger startle response than nonstressed AR–min rats, which performed similarly to their MR counterparts. Thus, the differences in startle between this study and Lovic and Fleming (2004) may also be explained by the addition of prenatal stress as a factor.

Enhanced startle responses are a common feature of anxiety disorders (Koch, 1999). This suggests that AR produces an increase in anxiety. However, there were no correlations between startle responses and plus-maze behavior. This suggests that these two endpoints are not measuring the same underlying construct (at least not in our study). In contrast, startle responses were positively associated with locomotor activity, suggesting that these two endpoints may be measuring the same thing (see below).

Consistent with previous research, prenatal stress had no effect on startle responses (Koenig et al., 2005; Lehmann et al., 2000). Also consistent with previous findings (Lehmann et al., 2000; Weiss, Domeney, Moreau, Russig, & Feldon, 2001) were observations of higher startle response in males.

*Prepulse inhibition.* Rearing conditions also affected PPI. In accordance with our predictions and previous findings (Lovic & Fleming, 2004), AR–min rats showed reduced PPI. Providing AR rats with tactile stimulation (AR–max) partially reversed this effect. Similar to previous studies (Lehmann et al., 2000; Weiss et al., 2001) we found no sex effects on PPI. As seen with the startle data, prenatal stress had no effect on PPI, which is consistent with most (Lehmann et al., 2000; Mintz, Yovel, Gigi, & Myslobodsky, 1998), but not all (Koenig et al., 2005), previous findings. Differences in reported effects of prenatal stress on PPI may be accounted for by different prenatal stress methodologies.

The restraint stress paradigm used in this study was originally selected because it was expected to produce the most extreme effects (Fujioka et al., 1999; Hashimoto et al., 2001). The most common paradigm of restraint stress is three 45-min sessions daily

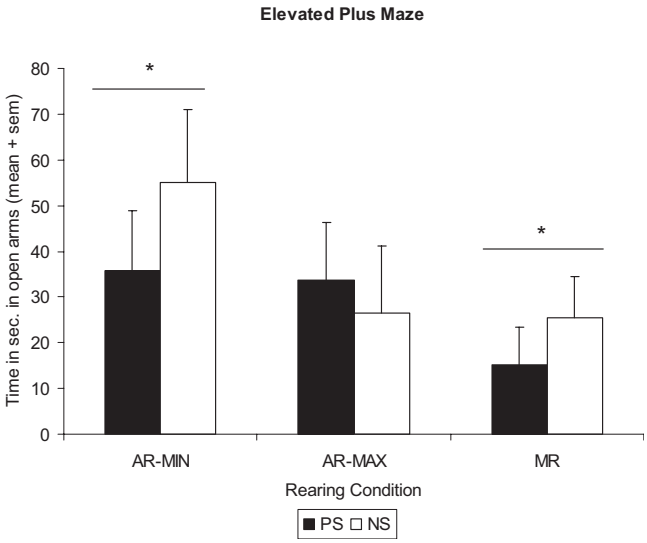


*Figure 4.* Artificial rearing with minimal stimulation increased time spent in open arms; maximal stimulation partially reversed this effect. PS = prenatally stressed; NS = nonstressed; AR–MIN = artificially reared with minimal stimulation; AR–MAX = artificially reared with maximal stimulation; MR = mother reared; sem = standard error of the mean. PS AR–MIN = 16; PS AR–MAX = 12; PS MR = 34; NS AR–MIN = 10; NS AR–MAX = 11; NS MR = 32. *$p < .05$.

from GD 14–21, which has been shown to increase levels of corticosterone and ACTH in the dam and her fetuses (Williams, Davis, McCrea, Long, & Hennessy, 1999). Unfortunately, the effects of our restraint stress protocol (a single 4-hr session occurring daily from GD 10 to 21) on the dam are at present unknown. Our assumption that this procedure would produce still greater stress effects than are found with shorter restraint periods may be unfounded. In fact, it may be the case that exposure to this repeated stressor produces adaptation and decreased HPA response to the stressor rather than an increase (see Bhatnagar & Dallman, 1998; Dhabhar, McEwen, & Spencer, 1997). Further, because our stressor was applied from GD 10 to 21, habituation may have occurred during the period when the offspring are most susceptible, that is, GD 14–21 (Koenig, Kirkpatrick, & Lee, 2002). In future studies, we plan to use variable stressors and stressors starting on GD 14, which may produce stronger prenatal stress effects (Koenig et al., 2005; Lehmann et al., 2000; Lemaire, Koehl, Le Moal, & Abrous, 2000; Vallée et al., 1996).

With the exception of PPI, the effects of prenatal stress on attention have not been extensively studied in the literature. Thus, the present finding that prenatal stress had no effect on PPI may not extend to all forms of attention. For example, Shalev and Weiner (2001) demonstrated that foot shock and corticosterone administration during pregnancy, but not restraint stress, produced deficits in latent inhibition, another measure of attention. Future studies should examine different animal paradigms of attention and use forms of prenatal stress other than repeated restraint. These studies are worth pursuing given that attentional deficits are a prominent feature of schizophrenia and ADHD, both of which have been linked to prenatal stressors (Kofman, 2002).

*Emotionality.* Prenatal stress had no effect on plus-maze behavior, which is not consistent with previous studies (Alonso, Arevalo, Afonso, & Rodriguez, 1997; Deminière et al., 1992; Lehmann et al., 2000). Differences in prenatal stress procedures possibly account for this incongruency. The aforementioned studies also used the restraint stress paradigm. However they commenced prenatal stress during the last week of gestation instead of on GD 10, as in our study (Alonso et al., 1997; Deminière et al., 1992; Lehmann et al., 2000)

In contrast, we found that artificial rearing had an effect on plus-maze behavior. AR–min rats spent significantly more time in the open arms than did mother-reared rats. AR–max rats spent an intermediate amount of time in the open arms. Maternal separation studies have reported inconsistent effects on behavior in the plus-maze. However, no studies have demonstrated increased time spent in open arms in maternally separated rats. In fact, Lomanowska, Rana, McCutcheon, Parker, and Wainwright (2006) found that juvenile AR rats spend significantly less time in the open arms.

Increased time in the open arms is commonly accepted to reflect reduced emotionality. However, we found that locomotor activity, and not startle response, was positively correlated with crossings into and time spent in the open arms. Therefore, the observation that AR–min rats spend more time in the open arms might be reflective of increased activity rather than reduced anxiety. Also, AR–min rats are more impulsive and behaviorally disinhibited (Lovic & Fleming, 2006). In this study, we casually observed that MR rats inspected the open arms before entering the arm. However, AR rats often proceeded into the open arms without much inspection. Further investigation with additional measures of anxiety, as well as inhibition, are required. Notably, these findings may complement studies of institutionalized children who have been shown to exhibit indiscriminate friendliness (Chisholm, 1998).

*Locomotor activity.* Multiple effects were observed on locomotor activity. Consistent with some studies (Deminière et al., 1992; Ordyan, & Pivina, 2003; Weller et al., 1988), prenatal stress was associated with increased locomotor activity. This effect was observed in both males and females (Elliot, Faraday, Phillips, & Grunberg, 2004; Lehmann et al., 2000). As predicted, AR enhanced locomotor activity (Gonzalez et al., 2001; Lovic, Fleming, & Fletcher, 2006).

*Memory.* There were no effects of either prenatal stress or artificial rearing on spatial working memory. This is consistent with some (Aleksandrov, Polyakova, & Batuev, 2001; Gue et al., 2004; Lemaire, Koehl, Le Moal, & Abrous, 2000) but not all (Vallée et al., 1997) previous studies.

Absence of artificial rearing effects on spatial memory is consistent with our previous findings (Levy et al., 2003). Testing order or handling effects may have affected our results, particularly in the water maze, as it was conducted last. However, this explanation is unlikely because this study replicates previous artificial rearing studies that counterbalanced testing order or consisted of a single dependent measure. Further, the lack of prenatal stress effects was consistent across tests despite their order.

### *Interaction Between Prenatal and Postnatal Factors*

A number of studies have demonstrated that postnatal manipulations can modify the effects of prenatal manipulations (Laviola et al., 2004; Wakshlak & Weinstock, 1990). Although prenatal stress can produce behavioral and physiological alterations, the brain

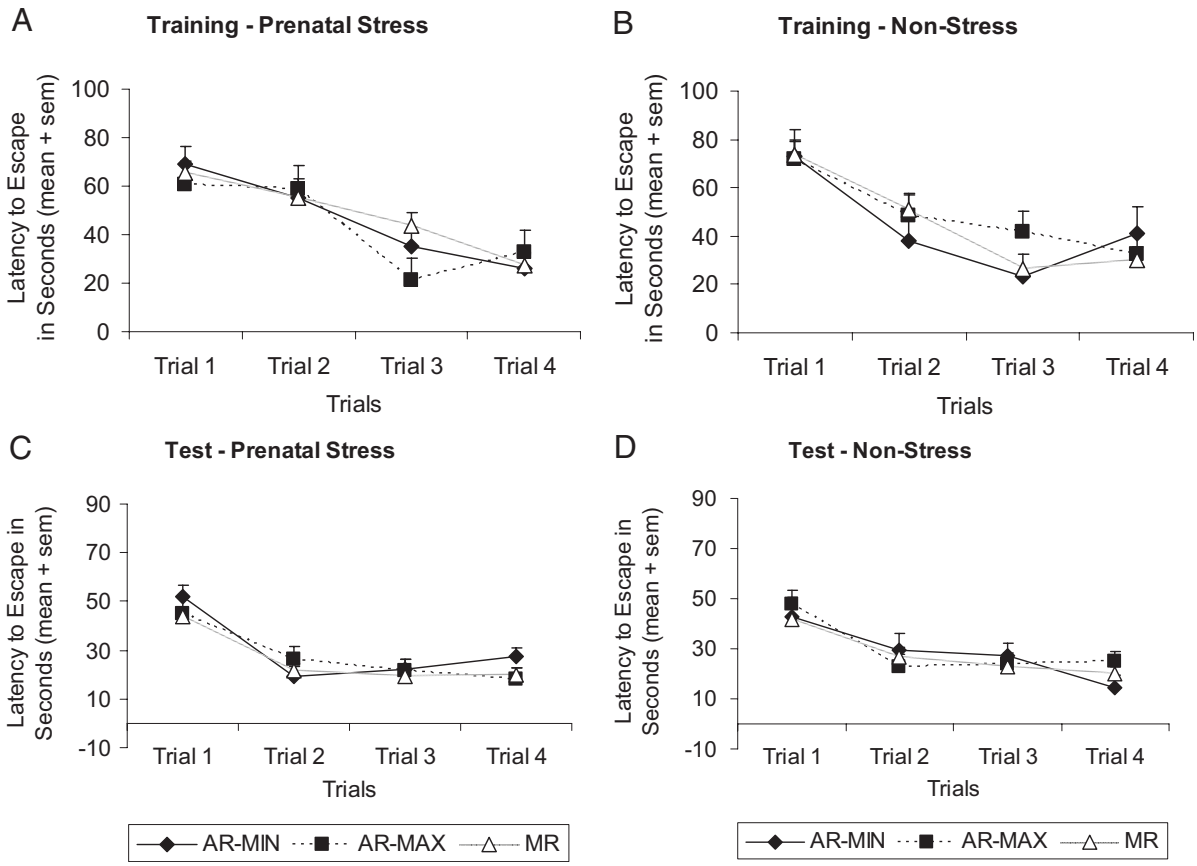


*Figure 5.* Spatial working memory task. Neither prenatal stress nor artificial rearing affected performance during spatial working memory water maze task training or testing. (A) Training, prenatally stressed (PS) condition. PS AR–MIN = 9; PS AR–MAX = 6; PS MR = 16. (B) Training, prenatally nonstressed (NS) condition. NS AR–MIN = 4; NS AR–MAX = 7; NS MR = 14. (C) Testing, prenatally stressed condition. PS AR–MIN = 9; PS AR-MAX = 6; PS MR = 16. (D) Testing, prenatally nonstressed condition. NS AR–MIN = 4; NS AR–MAX = 7; NS MR = 14. AR–MAX = artificially reared with maximal stimulation; AR–MIN = artificially reared with minimal stimulation; MR = mother reared; sem = standard error of the mean.

continues to develop postnatally and is still considerably plastic. Maternal behavior may serve as an ameliorating factor. A number of rodent studies have shown that maternal behavior manipulations, such as adoption, cross-fostering, and handling stimulation, can reverse some of the prenatal effects (Barros et al., 2004; Lehmann et al., 2000; Maccari et al., 1995; Vallée et al., 1996; Vallée et al., 1999). Thus, we anticipated that the combination of prenatal stress and maternal deprivation, with no additional tactile stimulation (AR–min), would act together to produce the most extreme effects. Our findings were not consistent with these predictions. However, with respect to locomotor activity, rather then seeing additive effects, we found an interaction between prenatal stress and AR.

Providing AR rats with additional licking-like stimulation (AR–max) had differential effects on locomotor activity according to the rat's prenatal experience. Typically, licking-like stimulation has reversed the effects produced by AR in a number of behavioral measures (Gonzalez et al., 2001; Lovic & Fleming, 2004). However, in this study, licking-like stimulation decreased locomotor activity in prenatally nonstressed rats but increased activity levels in stressed rats. Prenatal stress did not have any differential effects on AR–min or MR rats. This interaction between prenatal and postnatal manipulations suggests that licking-like stimulation normalizes locomotor activity levels in nonstressed rats, but, in fact, increases activity in offspring of mothers who were stressed during pregnancy. This combined prenatal stress/tactile stimulation produced a more extreme effect than being artificially reared. It is possible that the stroking stimulation was perceived by prenatally stressed AR rats as being too intense.

Prenatally stressed offspring may be more irritable or resistant to extra stimulation, as has been shown in rhesus monkeys that are exposed prenatally to alcohol. These animals are reported to be more resistant to handling attempts by researchers (Schneider, Moore, & Kraemer, 2004). Additionally, there may be a threshold in the amount of stimulation that is beneficial in prenatally stressed animals, and our eight stimulations per day may actually be perceived as aversive. Although dams presumably lick their offspring more than eight times per day, dams that were stressed during

pregnancy in fact lick their pups less (Patin et al., 2002), possibly because of the reduced vocalizations emitted by prenatally stressed pups (Takahashi, Turner, & Kalin, 1992). In the present context, we did not observe maternal behavior of the stressed dam or record the vocalizations of the stressed pup to help us understand the change that may have occurred in their behavioral interactions. An alternative explanation for the apparently negative responses to the stroking in prenatally stressed AR max pups, in comparison with nonstressed pups, may be that these pups are sensitized to the touch stimulation and perceive the stimulation as painful. This is a hypothesis that we are presently exploring.

What are the neurochemical mechanisms that mediate these prenatal and postnatal effects on behavior? The most consistent physiological effect of prenatal stress and maternal separation are alterations of the HPA axis. These HPA axis effects may be implicated in the stress-induced alterations in emotionality (Alonso et al., 1997; Deminière et al., 1992; Lehmann et al., 2000), activity (Deminière et al., 1992), attention (Lehmann et al., 2000), and cognition (Vallée et al., 1999). Further, the HPA axis is known to regulate the function of the dopamine system (Barrot et al., 2002; Biron, Dauphin, & Di Paolo, 1992), which has been implicated in the mediation of attention and locomotor activity (see Viggiano, Ruocco & Sadile, 2003). Thus, the disruption of HPA function produced by early adversity may indirectly affect behavior through modulation of the dopamine system, which is also known to be affected by early adversity (Barros et al., 2004; Berger, Barros, Sarchi, Tarazi, & Antonelli, 2002; Henry et al., 1995; Takahashi et al., 1992). Further, prenatal stress and AR may have differential effects on glutamate, which is known to be more sensitive to postnatal than to prenatal manipulations (Barros et al., 2004). Glutamate aids in the regulation of dopamine in a number of dopaminergic pathways (Barros et al., 2004) and is also modified by glucocorticoids (Cho & Little, 1999).

Despite the limitations, this study, examining the combined effects of prenatal stress and complete maternal deprivation, provides insights into the complex relationship between the two. The current results show that postnatal adversity can induce significant long-term alterations in attentional systems, activity levels, and emotionality. In addition, this study demonstrates that prenatal experiences can provide an important background for the potential effects of the postnatal environment for locomotor activity. Further, our results suggest that artificial rearing may have a greater impact on adult behavior than does prenatal stress (maternal restraint). The effects seen in this study can provide important background for animal models of psychopathology as well as the role of the mother in normal and healthy development.

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