

# Prenatal stress differentially affects habituation of corticosterone responses to repeated stress in adult male and female rats

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## Abstract

Environmental factors operating early in life have long-lasting and important consequences for the mental and physical health of the adult organism. In particular, prenatal exposure to stress represents one category of adverse early environmental events that are associated with development of depression and schizophrenia in adulthood. In the present studies, we examined whether prenatal stress alters the habituation of hypothalamic–pituitary–adrenal (HPA) activity that occurs with repeated stress exposure in adulthood. We compared corticosterone responses to the first vs. the eighth restraint, with lower responses to the eighth vs. the first considered evidence of habituation. In males, prenatal stress prevented the habituation of corticosterone responses to repeated restraint that was observed in non-prenatally stressed rats. Limited evidence of habituation was seen in either group of females and prenatally stressed females did not exhibit the enhanced corticosterone response during recovery from the eighth restraint that was seen in non-prenatally stressed females. Together, these results suggest a sex-specific interaction between prenatal stress and adult chronic stress on HPA activity.

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## Introduction

Considerable evidence suggests that environmental factors operating early in life have long-lasting and important consequences for the mental and physical health of the adult organism. Early environmental events that are adverse may render an individual more vulnerable to illness later in life (Kessler, 1997). In particular, prenatal exposure to stress represents one category of adverse early environmental events that are associated with development of depression and schizophrenia in adulthood (Kessler, 1997; Koenig et al., 2002; Quenstedt and Parshall, 1998). Various animal models of prenatal stress have been developed over the years. Adult animals that have been prenatally stressed exhibit many characteristics similar to those in humans with depression. These include dysregulation of the stress-responsive hypothalamic–pituitary–

adrenal (HPA) axis, anhedonic and anxiogenic behaviors, and alterations in circadian rhythms (e.g., Carroll et al., 1976; Mortola et al., 1987). Together, results from animal studies suggest that stress during late gestation affects fetal development which may lead to increased propensity to develop depression and schizophrenia in adulthood (Weinstock, 1997). Therefore, prenatal stress may represent a relevant animal model for the study of vulnerability to develop psychiatric illness related to exposure to adverse early environmental events.

There is strong evidence that episodes of schizophrenia and depression in adults can be precipitated or exacerbated by life stressors occurring in adulthood (Kessler, 1997; Post, 1992) such as physical abuse, low socioeconomic status, and lack of social support. Some evidence from animal models indicates that adverse early life events interact with chronic stress in adulthood to alter physiology and behavior (Bhatnagar and Meaney, 1995). However, little is known about changes in behavior and physiology of prenatally stressed animals when they are exposed to chronic stress in adulthood.

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Chronically stressed animals often exhibit decreased HPA responses to repeated exposure to the same or homotypic stressor. This decrement, termed habituation, has previously been shown with repeated exposure to restraint (Hauger et al., 1990; Cole et al., 2000; Viau and Sawchenko, 2002) as well as cold (Bhatnagar and Meaney, 1995), noise (Armario et al., 1986), water immersion (De Boer et al., 1990), immobilization (Garcia et al., 2000; Giralt and Armario, 1989), repeated ethanol injections (Spencer and McEwen, 1990), and repeated handling (Dobráková et al., 1993). In the present studies, we examined the effect of prenatal stress on HPA responses to repeated restraint in adult male and female rats. Smythe et al. (1996) have suggested that one of the primary effects of prenatal stress is to increase sensitivity to other manipulations, such as chronic stress. Little is known about the effects of prenatal stress on HPA responses to chronic stress in adulthood. We expected that prenatally stressed males and females would demonstrate disruptions in HPA responses to chronic restraint compared to chronically stressed rats that have not been prenatally stressed.

In addition, we monitored body weight gain from post-weaning to the onset of stress as an index of general growth and development. Since we administered prenatal stress during the third trimester, our discussion of other studies is limited to those that also administered stress during the third trimester, unless otherwise noted.

## Materials and methods

### *General housing conditions*

All rats were Sprague–Dawley rats and were housed in plastic tub cages. Rats were allowed ad lib access to food and water and maintained on a 12:12-h light–dark schedule (lights on at 07:00 h) and ambient temperature maintained at  $21 \pm 1^\circ\text{C}$ . All experiments were approved by the University Committee on Care and Use of Animals at the University of Michigan.

### *Prenatal stress*

Young adult female rats ( $n = 7$ ) were purchased from Harlan Sprague–Dawley (Indianapolis, IN). They were housed with males and monitored twice daily for presence of vaginal plugs. The day that vaginal plugs were detected was considered day 0 of pregnancy and the females were then separated and individually housed. Some pregnant female rats ( $n = 4$ ) were randomly assigned to the stress group and exposed to stress in the third trimester (days 15–21 of gestation). Stress consisted of placing the females in a Plexiglas restrainer for 45 min three times per day, beginning at 9 a.m., 12 p.m., and 4 p.m. The other pregnant females were assigned to the no stress, control group and these females were only exposed to weekly cage changes as were the stressed females. All litters were born within 12

days of one another. Litter sizes ranged from 8 to 14 at birth and litters were not culled. After birth, the female and her litters were undisturbed, except for cage changes until day 21. On this day, pups were weaned from their mothers and housed in same sex groups. Pups were tail marked and body weights were recorded at regular intervals. One week prior to onset of repeated restraint in adulthood, male and female rats were singly housed in plastic tub cages. Daily vaginal smears were conducted on the female rats starting 1 month prior to the onset of repeated restraint to determine stage of estrous cyclicity at the time of blood sampling for hormone responses to acute or repeated stress.

### *Adult stress paradigm*

Adult offspring from each litter were randomly assigned to one of two groups, acute restraint or repeated restraint. At approximately 85 days of age, some male prenatally stressed (PS) and non-prenatally stressed (NPS) rats were exposed to 7 days of repeated restraint and blood was sampled for corticosterone on days 1 and 8 (described further below). Other male PS and NPS rats were exposed to a single acute restraint only and also sampled as described below. PS and NPS females were similarly exposed to repeated or acute restraint but starting at approximately day 105 of age. Acute stress only groups were included to assess effects of acute stress on other measures not reported here.

Adult male and female NPS and PS rats were repeatedly stressed by placing them in a Plexiglas restrainer for 30 min a day (between 09:00 and 10:00) for 8 consecutive days. HPA activity habituates to repeated restraint exposure during this period (Bhatnagar et al., 2002a). On days 1 and 8, blood was collected via nicking of the tail vein within 60 s following placement in a Plexiglas restrainer. This is a standard procedure for collecting blood allowing for repeated sampling without the necessity of surgical implantation of indwelling catheters and provides consistent results (Akana et al., 1996; Bhatnagar et al., 2002a,b). Corticosterone levels in blood are not affected by placement in the restrainer if samples are collected within 60 s. On day 1, all animals were placed in a Plexiglas restrainer and a blood sample taken immediately from the tail vein (the 0-min sample), and also at 15 and 30 min during restraint. After collection of the 30-min sample, the animals were re-placed in their home cages. 30 min later (60 min after onset of 30 min restraint), samples were taken again from the tail vein by placement in a restrainer and the animals returned to their home cages. All blood samples were collected within 60 s of opening of the cage to remove the rat. The next day, day 2, animals were exposed to 30 min restraint, but not sampled. They were subsequently exposed to restraint every day until day 8 without being sampled. On day 8, rats were sampled as on day 1.

Plasma testosterone was also measured on days 1 and 8 of repeated restraint in NPS and PS male rats. After plasma was used for analysis of corticosterone, the leftover plasma

was pooled across the different time points for each animal and then analyzed for plasma testosterone.

Other groups of non-prenatally stressed and prenatally stressed male and female rats were exposed to a single, acute restraint and sampled as described above. Corticosterone responses in these rats are functionally the same as the day 1 responses in rats that went on to be repeatedly stressed (described above). There was considerable variability, especially in the NPS groups, in terms of responses to acute stress. Therefore, we combined data from these acutely stressed rats with data from day 1 in rats that went on to be repeatedly stressed.

#### Corticosterone radioimmunoassays

Blood was collected via nicking of the tail vein in tubes coated with sodium EDTA and kept on ice until centrifuged. After centrifugation, the plasma was aliquoted and kept frozen at  $-20^{\circ}\text{C}$  until assay. Plasma corticosterone was measured using a kit from ICN Biomedicals (NY). The minimum level of detection for corticosterone is  $0.6\ \mu\text{g}/\text{dl}$ . Integrated corticosterone responses to acute, day 1 or day 8 restraint were determined by calculating area under the curve and dividing by 60 min to obtain integrated corticosterone measures per minute over the duration of restraint and recovery from restraint. Plasma testosterone was measured using a kit from ICN Biomedicals.

#### Statistics

We found that the variability (standard deviations) for all rats in a given group that were littermates (variability within litters) was similar to or greater than the variability across litters on all our dependent measures. Therefore, the effects observed in the present studies were likely not due to within litter variations or the number of litters used. In addition, rats from each litter were randomly assigned to the adult acute or repeated stress groups. Finally, we analyzed our data using litter as a covariate and found no significant effects of litter. Therefore, litter as a variable was not considered in the final analyses and data from each individual animal were used.

Body weights in prenatally stressed (PS) and non-prenatally stressed (NPS) males and females were collected from day 34 until prior to onset of stress and were analyzed by two-way repeated measures ANOVA (prenatal stress (PS or NPS)  $\times$  age with age being the repeated factor) separately in males and females.

Plasma corticosterone responses to stress were analyzed separately in males and females. Plasma corticosterone responses to the first vs. the eighth restraint were analyzed by  $2 \times 2$  repeated measures ANOVAs (adult stress (acute or chronic stress)  $\times$  prenatal stress (PS or NPS) with repeated measures on the adult stress factor) at each time point. Integrated corticosterone was analyzed by  $2 \times 2$  ANOVAs (adult stress (acute or repeated stress)  $\times$  prenatal stress (PS or NPS)). Plasma corticosterone in acutely stressed rats was

analyzed by one-way ANOVAs (PS vs. NPS) at each time point.

Plasma testosterone was analyzed by a  $2 \times 2$  repeated measures ANOVA (adult stress (acute or chronic stress)  $\times$  prenatal stress (PS or NPS) with repeated measures on the adult stress factor). The change in plasma testosterone from day 1 to day 8 was also analyzed using a one-way ANOVA (PS vs. NPS).

## Results

### Body weights

Fig. 1 shows body weights across ages in male and female PS and NPS rats. In male rats, there was a significant

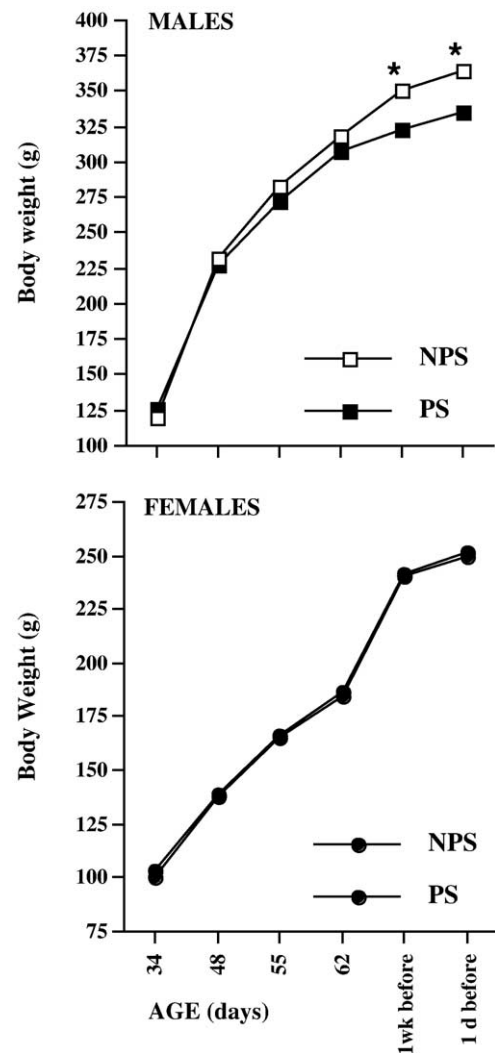


Fig. 1. Body weights in male and female rats that were exposed to prenatal stress (PS) or no prenatal stress (NPS) are shown at various ages and at the week before and 1 day before the onset of acute or repeated restraint. Data are expressed as Mean  $\pm$  SEM. Group sizes are PS female  $n = 11$ , NPS female  $n = 17$ , PS males  $n = 12$ , and NPS males  $n = 21$ . \* $P \leq 0.05$ ; PS and NPS significantly different from each other.

prenatal stress effect ( $F(1,31) = 4.73$ ;  $P < 0.03$ ), a significant age effect ( $F(1,5) = 1343$ ;  $P < 0.01$ ), and a significant prenatal stress  $\times$  age interaction ( $F(6,155) = 8.24$ ;  $P < 0.01$ ). Post hoc tests indicated that overall, PS animals weighed significantly less than NPS males and that body weights at day 48 and older were significantly higher than at the preceding age. Furthermore, PS males weighed significantly less than NPS males on the week before and the day before onset of repeated restraint. In female rats, there was a significant age effect ( $F(1,5) = 2119$ ;  $P < 0.01$ ). Post hoc tests indicated that body weights at day 48 and older were significantly higher than at the preceding age. No other significant effects were observed in female.

#### Plasma corticosterone and testosterone in males

A significant effect of prenatal stress was observed at the 0-min time point ( $F(1,16) = 9.39$ ;  $P < 0.01$ ; Fig. 2) with PS males exhibiting higher levels of corticosterone at

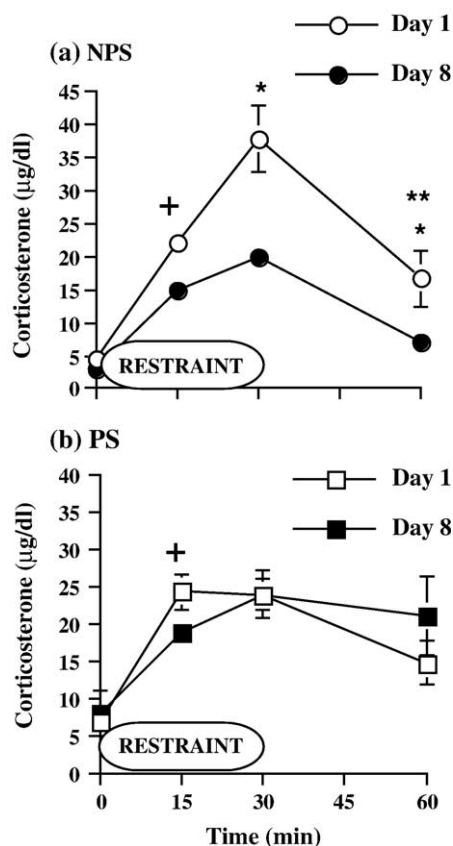


Fig. 2. Male rats were exposed to prenatal stress (PS) during the third week of gestation or not exposed to any stress (no prenatal stress; NPS). Adult NPS (a) and PS (b) males were exposed to 30 min of restraint for 8 consecutive days and blood was sampled only on days 1 and 8. Data are expressed as Mean  $\pm$  SEM. Group sizes are PS males  $n = 6-7$  and NPS males  $n = 9-11$ . Variations in  $n$  are due to insufficient plasma. \* $P \leq 0.05$ ; NPS rats significantly different on day 1 vs. day 8.  $^+P \leq 0.05$ ; day 8 values lower overall compared to day 1. \*\* $P \leq 0.05$ ; PS rats on day 8 significantly greater than NPS rats on day 8.

this basal time point compared to NPS males. At 15 min, there was a significant adult stress effect ( $F(1,16) = 18.1$ ;  $P < 0.01$ ) with all rats exhibiting lower levels of corticosterone on day 8 compared to day 1. At 30 min, there was a significant adult stress factor effect ( $F(1,13) = 5.5$ ;  $P < 0.03$ ) with overall lower corticosterone values on day 8 compared to day 1 of restraint. There was also a significant interaction effect ( $F(1,13) = 5.4$ ;  $P < 0.03$ ) and post hoc tests indicated that corticosterone levels were lower on day 8 compared to day 1 in NPS rats but no differences were found in PS rats. Finally, at 60 min, there was a significant interaction effect ( $F(1,13) = 9.4$ ;  $P < 0.01$ ). Post hoc tests showed that corticosterone levels NPS rats at day 8 were significantly lower than in all other groups at this time point. No other significant effects were observed.

Integrated corticosterone values were also calculated over the 60-min sampling period in all groups (Fig. 4). There was a significant adult effect ( $F(1,31) = 10.07$ ;  $P < 0.003$ ) and a significant interaction effect ( $F(1,31) = 4.6$ ;  $P < 0.04$ ) in integrated corticosterone levels. Post hoc tests indicated that corticosterone levels in NPS rats were lower on day 8 than those in all other groups and no other effects were observed.

We also analyzed corticosterone levels in PS and NPS rats that were exposed to only a single acute exposure to restraint (data not shown). Because this single exposure to restraint is similar to the day 1 responses to restraint, we combined corticosterone levels from rats on day 1 (that eventually went on to be chronically stressed) with corticosterone from rats exposed only to the single restraint. We found that PS rats exhibited higher levels of corticosterone ( $F(1,31) = 4.26$ ;  $P < 0.04$ ) at 60 min than NPS rats in response to an acute exposure to restraint. No significant differences were observed at any other time point and no significant effects in integrated corticosterone were observed in acutely stressed males.

In summary, NPS male rats exhibited lower levels of corticosterone on day 8 compared to day 1 at 30 and 60 min, evidence of habituation in these rats. In contrast, corticosterone levels were similar on days 1 and 8 in PS male rats at every time point, suggesting no habituation in PS males. The integrated corticosterone levels corroborated these individual time point results. Finally, in rats exposed to a single acute restraint, PS rats exhibited increased corticosterone at 60 min compared to NPS rats.

We also examined plasma testosterone levels taken on day 1 or day 8 of restraint in PS and NPS rats. We found that there was a significant adult stress  $\times$  prenatal stress interaction ( $F(1,32) = 4.26$ ;  $P < 0.05$ ). Post hocs only indicated a tendency ( $P < 0.07$ ) for NPS rats to have higher testosterone on day 8 compared to day 1 (data not shown). We then analyzed the change in testosterone from day 1 to day 8. We found a significant effect between NPS and PS rats ( $F(1,16) = 4.56$ ;  $P < 0.05$ ) with testosterone increasing over the repeated restraint period

in NPS rats ( $0.273 \pm 0.13$  ng/ml) but decreasing in PS rats ( $-0.217 \pm 0.2$  ng/ml).

#### Plasma corticosterone in females

We monitored estrous cyclicity in PS and NPS females for 1 month prior to the onset of stress in adulthood. Both PS and NPS females exhibited normal estrous cycles and were randomly cycling at the start of stress in adulthood. There were no significant effects related to plasma corticosterone at 0 min (Fig. 3). At 15 min, there was a significant adult stress effect ( $F(1,12) = 7.2$ ;  $P < 0.02$ ) with plasma corticosterone at day 8 overall lower than on day 1. At 30 min, there were no significant effects. At 60 min, there was a significant interaction effect ( $F(1,11) = 7.9$ );  $P < 0.02$ ). Post hoc tests indicated that corticosterone levels in NPS females on day 1 were significantly lower than in all other groups (Fig. 3). Integrated corticosterone values were calculated and no significant effects were observed (Fig. 4).

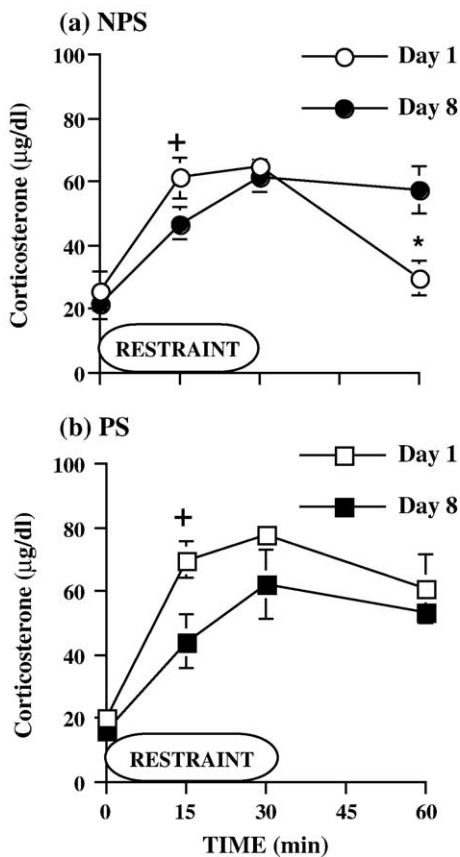


Fig. 3. Pregnant female rats were exposed to prenatal stress (PS) during the third week of gestation or not exposed to any stress (no prenatal stress; NPS). Adult NPS (a) and PS (b) females were exposed to 30 min of restraint for 8 consecutive days and blood was sampled only on days 1 and 8. Data are expressed as Mean  $\pm$  SEM. Group sizes are PS females  $n = 6-7$  and NPS females  $n = 7-9$ . Variations in  $n$  are due to insufficient plasma. \* $P \leq 0.05$ ; day 1 values in NPS rats significantly different from all other groups. † $P \leq 0.05$ ; day 8 overall lower than day 1.

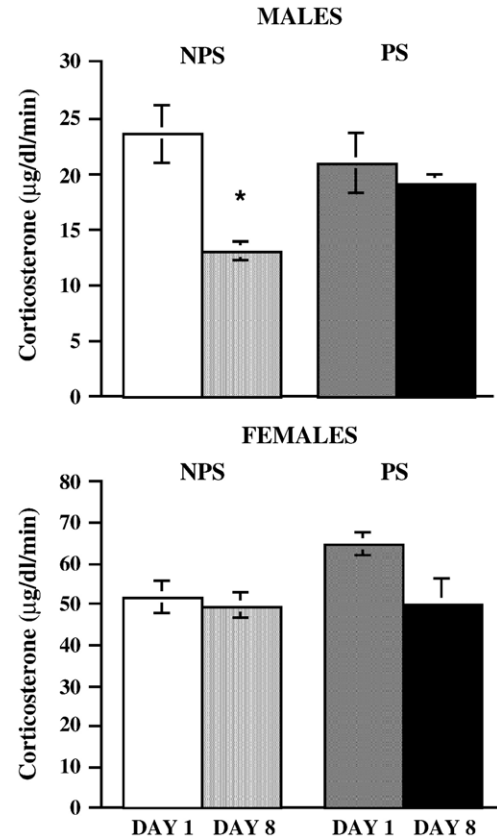


Fig. 4. Integrated corticosterone responses to restraint are shown for prenatally stressed (PS) and non-prenatally stressed (NPS) male and female rats. Integrated values for corticosterone responses of PS and NPS male rats exposed to restraint on days 1 and 8 are shown in a. In b, integrated values for corticosterone responses of PS and NPS female rats exposed to restraint on days 1 and 8 are shown. Data are expressed as Mean  $\pm$  SEM. \* $P \leq 0.05$ ; significantly lower than all other groups.

As with males, we also analyzed corticosterone levels in PS and NPS rats that were exposed to only a single acute exposure to restraint (data not shown). We combined corticosterone levels from rats on day 1 (that eventually went on to be chronically stressed) with corticosterone from rats exposed only to the single restraint. No significant effects were observed at 1, 15, or 30 min between PS and NPS rats exposed to a acute restraint. At 60 min, there was a significant effect ( $F(1,26) = 4.4$ ;  $P < 0.04$ ) with PS females exhibiting higher corticosterone than NPS females. No significant effect was observed with integrated corticosterone of acutely stressed female rats.

In sum, female rats, both NPS and PS, exhibited lower corticosterone levels on day 8 compared to day 1 at one time point (15 min) but no specific effect of PS was found at this time point or at 0 and 30 min. At 60 min, NPS females exhibited a higher response on day 8 than day 1 but no such difference was found in PS females. As with acutely stressed males, PS females exhibited increased corticosterone levels at 60 min following onset of acute stress compared to NPS females. However, none of these differ-

ences was large enough to be reflected in significant differences in integrated corticosterone.

## Discussion

We examined whether prenatal stress alters HPA responses to repeated stress in adult male and female rats. In males, we found that prenatal stress largely prevented the habituation of corticosterone responses to repeated restraint that was observed in non-prenatally stressed rats. Some habituation was seen in all females (PS and NPS) during restraint but, during recovery from restraint, prenatally stressed females did not exhibit the increased response seen in NPS females. Accordingly, the integrated corticosterone data showed that NPS males had overall lower corticosterone on day 8 vs. day 1 but PS males did not while there were no differences in integrated corticosterone amongst the PS and NPS females. Overall, our results suggest that prenatal stress has long-lasting effects on an animal's ability to respond to chronic stress in adulthood and that these effects are sex specific.

We also measured body weights at specific ages for both male and female rats. Over development from days 34 to 62 of age, body weights were not different between PS and NPS male rats. However, on the week and on the day prior to onset of acute/chronic stress (at approximately 85 days of age), PS males weighed less than NPS males (Fig. 1). Since litters were born at different days within 2 weeks of one another, it is possible that the differences between PS and NPS males in body weight prior to onset of acute/chronic stress are due to small differences in age. However, assignment of pregnant females to the PS and NPS conditions was done randomly and PS and NPS litters were born intermittently so that, on average, PS litters were not younger than NPS litters. Furthermore, variability due to different sizes of litters did not seem to affect body weights as growth rates were consistent and varied little in non-prenatally stressed rats. In support of our findings, *Fride et al.* (1986) have also reported that body weights at weaning were not affected by prenatal stress and *Zimmerberg and Blaskey* (1998) found no significant effects of prenatal stress on body weights in males and females up to day 65 of age. Our results suggest that prenatal stress effects on weight gain in males are slow to develop and only become significant at the time they approach adulthood. In contrast to the males, PS and NPS female rats did not exhibit any differences in body weight at any time point examined. Thus, prenatal stress differentially decreased body weight gain in males without affecting weight gain in female rats.

Earlier studies on the effects of prenatal stress on HPA responses to acute stress yielded conflicting results. Some studies have shown that prenatal stress elevates HPA responses to acute stress in both males and females (*Reznikov et al.*, 1999, 2001) particularly at recovery from

restraint (*Barbazanges et al.*, 1996; *Maccari et al.*, 1995), whereas other studies have shown no effects of prenatal stress (e.g., *McCormick et al.*, 1995). Here, we report that both male and female PS rats exhibited modest increases in corticosterone levels during recovery from acute restraint and that these increases were not of sufficient magnitude to alter the integrated corticosterone response to restraint. The plasma levels reported for acutely stressed animals were obtained by combining data from acutely stressed rats with responses on day 1 of rats that went on to be repeatedly stressed. The small difference in plasma corticosterone response to acute stress we observed in large groups of PS and NPS rats confirms the suggestion that the effects of prenatal stress on HPA responses to acute restraint are modest.

NPS male rats exhibited habituation of corticosterone responses to repeated stress (Figs. 2 and 4). Corticosterone responses during and at recovery from the eighth restraint were significantly lower than to the first restraint specifically in NPS rats. The integrated corticosterone data corroborate these findings with lower integrated corticosterone levels at day 8 compared to day 1 in NPS rats. Habituation to repeated restraint (from 30 min up to 2.5 h per day for 6 to 16 days) in previously non-stressed male rats has been previously demonstrated (*Bhatnagar et al.*, 2002a; *Cole et al.*, 2000; *Viau and Sawchenko*, 2002). Importantly, habituation was not seen in prenatally stressed rats (there was only an overall effect of adult stress in all rats at 15 min). Furthermore, there was no difference in integrated corticosterone responses to the first vs. the eighth restraint in PS rats. Overall, these data indicate that prenatal stress prevents the habituation of corticosterone responses to repeated restraint in male rats.

In female rats, regardless of prenatal stress condition, significantly lower corticosterone responses to restraint on day 8 compared to day 1 were observed only during restraint (significant adult stress effect only; Fig. 3). The limited habituation of HPA activity during restraint in normal female rats has been found by some (*Galea et al.*, 1997) though others have shown that female rats exhibit habituation when corticosterone is measured at 1 h (*Bowman et al.*, 2001) using 6 h of restraint per day for 21 days and in response to repeated forced swimming (*Szuran et al.*, 2000). Together, these studies and our results suggest that, in contrast to males, limited habituation to repeated restraint is observed in female rats. One caveat is that in the present and above studies, females were gonadally intact and randomly cycling at the time of sampling. HPA responses to acute stress vary over the estrous cycle (*Viau and Meaney*, 1992) and it is possible that variations in estrous cyclicity within and across groups prevents habituation from being clearly observed. Furthermore, the possibility remains that the daily handling in females (in order to determine stage of estrous) could have affected HPA responses to acute and repeated stress in our studies. Overall, how gonadal hormones may regulate habituation to repeated restraint in females is unclear and will be an important consideration in

determining how prenatal stress alters neuroendocrine function in adult females.

As in males, little is known about the interaction of prenatal stress with adult chronic stress in females. During recovery from restraint, we found that NPS females exhibited a higher response on day 8 than on day 1, indicating a sensitized response to repeated restraint (Figs. 3 and 4). Alternatively, this higher response could be due to impaired negative feedback in NPS female rats. No such enhanced response was observed in PS females even though PS females exhibit lower densities of glucocorticoid receptors in the hippocampus, a region important for negative feedback inhibition of HPA activity (Szuran et al., 2000). In support of our findings, Pollard (1984) found that no habituation was seen in either prenatally stressed (stressed throughout gestation) or non-prenatally stressed females to repeated footshock though the prenatally stressed females exhibited enhanced corticosterone with repeated footshock. In sum, we found limited evidence for habituation to repeated restraint in all female rats and no difference in the corticosterone response at recovery to the first vs. eighth restraint in PS females. These more complex findings in female rats stand in sharp contrast to the clear blockade of habituation produced by prenatal stress in male rats. The differing effects of prenatal stress on habituation of HPA activity in male and female animals suggest that manipulations of the early environment have long-term sex-specific effects on habituation of HPA activity.

These differing effects of prenatal stress responses to repeated stress in male and female rats could be due to a number of factors that are discussed briefly below. In general, testosterone tends to inhibit corticosterone responses to stress, with gonadectomy in male rats producing enhanced corticosterone responses to stress (for a review, see Viau, 2002; Young, 1998). In part, testosterone may decrease responses to stress by inhibiting plasma and intrapituitary levels of corticosteroid binding globulin (CBG; Viau and Meaney, 2004a). CBG serves to sequester corticosterone away from binding to its receptors, thereby decreasing the functional consequences of corticosterone release. The net result of lowering CBG may be to enhance the ability of corticosterone to exert its inhibitory negative feedback effects. However, CBG does not seem to be altered by PS in males (McCormick et al., 1995) although the possibility remains that it is altered by repeated restraint. In males, third trimester prenatal stress is known to decrease testosterone levels in males in utero resulting in feminization and demasculinization of certain behaviors (Ward, 1984; Ward et al., 1996). Plasma testosterone has been reported to be unaffected in the adult offspring by prenatal stress (Ward, 1984; Ward et al., 1996) and our finding that there was no difference in plasma testosterone between PS and NPS males on either day 1 or day 8 corroborates these earlier reports (see Results). However, in the present study, plasma testosterone increased over the week of repeated restraint in NPS rats but

decreased in PS rats resulting in a significantly different change in testosterone in NPS compared to PS rats. Why testosterone increased in repeatedly restrained rats in our study but is generally decreased by repeated or chronic stress in other studies is not clear (Bell et al., 2002; Bonilla-Jaime et al., 2003; Tamashiro et al., 2004). This discrepancy may be due type and/or duration of the repeated/chronic stress. Given the inverse nature of the relationship between testosterone and corticosterone, the increase in testosterone in NPS rats may be partially responsible for the habituation seen in these animals and the decrease in testosterone in PS animals may help explain the lack of habituation in this latter group.

In females, estrogen has stimulatory effects on ACTH secretion and expression (for a review, see Viau, 2002; Young, 1998). In part, estrogen could work through actions on the CRH cell population in the PVN or in other parts of the brain that contain sex steroid receptors. Sex steroids can act in multiple sites in the brain to alter HPA responses to stress, including the medial preoptic area, brainstem catecholaminergic regions, amygdala, and/or hippocampus (Kerr et al., 1996; Simerly, 1993; Stumpf and Sar, 1976; Viau and Meaney, 1996; Viau and Meaney, 2004b). Therefore, prenatal stress may exert sex-specific effects on HPA responses to repeated restraint through a number of potential mechanisms. These sex-specific effects could occur through prenatal stress-induced alterations in sex steroid hormone levels in circulation or sex steroid hormone receptors in specific brain regions important in regulating the HPA response to repeated stress. Sex-specific effects of prenatal stress could also occur through changes in CBG or sex steroid regulation of ACTH secretagogue gene expression in the PVN.

Regardless of the mechanisms that produce sex-specific effects of prenatal stress on HPA responses to repeated stress, prenatal stress clearly modifies HPA responses to repeated restraint in males. Some brain regions important for regulating HPA responses to repeated stress have been identified, primarily in male rats. Some of these regions may also be important for the sex-specific effects of prenatal stress, but this remains to be determined. Our previous studies have indicated a role for the paraventricular nucleus of the thalamus, specifically its posterior division (pPVTh), in habituation of HPA activity to repeated restraint (Bhatnagar et al., 2002b). Thus, prenatal stress may alter habituation of HPA responses to repeated stress by altering activity in the circuitry related to the pPVTh. Ultimately, any such circuitry will have to modulate the medial parvocellular PVN, the site of neuroendocrine cells that regulate ACTH synthesis and release. In particular, the ventral aspect of the medial parvocellular PVN will likely be activated since repeated restraint specifically activates this ventral aspect medial parvocellular PVN while the dorsal aspect is activated by acute stress (Viau and Sawchenko, 2002). The divergent effects of prenatal stress in males and females suggest that any alterations in such circuitry are sex

specific and could occur through one or more of the mechanisms discussed earlier. Alternatively or in addition, negative feedback systems may be altered by prenatal stress particularly since prenatal stress blocks the enhanced response to recovery from restraint found in NPS females. Peripheral injections of a mineralocorticoid receptor antagonist or a combination of a mineralocorticoid and glucocorticoid receptor antagonist have been shown to block the habituation of corticosterone in response to repeated restraint (Cole et al., 2000). Therefore, alterations in negative feedback functions over the course of repeated stress exposure may represent another way that prenatal stress alters habituation of HPA activity.

The divergent effects of late gestational stress on adult males and females could be due to processes acting during gestation, early development, or in adulthood. Whether these processes act through modulatory neural circuitry or negative feedback effects of corticosterone or both is not known. Nonetheless, these results suggest that prenatal stress represents one important category of early environmental events that affects vulnerability to the potentially adverse effects of repeated stress in adulthood.

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