Effects of Early Deprivation and Maternal Separation on Pup-Directed Behavior and HPA Axis Measures in the Juvenile Female Rat

ABSTRACT: Juvenile female rats show maternal-like behavior toward pups. The purpose of the following experiment was to investigate whether the HPA axis, through the use of early separation manipulations that alter HPA functioning in rats, plays a role in the juvenile response to foster pups. Female rats were early deprived or maternally separated for 5 hours daily from PND 2 to 14 and compared to animal facility-reared rats. Deprivation or separation increased CRH-R1 IR in the juvenile PVN, but had no other effects on other HPA measures or on maternal behavior. Pup-exposure during the juvenile period blunted corticosterone levels after acute and repeated pup-exposures when compared to exposure to novelty and conspecifics respectively. Repeated exposures to pups also increased CRH-R1 IR relative to isolation during the juvenile period. Overall, the data suggest that although pup-exposure affects corticosterone levels, the HPA axis does not relate to juvenile maternal behavior in the present experiments. © 2008 Wiley Periodicals, Inc. Dev Psychobiol 50: 315–321, 2008.

Keywords: early deprivation; maternal separation; maternal behavior; juvenile period; HPA axis; Sprague–Dawley rat

INTRODUCTION

If exposed to pups, female juvenile rats will show maternal behaviors soon after weaning (Bridges, Zarrow, Goldman, & Denenberg, 1974; Brunelli, Shindledecker, & Hofer, 1985; Gray & Chesley, 1984; Lovic, Gonzalez, & Fleming, 2001; Mayer & Rosenblatt, 1979; Oxley & Fleming, 2000; Rees & Fleming, 2001). Juvenile maternal sensitization is unlike the adult virgin sensitization in that juvenile rats show shorter latencies to become maternal (Brunelli et al., 1985; Mayer, Freeman, & Rosenblatt, 1979; Mayer & Rosenblatt, 1979) which may be a result of a still developing juvenile fear and/or stress system (Numan & Insel, 2003).

Corticosterone, a stress hormone of the hypothalamic-pituitary-adrenal (HPA) axis, increases maternal behavior in the postpartum rat but decreases maternal behavior in the sensitized virgin rat (Rees, Panesar, Steiner, & Fleming, 2004; Rees, Panesar, Steiner, & Fleming, 2006a). The HPA axis may contribute to the onset and expression of juvenile maternal behavior although the direction of effects remains unclear as juvenile rats do not experience pregnancy unlike postpartum rats, yet show a rapid onset of maternal behavior unlike adult virgin rats. Olazabal and Morrell (2005) found decreased c-fos activation in fear- and maternally-related areas during pup-exposure in juvenile rats, but whether this relates to the HPA axis is also unclear. The purpose of the following experiments was to utilize the standard manipulations of early deprivation and maternal separation to alter HPA functioning (Pryce & Feldon, 2003; Rees, Steiner, & Fleming, 2006b) in order to investigate how pup-exposure affects maternal responsiveness and HPA axis in the juvenile female rat.
Early deprivation (separated from mother and littermates for 5 hours daily from PND 2 to 14) but not maternal separation (separated from mother, but not littermates for 5 hours daily from PND 2 to 14), blunts the corticosterone response to stress in juvenile and adult female rats (Rees, Steiner, et al., 2006b). Maternal separation disrupts adult postpartum maternal behavior (Boccia & Pedersen, 2001; Lovic et al., 2001), but has only subtle effects on juvenile maternal behavior (Lovic et al., 2001; Rees & Fleming, 2001). Whether early deprivation affects maternal behavior has not been investigated, although early deprivation transiently increases maternal behavior during maternal sensitization of the adult virgin rat (Rees & Fleming, unpublished observations) and may have the same effect on juvenile maternal behavior.

The CRH system, a system involved in the expression of fear-related behaviors (Dautzenberg, Kilpatrick, Hauger, & Moreau, 2001; Smagin, Heinrichs, & Dunn, 2001), may also be involved. The administration of CRH to virgin female rats inhibits maternal behavior (Pedersen, Caldwell, McGuire, & Evans, 1991) although what its role is in postpartum maternal behavior remains unclear. While little research has addressed the effects of early separation on CRH systems in the female rat, separation in male rats increases CRH mRNA, CRH-1R, and CRH content in the PVN in adulthood (Ladd, Owens, & Nemeroff, 1996; Ladd, Huot, Thrivikraman, Nemeroff, Meany, & Plotsky, 2000; Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; Plotsky & Meaney, 1993). The effects of separation may be specifically due to the CRH-R1 system given that separation has no effect on corticosterone levels in CRH-R1 deficient mice (Schmidt, Oitzl, Muller, Ohl, Wurst, Holzboer, Levine, & de Kloet, 2003). Separated male rats also show increased CRH-R1 mRNA and binding in the PVN and locus coeruleus and decreased CRH-R1 mRNA and binding in the anterior pituitary gland, amygdala, and cortex in adulthood (Ladd et al., 2000; Plotsky, Thrivikraman, Nemeroff, Caldji, Sharma, & Meaney, 2005). How maternal separation or early deprivation affects the CRH system of the juvenile female rat remains to be investigated.

In experiment one, we tested the effects of early deprivation and maternal separation on maternal responsiveness and HPA hormones (corticosterone and ACTH) following a short pup-exposure in juvenile female rats. We predicted that early deprivation would blunt the corticosterone response to pups and lead to an increase in maternal responsiveness. In experiment two, we again tested the effects of early deprivation and maternal separation on juvenile maternal behavior and HPA hormones, but this time following repeated pup-exposure during the juvenile period. To determine whether pup-exposure is more similar in its effects on the HPA axis to conspecific exposure or to isolation, two additional groups experiencing either conspecific exposure or isolation were added. In experiment three, the possibility of an early or juvenile experience-induced change to the CRH system was investigated through measurement of CRH-R1 densities in the paraventricular nucleus (PVN) of the hypothalamus and the amygdala. Little research has investigated CRH-R1 in juvenile rats; however, we predicted that early separation would decrease CRH-R1 in the basolateral amygdala and increase CRH-R1 in the PVN similar to what is seen in adult male rats (Sanchez, Ladd, & Plotsky, 2001).

**GENERAL METHODS**

**Subjects**

Rats (Sprague–Dawley) were born at the University of Toronto at Mississauga, from a stock originally obtained from Charles River Farms (Quebec, Canada). They were maintained on a 12:12-hour light:dark cycle (on at 8:00 am). Room temperature and humidity were maintained at 24.0°C and 40%, respectively. All procedures involving animals were approved by the University of Toronto Animal Care Committee.

**Separation Procedures**

Litters were culled to six female and six male pups on the day after birth and assigned to separation groups. There were three groups: (1) Separated from both mother and peers for 5 hours daily from PND 2 to 14 (early deprivation; ED); (2) Separated from mother, but not peers for 5 hours daily from PND 2 to 14 (maternal separation; MS); (3) Left undisturbed until weaning except for standard animal facility procedures, from PND 2 to 21 (animal facility rearing; AFR).

Pups were removed at approximately 9:00 am and returned at approximately 2:00 pm for a 5-hour separation period. Only female pups were removed during the separation period meaning that mother rats always had male pups in the home cage. All separation chambers were maintained at approximately 32°C.

**Juvenile Exposure Groups**

In experiment 1, all rats were weaned on PND 22 and housed with a same-sex, same-age rat until PND 30. On PND 30, rats were further subdivided into five groups: (1) No pup-exposure (baseline sample); (2) Blood sampled 5 minutes after pup-exposure; (3) Blood sampled 60 minutes after pup-exposure; (4) Blood sampled 5 minutes after novel environment exposure; (5) Blood sampled 60 minutes after novel environment exposure. For all groups, the same-sex cage mate was removed prior to either baseline sampling or pup/novelty exposure. Rats
were not habituated to cage mate removal, but all groups received the same cage mate removal procedure. Cage mates were then used in another set of experiments. Rats in the pup exposure group were exposed to pups in their home cage in the colony room for 5 minutes. Rats in the novel environment group were exposed to a large novel arena (150 cm × 150 cm × 49.5 cm) for 5 minutes in a room separate from the colony room. Finally, a separate set of rats was tested to determine the effects of early separation on maternal responsiveness during a 5-minute pup-exposure.

In experiment 2, rats were weaned on PND 21 and placed into three groups: (1) Pup exposure (continuous exposure from PND 22 to 30); (2) Conspecific exposure (continuous exposure to age-matched, same sex, non-sibling peer from PND 22 to 30); 3. Isolation—no exposure (isolated from PND 22 to 30). These groups were further subdivided into two groups on PND 30: (1) No Stress; (2) Isolation + Stress. Rats in the non-stressed group were immediately sacrificed to provide a baseline sample. Rats in the stressed group were isolated through either removal of pups or of the conspecific and given a saline injection (10 cc) 20 minutes prior to sacrifice.

In experiment 3, rats were weaned on PND 21 and placed into two groups: (1) Pup exposure (continuous exposure from PND 22 to 30); (2) Isolation exposure (isolated from PND 22 to 30). Rats were sacrificed on PND 30 and brains were taken for analysis of CRH-R1 densities.

**Pup Exposure and Maternal Behavior**

For all pup exposures, juvenile rats were individually exposed to a litter of six 3- to 8-day-old foster pups obtained from donor mothers. On the day prior to first exposure (PND 29 in experiment 1 and PND 21 in experiments 2 and 3), nesting material was placed in each rat’s cage. On PND 30 in experiment 1 and during pup replacement in experiments 2 and 3 (PNDs 22–30), the foster litter was placed in the cage in the diagonally opposite corner of the nest site. In experiment 1, maternal testing took place during a 5 minute pup exposure after which pups were removed. In experiments 2 and 3, 24 hours after the initial pup exposure, the position of the juvenile rat and pups was noted and the pups were removed. Within approximately 10 seconds, a fresh set of six donor pups was introduced. This occurred each day from PNDs 22 to 30 and maternal latency was determined over this period. The latency to become maternal was defined as reaching the maternal criterion of 2 days of full retrieval (all pups retrieved to the nest during the first 10 minutes after pup replacement). On PND 29, maternal behaviors were observed and recorded during an 8-minute maternal test following pup replacement.

For maternal tests, frequency and duration of the following behaviors were recorded (BEST, Behavioral Evaluation Strategies and Taxonomies, S and K Computer Products, Toronto, Ontario): (a) retrieving (carrying a pup to the nest site); (b) licking, with separate observation of body licking and genital licking; (c) sniffing pup; (d) nest building; (e) over pups, with separate observations of hovering (being over pups and engaged in licking or other behaviors) and of crouching (kyphosis or nursing posture) (f) mouthing (carrying pups in the cage after retrieval); (g) sniffing air; (h) self grooming.

**Blood Sampling and Radioimmunoassay**

For each experiment, blood samples were collected after decapitation on PND 30 between 9 and 11 am. Corticosterone (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA; interassay variability = 8.5%; intra-assay variability = 6.83%) and ACTH (ICN Biomedicals, Inc., Costa Mesa, CA; interassay variability = 7.35%; intraassay variability = 5.05%) were determined by radioimmunoassay.

**CRH-R1 Receptor Immunocytochemistry (ICC)**

Rats were anesthetized with a lethal dose of Sodium Pentobarbital and transcardially perfused with ice cold phosphate buffer followed by 4% paraformaldehyde (pH 7.4). Brains were extracted, post fixed in the same fixative for 4 hours and transferred into 30% sucrose for 48 hours. Brains were sliced at 40 μm and processed for ICC.

Sections were incubated in 30% w/w hydrogen peroxide in trizma buffered saline (TBS) for 30 minutes at 4°C. Slices were washed 3 × 5 minutes in TBS and then pre-blocked in 3% normal goat serum (NGS, Vector Laboratories, Mississauga, ON, Canada) in 0.05% Triton TBS for 90 minutes at 4°C. Slices were then incubated for 48 hours in anti-cRFR anti serum (Santa Cruz Biotechnology, Inc., Santa Cruz, CA; diluted at 10 μg/ml) in 0.05% Triton TBS with 3% NGS at 4°C. Following 48 hours of incubation, slices were washed and incubated in biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlington, ON, Canada; 1:200) in 0.05% Triton TBS with 3% NGS for 1 hour at 4°C. Slices were then washed and placed in avidin-biotinylate-peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories; 1:50) for 2 hours at 4°C. The peroxidase complex was visualized by sequential incubation at room temperature with 50 mM Tris for 10 minutes, 3′,3′ diaminobenzidine (DAB) in 50 mM Tris (pH 7.8) for 10 minutes on an agitator, and DAB/3% hydrogen peroxide/8% Nickel Chloride in 50 mM Tris. Slices were then mounted on gel-coated slides and coverslipped.

All slides were coded for group identification such that experimenters completing the microscopy, imaging, and
Images were captured using an Olympus camera (OLYMPUS BX60, Tokyo, Japan) and through 10× objective lens using standard factory settings. The number of nuclei was counted using Image Pro System (Media Cybernetics, Inc., Silver Spring, MD) and counts were taken from the right and left sides of the brain and averaged for a total count. From one section per subject for each given brain area, CRH receptors densities were analyzed in the paraventricular nucleus (PVN) of the hypothalamus and the central (CeA) and basolateral (BLA) nuclei of the amygdala. These brain sites were identified on the basis of surrounding landmarks and a counting box was fitted to the site to standardize how much of the area was counted (see Fig. 1). Parameters were defined based on stereotaxic coordinates from Paxinos and Watson (2007) (PVN: bregma—1.80 mm; interaural 7.20 mm; 0 to 1 from midline; 7.8 to 8.4 from dorsal surface; CeA: bregma—1.80 mm; interaural 7.20 mm; 3.8 to 4.4 from midline; 7.8 to 8.4 from dorsal surface; BLA: bregma—1.80 mm; interaural 7.20 mm; 4.4 to 5.2 from midline; 7.4 to 8.4 from dorsal surface).

RESULTS

Experiment 1

Using a 3 (early separation) × 5 (juvenile experience) analysis of variance (ANOVA), a main effect of juvenile exposure was found for corticosterone \( F(4, 65) = 31.26, p < .001 \) (see Tab. 1). Regardless of stressor, corticosterone levels at 5 minutes post-stress were higher than levels at baseline and 60 minutes post-stress (Tukey’s, \( p < .05 \)). Additionally, rats exposed to a novel environment had higher levels of corticosterone than rats exposed to pups at both 5 and 60 minutes post-stress (Tukey’s, \( p < .05 \); see Tab. 1). There were no effects of early separation on corticosterone or ACTH levels nor were ACTH levels affected by juvenile experience.

Using an One-way ANOVA, no effects of early separation were found for maternal behaviors during a 5-minute pup exposure.

Table 1. The Effects of Acute and Repeated Pup Exposure on ACTH (pg/mL) and Corticosterone (ng/mL) Levels (mean ± SEM)

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Post-Stress</th>
<th>5 Minutes</th>
<th>60 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CORT</td>
<td>ACTH</td>
<td>CORT</td>
</tr>
<tr>
<td>Pups exposure</td>
<td>242.88 (55.42)</td>
<td>95.28 (18.27)</td>
<td>560.96(^a) (74.76)</td>
</tr>
<tr>
<td>Novel environment</td>
<td>1138.01(^a,b) (75.00)</td>
<td>110.37 (18.30)</td>
<td>461.38(^a) (75.71)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 2</th>
<th>Basal</th>
<th>20 Minutes Post-Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CORT</td>
<td>ACTH</td>
</tr>
<tr>
<td>Pup exposure</td>
<td>184.84 (45.00)</td>
<td>47.13 (6.95)</td>
</tr>
<tr>
<td>Conspecific exposure</td>
<td>230.13 (54.7)</td>
<td>76.50 (8.30)</td>
</tr>
<tr>
<td>Isolation—no exposure</td>
<td>187.05 (44.32)</td>
<td>48.98 (6.73)</td>
</tr>
</tbody>
</table>

Experiment 1: CORT—\(^a\)Novel Environment group is greater than Pups group \( p < .05 \); \(^b\)5 minutes post-Novel Environment group is greater than all other groups \( p < .05 \).

Experiment 2: CORT—Main effect of stress with stress groups being greater than basal groups \( p < .05 \); Main effect of juvenile experience with pup and isolation groups being lower than conspecific group \( p < .05 \).

Experiment 2: ACTH—Main effect of juvenile experience—pup and isolation groups are less than conspecific group \( p < .05 \).
Experiment 2

To analyze HPA measures, an initial 3 (early separation) × 3 (juvenile experience) MANOVA was computed for both stressed and non-stressed rats. However, since there were no early separation effects, 3 (juvenile experience) × 2 (stress vs. no stress) ANOVAs were then computed. A significant main effect of juvenile experience was found for both corticosterone (F(2, 134) = 4.85, p < .009) and ACTH (F(2, 134) = 4.59, p < .012). Rats exposed to conspecifics had higher levels of both corticosterone and ACTH than rats exposed to pups and rats exposed to isolation (Tukey’s, p < .05; see Tab. 1). There was also a main effect of stress for both corticosterone (F(1, 134) = 83.91, p < .001) and ACTH (F(1, 134) = 33.16, p < .001) with stressed rats having higher levels of both hormones than non-stressed rats (see Tab. 1).

There were no effects of early separation on maternal latency or maternal behavior. As well, there were no significant correlations between maternal measures and baseline or stress-induced hormone levels.

Experiment 3

Due to low sample sizes, non-parametric statistics (Kruskal–Wallis test followed by Mann–Whitney U tests for post-hoc comparisons) were used to determine the effects of early experience on CRH-R1 densities. There was an effect of early experience on CRH-R1 in the PVN (Kruskal–Wallis χ² = 11.41, df = 2, p < .003), with ED and MS increasing CRH-R1 when compared to AFR (ED vs. AFR: Mann–Whitney U = 0, p < .004; MS vs. AFR: Mann–Whitney U = 5, p < .008; see Tab. 2). Again, due to low sample sizes, a Kruskal–Wallis test was used to determine the effects of juvenile experience on CRH-R1 densities. There was an effect of juvenile experience on CRH-R1 in the CeA (Kruskal–Wallis χ² = 7.20, p < .007) with pup-exposed increasing CRH-R1 when compared to isolation (see Tab. 2). There were no effects of early experience on maternal measures or any correlations between maternal measures and CRH-R1.

DISCUSSION

In summary, early separation had no effect on maternal behavior and had little effect on the HPA axis of the juvenile female rat, although both separation manipulations increased CRH-R1 densities in the PVN. Pup exposure during the juvenile period did; however, affect the HPA axis. Short and repeated pup-exposures blunted corticosterone levels when compared to exposure to a novel environment and to a social conspecific respectively. Corticosterone levels after pup-exposure over 8 days; however, did not differ compared to corticosterone levels after 8 days of isolation despite pup-exposure increasing CRH-R1 in the central amygdala when compared to isolation.

The limited effect of early experience on HPA measures was surprising given that we (Rees, Steiner, et al., 2006b) have recently shown that early deprivation blunts the corticosterone response to a novel environment in juvenile female rats. The fact that separation increased CRH-R1 in the PVN supports findings in adult male rats (Sanchez et al., 2001). Both stress and CRH administration not only increased CRH mRNA in the PVN, but also upregulated CRH-R1 in the PVN (Imaki, Naruse, Harada, Chikada, Imaki, Onodera, Demura, & Vale, 1996; Luo, Kiss, Makara, Lolia, & Aguilera, 1994; Makino, Schulkin, Smith, Pacak, Palkovits, & Gold, 1995) suggesting that separation was stressful. Makino, Tanaka, Nazarloo, Noguchi, Nishimura, and Hashimoto (2005) propose that upregulation of CRH-R1 in the PVN allows CRH to improve its own biosynthesis during stress. Perhaps different stressors and/or different sampling regimens than those used in the present experiments would elicit deprivation/separation-induced effects on corticosterone and ACTH levels.

In the present experiments, separation did not affect juvenile maternal behavior, similar to the findings of Lovic et al. (2001) and Rees and Fleming (2001). Conversely, maternal separation as well as artificial rearing decrease maternal licking and crouching behavior in adulthood; however, only artificial rearing has a robust disruptive effect on juvenile maternal behavior (Gonzalez & Fleming, 2002; Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001; Lovic et al., 2001; Rees and Fleming, 2001). Whether these effects of artificial rearing on maternal behavior are due to changes in the HPA axis remain unclear, although research has shown that artificial rearing does not affect baseline or stress-induced levels of corticosterone (Burton, Chatterjee, Chatterjee-Chakraborty, Lovic, Grella, Steiner, & Fleming, 2007; Ward, Xing, Carnide, Slivchak, &

Table 2. Effects of Early Separation and Juvenile Experience on CRH-R1 Densities (Median) in the PVN of the Hypothalamus and the Amygdala (Central Nucleus)

<table>
<thead>
<tr>
<th></th>
<th>PVN</th>
<th>CeA</th>
</tr>
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<tbody>
<tr>
<td>Early separation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early deprivation (n = 5)</td>
<td>15.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.10</td>
</tr>
<tr>
<td>Maternal separation (n = 8)</td>
<td>12.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.80</td>
</tr>
<tr>
<td>Animal facility reared (n = 7)</td>
<td>4.71&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>11.55</td>
</tr>
<tr>
<td>Juvenile experience</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With pups (n = 13)</td>
<td>9.88</td>
<td>21.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isolation (n = 18)</td>
<td>10.92</td>
<td>12.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>ED is greater than AFR (p < .05).
<sup>b</sup>MS is greater than AFR (p < .05).
<sup>c</sup>With pups is greater than isolation (p < .05).
While a link between early experience’s effects on maternal behavior and the HPA axis now seems unlikely, juvenile exposure to pups does affect measures of the HPA axis. An acute pup-exposure of 5 minutes increased corticosterone levels relative to baseline; however, whether this is due to the pups being novel or the pups per se remains unclear. This increase was significantly less than that seen in juvenile rats exposed to a novel environment for 5 minutes. A more meaningful comparison might have been to measure the corticosterone response to a novel object placed into the home cage; future studies will explore this idea.

Repeated pup-exposure during the juvenile period also affected activation of the HPA axis, although this effect may simply be due to being isolated from conspecifics. Pup-exposure or isolation decreased corticosterone levels when compared to conspecific exposure. It is possible that conspecific exposure increased corticosterone relative to the other groups due to the increased activity that occurs when two juvenile rats interact; however, juvenile rats exposed to pups are also active while they respond to pups. How activity levels contrast among the three juvenile groups was not investigated.

As discussed above, pup-exposure does not differ from isolation in terms of its effects on corticosterone and ACTH levels. However, in terms of CRH-R1, pup-exposure increased CRH-R1 in the amygdala when compared to isolation. This represents a change in the amygdalar-CRH system which may relate to fear/anxiety-related behavioral systems (Dautzenberg et al., 2001; Smagin et al., 2001). Makino et al. (1995) found that psychological stress does not change CRH-R1 mRNA in the CeA of adult rats, suggesting that increased CRH-R1 in the CeA after pup-exposure may not be a result of stress per se. Merali, Khan, Michaud, Shippy, and Anisman (2004) propose that CRH in the amygdala is important for drawing attention to relevant stimuli in the environment. CRH in the CeA is activated in response to both positive and negative stimuli and may not be involved in behavior per se, but rather in the preparation for future situations (Merali, Michaud, McIntosh, Kent, & Anisman, 2003). In the present context, this suggests that juvenile rats may have a changed sensitivity to pup cues after repeated exposure to them. In virgin rats, CRH inhibits maternal behavior, but less so if rats had been exposed to pups prior to administration of CRH (Pedersen et al., 1991). This again suggests an experience-based modulation of CRH systems with pup exposure.

In conclusion, early separation manipulations had little effect on the HPA axis and maternal behavior. Early separation disrupts maternal behavior in adulthood (Boccia and Pedersen, 2001; Lovic et al., 2001), suggesting that the juvenile period may be a period where effects of early separation are latent or in transition. Despite a lack of relation between maternal and HPA measures, pup-exposure did affect the HPA axis. Pups are stressful, but not as stressful as novelty. Repeated exposure to pups decreased corticosterone, although these effects may be due to isolation rather than pup-exposure per se. However, pup-exposed rats had higher densities of CRH-R1 in the CeA when compared to levels in isolated rats, possibly reflecting an effect of pup-exposure on the CRH-related fear system rather than on the HPA stress system per se.

NOTES

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factor up-regulates its own receptor mRNA in the paraventricular nucleus of the hypothalamus. Molecular Brain Research, 38, 166–170.


