Sex differences in the long-term effect of preweanling isolation stress on memory retention

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Abstract

Social experiences during development can powerfully modulate later neuroendocrine and behavioral system. In the present study, male and female rat pups experienced daily bouts of social isolation for 6 h per day or control conditions during the third postnatal week. Performance on a 12-arm radial maze with 8 arms consistently baited with food reward was examined in adulthood. During the social isolation, both male and female pups exhibited a significant increase in plasma corticosterone levels. When tested on the radial arm maze as adults, the performance of female rats that had experienced social isolation during development was not affected; however, male rats in the isolation condition initially exhibited impairments in working memory but not reference memory. Despite achieving comparable asymptotic levels of performance on the maze, male rats that experienced social isolation during the third week demonstrated disruption in working memory retention when radial arm maze trials were interrupted after the fourth arm choice. Thus, while male rats that experience social isolation during the third week of life eventually perform comparably to controls on the standard radial arm maze task, their ability to retain information over a delay remains impaired. These findings highlight an important sex difference in the long-term effects of stress during this period of late preweanling development.

Keywords: Stress; Learning; Memory; Radial arm maze; Cognition; Development; Hippocampus; Corticosterone; Retention; Sex difference

Severe and prolonged exposure to environmental stressors during periods of early development significantly influences the organization of several neuroendocrine systems. The effects of such developmental manipulations can persist throughout the lifespan of the animal. For example, 24 h of maternal deprivation during the first weeks of postnatal life, sufficient to cause acute activation of the hypothalamic–pituitary–adrenal (HPA) axis, causes changes in basal corticosterone levels and stress responsivity that are evident in adulthood (Lehmann et al., 2002; Penke et al., 2001; Rots et al., 1996). Six hours of daily isolation stress during the third postnatal week is also sufficient to cause elevation in baseline corticosterone levels in adulthood (Sandstrom and Hart, 2005). One mechanism through which such a developmental stressor may permanently alter HPA axis function and stress responsivity involves the down-regulation of glucocorticoid receptor expression in the hippocampus (Meaney et al., 1996). The hippocampus has been identified as a site of negative feedback regulation of corticotropin releasing hormone (CRH) secretion from the hypothalamus (Jacobson and Sapolsky, 1991) and such a down-regulation of glucocorticoid receptor expression in the hippocampus limits this source of regulation, resulting in persistent elevations in glucocorticoid secretion, greater HPA activation in response to acute stress, and a slower return to baseline levels following the termination of a stressor.

The hippocampus is critically involved in performance of spatial learning and memory tasks. Lesions of the hippocampus impair acquisition on the Morris water maze task (Gallagher and Holland, 1992; Morris et al., 1982; Whishaw and Jarrard, 1995) and disrupt spatial working memory as
assessed on the radial arm maze task (Becker et al., 1980; Cassel et al., 1998; Jarrard, 1993, 1995; Jarrard et al., 1984; Olton and Papas, 1979; Whishaw and Jarrard, 1995). Performance on these tasks can be adversely affected by chronic elevation of glucocorticoid levels similar to those that result from developmental stress (Bodnoff et al., 1995; Luine, 1994; Luine et al., 1994b). Interestingly, several studies have reported marked sex differences in the effects of repeated stress during early periods of development. For example, male rats are more susceptible to the adverse effects of prenatal stress when tested as adults on the water maze or radial arm maze tasks (Bowman et al., 2004; Szuran et al., 2000). In contrast, both male and female rats that experienced repeated isolation stress during the third postnatal week show impairments in acquisition on the water maze task when tested as juveniles but not when tested in adulthood (Frisone et al., 2002). Together, these findings suggest that the developmental stage at which rat pups experience repeated stressors may significantly influence the long-term cognitive consequences and that these consequences may be sexually dimorphic. Furthermore, the persistence of cognitive deficits resulting from developmental stress may be related to age at which the stress is experienced.

Recently, we have shown that complete social isolation for 6 h per day during the third postnatal week results in transient impairment in working memory assessed with the radial arm maze task in male rats (Sandstrom and Hart, 2005). It is not known, however, whether female rats are similarly susceptible to this adverse consequence of this developmental stressor. The present study examines sex differences in the effects of 6 h of daily isolation stress during the third postnatal week on spatial memory assessed with a 12-arm radial maze. For each rat, only 8 of the 12 arms were baited with food reward to allow the assessment of memory for information that is consistent across all trials (i.e., reference memory) and memory for trial-specific information (i.e., working memory) (Olton, 1983). In addition to examining performance across standard training trials, the ability of the rats to maintain information across a delay was assessed by removing the rat from the maze during the middle of the trial and replacing it after a 1- or 15-min interval had elapsed (memory retention). The results of this study indicate that isolation stress during the late preweanling period causes changes in working memory during acquisition as well as working memory retention in a sex-specific manner.

Methods

Subjects

Sixty-eight Long–Evans rat pups (34 male and 34 female) derived from 17 litters generated in our breeding colony were used to assess the hormonal response to social isolation and the long-term cognitive consequences of social isolation. One male and one female pup from each litter were assigned to the social isolation condition. A second male pup and a second female pup from each litter were assigned to the control condition. The remaining pups in each litter were not disturbed. All litters had free access to rat chow and water and were maintained on a 12-h light–dark cycle with lights on at 7:00 am. All treatments were in accordance with NIH guidelines and were approved by the IACUC of Williams College.

Social isolation and blood sampling

On day 15, each dam was removed from her litter and placed in a temporary holding cage. The four experimental pups from each litter were marked on the tail with permanent marker. The control pups were then returned to their home cages along with the dams and the rats in the social isolation condition were placed in isolation cubicles in an adjacent room. The isolation cubicles (9.8 cm × 8.3 cm × 10.2 cm) consisted of white wooden walls, a wire mesh floor, and Plexiglas lid. The cubicles were maintained at 24°C and were wiped clean after each isolation session.

Daily isolation periods lasted from 10:00 am to 4:00 pm beginning on postnatal day 15. Pups in the control condition remained in the home litter during this 6-h period. To confirm that this manipulation caused an elevation in plasma corticosterone, seven male and seven female rats in each treatment group (control and isolated) were sacrificed 3 h into the social isolation period on the fifth day of the isolation manipulation (postnatal day 19). Trunk blood was collected into heparin-treated microcentrifuge tubes and samples were centrifuged at 1,000 × g for 10 min at 4°C. The plasma was then transferred to a clean tube and stored at −80°C until assayed.

Animals assigned for behavioral testing were not sacrificed for corticosterone assessment and continued to experience isolation or control conditions daily through day 21. These animals were weighed prior to the isolation period on days 15 and 21 (the first and last days of the manipulation). Importantly, isolation was not associated with any significant change in body weight for either males or females. Animals continued to live in the home litter until day 25 when they were rehoused in same-sex pairs in hanging cages (18 × 18 × 25 cm) with free access to food and water. Lights were maintained on a 12:12 light/dark cycle with lights on at 7:00 am.

Corticosterone radioimmunoassay

Plasma corticosterone levels were determined for samples collected on day 19 during the social isolation or control manipulation. Samples were assayed using a commercially-available double-antibody corticosterone radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA, USA). All standards and samples were run in duplicate.
Assay sensitivity is approximately 15 ng/ml, with an interassay variability of less than 8%.

Radial arm maze testing

The white, wooden radial arm maze consisted of 12 identical arms (8 cm × 75 cm) extending out from a circular central platform (diameter = 33 cm) elevated 75 cm above the ground. A short wall (5 mm) ran along the edge of each arm and a circular food well (diameter = 3 cm, depth = 7 mm) was located at the end of each arm. The maze was positioned in a rectangular room with a variety of extramaze cues. A video camera was suspended above the maze and the experimenter sitting in an adjacent room observed each trial on a monitor and recorded the sequence of arm choices over the course of each trial.

Pretraining

At 3 months of age, the rats were individually housed in hanging cages with free access to food and water. Each rat was then food-restricted to 85% of its free-feeding body weight. After reaching their target weights, rats were pretrained on the radial arm maze by placing them on the maze in groups of 4–5 for 10 min on each of five consecutive days. During pretraining, 45-mg Noyes food pellets were scattered over the arms of the maze. By the last day of pretraining, rewards were placed only at the ends of the arms and the animals readily traversed the maze, consuming the food pellets at the end of the arms.

Training trials

Following pretraining, each rat was randomly assigned one of nine possible baiting patterns consisting of 8 arms that were consistently baited with a single 45-mg pellet (S⁺) and 4 arms that were consistently left unbaited (S⁻). Each individual rat experienced the same baiting pattern on each trial for the remainder of behavioral testing. Training was performed 5 days per week during the light part of the cycle between approximately 10 am and 4 pm and continued for a total of 30 trials.

On each daily trial, the rat was placed in the center of the maze in a random orientation. The experimenter then returned to the monitor and computer in the adjacent room and recorded the sequence of arm choices. A choice was operationally defined as the rat traveling at least halfway down the arm. Each trial lasted until all eight baited arms had been selected. Each choice of an S⁻ arm was recorded as a reference memory error and each choice of an arm that had previously been selected on that particular trial was recorded as a working memory error.

Delay trials

After 30 trials of standard training on the radial arm maze, six trials were conducted on which a delay was introduced after the fourth arm choice. On these trials, the maze was baited according to the assigned baiting pattern for each rat and the rat was placed on the maze in a random orientation. When the rat returned to the central platform following the fourth choice, it was removed from the maze and returned to a holding cage (44 cm × 22 cm × 20 cm) with wood chip bedding where it remained for an interval of 1 min or 15 min. During this interval, the maze was wiped clean to eliminate any odor trails. After the retention interval had elapsed, the rat was returned to the central platform in a random orientation and was allowed to continue making arm choices until the remaining S⁺ arms had been selected at which time it was removed from the maze and returned to its home cage. Over 6 days, each rat completed three trials with each delay interval in the following order: 1, 15, 1, 1, 15 min. As during training, the number or working memory and reference memory errors were recorded for each trial.

Results

Plasma corticosterone

Corticosterone levels in plasma from animals sacrificed 3 h into the isolation or control manipulation on day 19 were analyzed with a 2 (Sex) × 2 (Treatment) ANOVA. Social isolation was associated with a significant increase in corticosterone levels, \( F(1, 24) = 49.21, P < 0.001 \) (Fig. 1). There was no effect of sex nor was there an interaction between sex and treatment. Analysis of the simple main effects of treatment confirmed a significant isolation-induced elevation in corticosterone levels for both male pups (Control = 36.0 ng/ml ± 7.0; Isolated = 136.4 ng/ml ± 20.4) and female pups (Control = 40.1 ng/ml ± 6.1; Isolated = 126.0 ng/ml ± 14.3).

Radial arm maze testing

Training trials

The mean number of working memory errors (selection of an arm that had previously been selected on that particular trial) for rats in the social isolation and control condition was plotted as a function of blocks of five training trials (Fig. 1). A 3-factor ANOVA with treatment and sex as between-subjects factors and trial block as a repeated-measure revealed a main effect of block, \( F(5, 180) = 97.28, P < 0.001 \). The main effects of sex and treatment were not statistically significant. The interaction between sex and treatment approached statistical significance, \( F(1, 36) = 3.65, P = 0.06 \). There was a significant interaction between block and treatment, \( F(5, 180) = 2.95, P < 0.05 \), but not between block and sex. The 3-way block × treatment × sex interaction was also statistically significant, \( F(5, 180) = 2.60, P < 0.05 \). A linear contrast analysis was conducted to determine whether the rate of improvement across the training trials varied as a function of sex and/or treatment. This analysis revealed a significant block × sex × treatment interaction, \( F(1, 36) = 4.23, P < 0.05 \), indicating that the difference in rates of improvement...
between rats in the isolation condition and rats in the control condition varied as a function of sex with a greater difference between isolated and control males than between isolated and control females.

As shown in Fig. 1, the largest differences among the groups occurred early in training with all groups performing comparably by the last block of trials. The mean number of working memory errors on the first trial block was analyzed with an ANOVA with sex and treatment as between-subjects factors. This analysis revealed a significant main effect of treatment, $F(1, 36) = 4.91$, $P < 0.05$, and a significant treatment × sex interaction, $F(1, 36) = 8.63$, $P < 0.01$, indicating that the performance of males was more adversely affected by the social isolation manipulation than was the performance of females during this early period of acquisition. The main effect of sex was not statistically significant.

Analysis of performance during the last trial block revealed a main effect of treatment, $F(1, 36) = 4.77$, $P < 0.05$, with rats in the social isolation condition committing more working memory errors than rats in the control condition. The main effect of sex was also statistically significant, $F(1, 36) = 3.98$, $P = 0.05$, with female rats making more errors than males at the end of training. The interaction between sex and treatment, however, was not statistically significant.

The mean number of reference memory errors for male and female rats in the isolation and control conditions was similarly analyzed with a 2 (sex) × 2 (treatment) × 6 (trial block) ANOVA. The main effect of trial block was significant with fewer errors occurring as training progressed, $F(5, 180) = 71.03$, $P < 0.001$. However, there were no significant main effects of sex or treatment and no significant interactions (data not shown).

**Delay trials**

The mean number of working memory errors during trials on which a delay of 1 or 15 min was inserted following the fourth choice was analyzed with a 2 (sex) × 2 (treatment) × 2 (delay) ANOVA (Fig. 2). This analysis revealed main effects of sex, treatment, and delay, $F_s(1, 36) > 4.64$, $Ps < 0.05$. Furthermore, the sex × delay and the
treatment \times delay interactions were statistically significant, Fs(1, 36) > 5.76, Ps < 0.05. The three-way sex \times treatment \times delay approached statistical significance, F(1, 36) = 3.06, P = 0.09. A series of paired-samples t tests with delay interval as the repeated measure confirmed that only males in the isolated condition demonstrated a delay-dependent increase in working memory errors, t(9) = 3.07, P < 0.05. Males in the control condition, females in the isolated condition, and females in the control condition failed to show such a delay-dependent effect in the number of working memory errors.

The mean number of reference memory errors during delay trials was similarly analyzed revealing no significant main effects of sex, treatment, or delay. Furthermore, there were no significant interactions among any of these factors (data not shown).

Discussion

The data reported here describe a sex difference in the long-term effect of 6 h of daily isolation stress during the third postnatal week on radial arm maze performance in adulthood. This developmental manipulation was associated with a significant elevation in corticosterone levels during the isolation period in both male and female pups. As adults, only males showed evidence of a significant impairment in radial arm maze performance including an increase in working memory errors early in training and a significant delay-dependent increase in working memory errors when a retention interval was inserted following the fourth choice; reference memory errors during acquisition and retention tests were not affected. Despite exhibiting a significant increase in corticosterone levels during the isolation periods, females that experience social isolation during development and Hart, 2005) which reported a transient impairment in MWM task in adulthood (Frisone et al., 2002). Interestingly, this study reported that isolation stress led to more rapid acquisition on the water maze for both males and females but that isolated and control animals all reached comparable levels of asymptotic performance. Performance on these two hippocampally-dependent tasks is differentially affected by isolation stress during the third week. These differential effects may be due to differences in the motivational characteristics of the tasks (water escape versus food rewards) or differences in the cognitive demands of the tasks.

Despite the finding that isolated males eventually perform comparably to controls on the standard radial arm maze, the present findings indicate that they continue to display delay-dependent impairments in working memory retention. Previous studies have demonstrated that a 15-min interruption between choices impairs accuracy on a 12-arm radial maze task and that the effect of such an interpolated delay is dependent on the point during the trial at which the delay is interpolated (Cook et al., 1985; Williams et al., 1990). We utilized 1-min and 15-min delay durations following the fourth choice in the present study. Simple removal from the maze with return after 1-min in the holding cage did not dramatically affect performance of any groups. When the delay was extended to 15 min, however, only male rats that had experienced social isolation during the third postnatal week showed a significant increase in the number of working memory errors; neither group of females was affected by the 15-min delay. It is possible that increasing the memory demands of the task by using a longer delay period may reveal a difference in retention ability of female rats in the control and isolated conditions. The present data only indicate that neither isolated nor control females are adversely affected by a 15-min retention interval.

In contrast to males, the radial arm maze performance of females was largely unaffected by the isolation manipulation. Females showed no impairment during radial arm maze acquisition when tested as adults, nor did they show any delay-dependent deficit in memory retention. These findings are similar to those of Bowman et al. (2004) who have recently reported that prenatal stress slightly impairs working memory of adult males but slightly improves performance of females, and Szuran et al. (2000) who have reported adverse effects of prenatal stress on water maze performance of males but not females.

The nature, duration, and timing of developmental stress can profoundly shape the long-term cognitive consequences. In contrast to the sexually dimorphic long-lasting effects of prenatal stress and isolation stress during the late preweaning period, more subtle stressors such as exposure of the dam to elevated levels of corticosterone via the drinking water from parturition through weaning throughout lactation as well as between postnatal days 5 and 9 (but not between days 13–17) can actually lead to improvements in performance on some hippocampally-mediated tasks in both males and females (Casolini et al., 1997; Catalani et al., 1993, 2000, 2002; McCormick et al., 2001). Together, these findings indicate that increases in stress hormones can have very different effects at varying points in development and suggest that the late preweaning period may be a time of significant vulnerability for male rats.

The apparent vulnerability of male rats to the adverse effects of isolation stress during the third postnatal week may be due in part to the fact that the hippocampus undergoes extensive maturation during the third postnatal week including increases in glucocorticoid receptor expression (Bohn et al., 1994; Meaney et al., 1985; Rosenfeld et al., 1988;
Sarrieau et al., 1988). Elevated glucocorticoid levels during this period are associated with both a decrease in glucocorticoid receptor expression in adulthood and an increase in basal corticosterone levels in adulthood (Meany et al., 1996; Ordyan et al., 2001; Sapolsky et al., 1984). In fact, adult male rats that experienced isolation stress during the third postnatal week exhibit chronic elevations in corticosterone (Sandstrom and Hart, 2005). Such chronic elevations have been associated with atrophy of CA3 pyramidal neurons (Luine et al., 1994a; Magarinos and McEwen, 1995a,b; Magarinos et al., 1998; McKittrick et al., 2000; Watanabe et al., 1992a,b,c; Woolley et al., 1990) as well as an impairment in performance on hippocampally-mediated learning and memory tasks such as the radial arm maze, Morris water maze, and T-maze (Arbel et al., 1994; Bodnoff et al., 1995; Conrad et al., 1996; Luine et al., 1994a,b). Interestingly, male rats are more susceptible than females to the adverse cognitive effects of chronic stress hormone elevations in adulthood (Beck and Luine, 2002; Bowman et al., 2001; Conrad et al., 2003; Luine, 2002; Luine et al., 1994a). We do not currently know whether female rats that experience isolation stress during the third postnatal week show any changes in baseline corticosterone levels in adulthood. It is possible that either (a) females show no long-term effects of isolation stress on basal corticosterone levels, or (b) females do show a lasting increase in corticosterone but this increase does not adversely affect radial arm maze performance.

In summary, the present data reveal a sex difference in the long-term effects of daily bouts of isolation stress during the third postnatal week with males but not females showing both an impairment in working memory during acquisition as well as a delay-dependent impairment in working memory retention. These findings contribute to a growing body of literature that suggests that exposure to stress during development can significantly impact later cognitive function. Furthermore, these findings highlight the need to carefully consider sex as one factor that influences the long-term outcome of developmental stress.

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