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Research report

Early deprivation, but not maternal separation, attenuates rise in corticosterone levels after exposure to a novel environment in both juvenile and adult female rats

Stephanie L. Rees [a], Meir Steiner [b], Alison S. Fleming [a,*]

[a] *Department of Psychology, University of Toronto at Mississauga, 3359 Mississauga Road North, Mississauga, Ont. L5L 1C6, Canada*
[b] *McMaster University, St. Joseph's Hospital, Hamilton, Ont., Canada*

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Abstract

Separation from the maternal nest alters the hypothalamic-pituitary-adrenal (HPA) axis stress response in adult male rats, but little research has addressed how separation affects female rats. The following experiments investigated how early maternal separation from postnatal day (PND) 2 to 14 affected stress-induced corticosterone and ACTH after exposure to an open field in juvenile and adult female rats. Female rats were separated for 5 h daily from mother and littermates (early deprivation: ED), separated from mother but not littermates (maternal separation: MS), or animal facility reared (AFR). Male siblings were left with the mother rat during separation. Female rats were exposed to an open field arena either during the juvenile period (PND 30) or during adulthood (PND 80–100). Results show that ED juvenile female rats showed a lower corticosterone stress response than MS and AFR female rats when measured at 5 min post-stress, but no difference at 20 or 60 min post-stress. In adulthood, ED female rats showed comparable elevations of corticosterone as MS and AFR rats at 5 min post-stress but lower elevations at 20 min. In terms of behavior, there were no significant effects of early experience. However, in adulthood, ED and MS rats tended to show a decreased proportion of inner grid crossings of the open field compared to AFR rats, suggesting a tendency for increased anxiety in these two separation groups.

Keywords: Separation; Juvenile; Adult; Female rat; Open field; HPA axis

1. Introduction

Early experience during the neonatal period can have effects on behavior and physiology that are evident at multiple developmental stages. For example, acute maternal deprivation during the stress hyporesponsive period (SHRP; postnatal days 2–14) increases corticosterone, an adrenal hormone released in response to stress, in pups and repeated deprivation further sensitizes this response during the neonatal period [38,61]. Being separated repeatedly from the mother rat but not littermates during the SHRP can lead to a hyperresponsive hypothalamic-pituitary-adrenal (HPA) response to stress in adulthood ([3,25,26,31,47]; other refs; also see Table 1). However, the effects of early repeated maternal separation on adult HPA axis response are inconsistent at best ([30,45,53]; also see Table 1). These inconsistencies may be due to the fact that maternal separation may have stressor-specific, manipulation-specific, age-specific, and/or gender-specific effects on the HPA stress response. At present, most maternal separation research has investigated effects on adult male rats. The purpose of the following experiments was to investigate the effects of early maternal separation on female rats in terms of behavioral and HPA responses to novelty (open field arena) at two different developmental periods, specifically during the pre-pubertal juvenile period and during adulthood.

In adult male rats, repeated maternal separation from mother and nest during the SHRP increases CRH mRNA, CRH content, ACTH, and corticosterone levels after stress ([3,25,26,31,47]; also see Table 1); however, the consistency of these effects depends on the type of stressor used, conditions of the separation procedure, and the age of testing [53]. For instance, in comparison to non-separated rats, male rats separated from the mother rat but not from littermates showed increased behavioral activity but lowered corticosterone in response to novelty during

* Corresponding author. Tel.: +1 905 828 3961; fax: +1 905 569 4326.
E-mail address: fleming@psych.utoronto.ca (A.S. Fleming).

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Table 1
A sample of the effects of early repeated maternal separation on corticosterone and anxiety-related behaviors in rats

Group	Gender	Effect	Reference (age of testing)
Corticosterone			
ED	Males	CORT: ED > H&NH basal and post-stress	3 (PND 45)
		No effect on CORT	25 (PND 105); 62 (∼PND 300)
ED	Males and females	Males, H > ED, NH&AFR for CORT post-stress, but no effect on females (restraint)	50 (∼PND 110)
		Males, no effect, but ED < NH for CORT post-stress (fear conditioned stimulus) for females	52 (adulthood)
MS	Males	MS > H for CORT post-stress	22 (PND 60–75); 24 (PND 90?); 31 (PND ∼90–120); 33 (∼PND 80); 48 (PND 60–75)
		MS > NH for CORT post-stress	31 (PND ∼90–120); 48 (PND 60–75); 58 (PND 40)
		MS < AFR for CORT post-stress	45 (PND 49)
		No effect on CORT	8 (PND 60); 28 (PND 600); 47 (∼PND 90–120)
MS	Males and females	Males, MS > AFR for CORT post-stress, no effect on females	74 (PND 120–150)
Anxiety-related behaviors			
ED	Males	ED&NH > H in exploratory test	3 (PND 45)
		No effect on OFA	43 (adulthood)
ED	Males and females	ED > NH activity in OFA in males	50 (∼PND 110)
		No effect of ED on males, but ED < NH anxiety in EPM for females	39 (PND 35)
		No effect on anxiety-related measures	62 (adulthood)
MS	Males	MS > AFR anxiety in EPM	8 (PND 60); 14 (PND 60); 22 (PND 60)
		MS < AFR anxiety in OFA	45 (PND 42)
		No effect on OFA	65 (∼PND 80)
MS	Males and females	Males, MS > NH&AFR anxiety in EPM, but females, MS < H&NH&AFR anxiety in EPM	24 (PND 90?); 74 (PND 120–150)
		Males, MS > NH activity in OFA, but females, only trend for same pattern	73 (∼PND 100)
		No effect on OFA	1 (PND 15); 28 (∼PND 85); 58 (PND 40)

Abbreviations: ED = early deprivation; separated from both mother and littermates. MS = maternal separation; separated from mother, but not littermates. H = handled; separated from mother, but not littermates for 15 min; also referred to as early handling. NH = non-handled; no handling/manipulations from birth to weaning. AFR = animal facility rearing; includes normal cage changing as per schedule within animal facility. PND: postnatal day. EPM = elevated plus maze. OFA = open field activity. CORT = corticosterone.

the juvenile period. In adulthood, similar increases in corticosterone after stress were seen for all groups [35]. This suggests an age-dependent effect of separation. In terms of anxiety-related behavior, the effects of repeated separation in male rats have been investigated using many tasks and are age-dependent, but generally, separation increases anxiety in the elevated plus maze and activity in the open field [1,8,14,24,28,39,60,65,73–75]. Some studies, however, have found no effect of separation on anxiety-related tasks [13,21,34,39,65,75] or a decrease in anxiety [46].

In terms of female rats, behavioral and endocrine effects are again inconsistent, but little research has addressed the effects of separation (from mother and nest, but not littermates; hence, 'maternal separation') or deprivation (of mother, nest and littermates; hence, 'early deprivation'). Early deprivation decreases the corticosterone response to some, but not all, stressors in female rats [50,52] while maternal separation has been found to be ineffective [67,74]. In female rats, maternal separation has been found to have no effect on anxiety-related behaviors [67], decrease anxiety-related behaviors [9,13,39,70], or increase anxiety-related behaviors [22,24,25,74].

Another factor that may contribute to these inconsistencies is the fact that corticosterone levels fluctuate over the estrus cycle with the highest levels seen during proestrus [2]. However, limited consideration has been given to fluctuations across the estrus cycle when looking at the effects of separation on the HPA axis and/or anxiety-related behavior in adult female rats [13,50,52,67,74].

As well as manipulation-specific effects, maternal separation may have effects that differ as a function of development. Male and female rats show an increase in gonadal hormones following puberty [7,10,54,72], with sex differences in open field activity appearing after puberty [36]. Responses to novelty also change with age [16,68,69]. Female rats, but not male rats, show an increase in basal corticosterone release and CRH mRNA levels with age [72] creating gender differences in HPA axis function [23,29,59,71,72]. Since maternal separation affects stress and the HPA axis changes with age, it is likely that the effects of separation will also change with age. This pattern was, in fact, found by Marin and Planeta [35] with separated male rats showing increased activity and lowered rise in corticosterone in response to novelty during the juvenile period. No separation effect was found in adulthood. Separation also affects juvenile female rats, but not juvenile male rats, by decreasing anxiety in the elevated plus maze in comparison to non-separated rats [39]. This suggests that there are sex-specific effects of maternal separation that may change over development, possibly implicating an influence of gonadal hormones.

With this background in mind, the purpose of the following experiments was to determine: (1) The effect of different early experience manipulations (being separated from both mother and littermates (early deprivation: ED, as in [50,52]); being separated from mother, but not littermates (maternal separation: MS, as in [74]); being animal-facility reared (AFR)) on open field activity and HPA axis response to novelty in the female rat. (2) The effect of maternal separation and early deprivation on behavior at different ages (juvenile (experiment one) versus adult (experiment two)) using the same measures. In terms of effects of early experience on corticosterone and ACTH levels after exposure to the open field, being deprived of both mother and litter (ED) was predicted to reduce the elevations in corticosterone levels after stress in adult female rats when compared to AFR (similar to what was found by Pryce et al. [52]). Predictions regarding effects in juvenile rats were less clear; however, it was expected that the effects of the different separation paradigms on the expression of the HPA axis would differ as a function of age due to basic developmental changes in HPA axis expression of female rats [72]. Based on previous research, it was also hypothesized that in female rats, deprivation/separation would affect open field behavior in juvenile but not in adult rats with a decrease in 'anxiety behavior' when compared to AFR rats expected (similar to what was found by 1 and 39). In order to account for the estrus cycle, adult female rats were tested and sampled during estrus.

Research using separation paradigms have utilized various methods of separating pups from their mothers. Two separation manipulations were used in the following experiments: being separated from mother and littermates (early deprivation: ED), similar to that used by Pryce et al. [50,52] and being separated from mother, but not littermates (maternal separation: MS), similar to paradigm used by Wigger and Neumann [74]. This allowed for a direct comparison of two established separation manipulations on behavior and the HPA axis response to exposure to a novel environment (e.g. open field arena) in juvenile and adult female rats.

As well, periods of separation have ranged from 15 min to 24 h. Separation of 1 h has been favored by several researchers [17,21,11,24,25,31,46] while others have used 4 to 6 h of deprivation [27,43,50–52,62,63]. For the present study, 5 h of separation was chosen because we have previously found that 5 h, but not 3 h, of repeated separation during the neonatal period leads to disruptions in adult postpartum maternal behavior [32,55] and we were interested in comparing the results of the present study to those on adult maternal behavior. As well, in the present experiment, male pups were left with in the mother rat while female pups were removed during the separation period. Most research removes all pups during the separation period, however, there are some examples of studies where only part of the litter is taken away during the separation period [32]. As well, if the mother rat is provided with a foster litter during the separation period, effects of separation on the stress response are partially reversed [21]. This suggests that the effects of separation on the pup's adult stress response are at least partially mediated by maternal care received upon reunion from separation (although see [33]). By leaving some pups with the mother rat during the separation period, the effects of separation per se were determined by presumably decreasing some of the influence of changes in maternal care received by separated pups.

2. General methods

2.1. Subjects and housing

All subjects were female pups that were tested as juvenile ($n = 140$) or adult rats ($n = 80$). Sprague–Dawley rats used in these experiments were born at University of Toronto at Mississauga, from a stock originally obtained from Charles River Farms in Quebec. The subjects were maintained on a 12:12-h light:dark cycle, with lights on at 08:00 h. 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.

2.2. Separation groups

There were three early separation groups:

1. Separated from both mother and peers for 5 h a day from postnatal day (PND) 2 to 14 (early deprivation: ED).
2. Separated from mother, but not peers for 5 h a day from PND 2 to 14 (maternal separation: MS).
3. Disturbed only by animal facility practices until weaning, from PND 2 to 21 (animal facility reared: AFR).

Only female pups were separated during the separation period; therefore, mother rats always had male pups in the home cage during the separation period. From PND 2 to 14, female pups in the ED group were removed from their mothers and peers daily and placed individually in a separation chamber with bedding. These chambers were kept warm at approximately 32 °C. After the separation period, separated pups were returned to their home cage. For the MS group, all female pups were removed from their mothers daily and placed together in a large separation chamber with bedding. These large chambers were also maintained at approximately 32 °C. For each of the separation manipulations, pups were removed at approximately 9:00 am and returned at approximately 2:00 pm for a 5 h separation period.

2.3. Open field activity testing

On PND 22, pups were weaned into same sex pairs until testing. Juvenile rats were tested on PND 30 (experiment one). Adult rats were also weaned into same sex pairs on PND 22 and kept paired until testing (experiment two) between PNDs 80 and 100. Both juvenile and adult rats were tested in the open field between 9:00 and 11:00 am. Using an estrous cycle monitor (Fine Science Tools Inc.), the afternoon of proestrus was determined and all adult female rats were tested the following morning (presumably during the estrous phase of the cycle). As well, blood samples were sampled for estrogen levels to account for any individual variations between cycling adult rats.

During a 5 min observation period, rats were exposed to an open field (150 cm × 150 cm × 49.5 cm) divided into a grid with 100 squares (each square: 15 cm × 15 cm). During the test, the path of the rat in the open field was traced on an observation sheet by an observer blinded to the experimental groups of the rats being tested (recorded in real time). Outer crossings (total crossings of all grid lines around the perimeter of the open field in the first two rows) and inner crossings (total crossings of all grid lines other than the first two rows around the perimeter) were recorded. Grid crossings are a general measure of activity levels, while inner grid crossing are used as a measure of anxiety-related behavior. The proportion of inner grid crossings over total grid crossings was computed (inner crossings divided by total crossings) to give a measure of anxiety relative to activity. Also, the number of boli, frequency of grooming, and frequency of rearing were recorded during the exposure to the open field. The number of boli and the frequency of grooming are also measures of anxiety-related behavior, while rearing is used to measure exploratory behavior. The open field was cleaned with ethyl alcohol between each test.

2.4. Administration of stressor, blood sampling, and radioimmunoassays

Exposure to the open field was used as the stressful stimulus in these experiments. Rats used to collect blood samples for baseline analysis were not exposed to the open field. Rats were separated into four groups: no stress, 5 min post-stress, 20 min post-stress, and 60 min post-stress. Rats in the stressed groups were isolated and exposed for 5 min to the open field 5, 20, and 60 min prior to sacrifice. Rats in the non-stressed group were isolated and immediately sacrificed to provide a basal sample.

Blood samples were taken, through decapitation, to determine the levels of corticosterone and ACTH, as well as levels of estrogen for a subset of rats. Decapitation was used as a method of blood sampling so that brains could also be taken for future analysis. Corticosterone was determined by solid phase (I-125) radioimmunoassay (Coat-a-Count, Diagnostic Products Corporation; inter-assay variability = 8.5%; intra-assay variability = 6.83%) and ACTH was determined by solid phase (I-125) radioimmunoassay (ICN Biomedicals Inc.; inter-assay variability = 7.4%; intra-assay variability = 5%). Estrogen levels were also measured in the adult female rats to determine if separation and stress had any effect on estrogen levels in the cycling virgin female rat and whether changes in estrogen were related to other measures (Ultra-Sensitive Estradiol RIA, Diagnostic Systems Laboratories, Inc.; inter-assay variability = 9.35%; intra-assay variability = 7.49%).

2.5. Statistical analysis

To analyze blood samples, a 3 (group: ED versus MS versus AFR) × 4 (time: 0 min post-stress (baseline) versus 5 min post-stress versus 20 min post-stress versus 60 min post-stress) two-way ANOVA was undertaken for each hormone (ACTH and corticosterone). A repeated measures ANOVA was not used as separate groups of rats were used for each time point. To analyze open field behavior, a one-way ANOVA was used with early separation as the between group factor, while total, outer, inner, and proportional (inner/total) crossings and frequencies of boli, grooming, and rearing were the dependent measures. Correlations between hormone levels at 5 min post-stress and open field measures were computed. Five minutes post-stress would represent the closest time to exposure to the open field and thus should best reflect the relation between hormones and behavior in these experiments. As well, ACTH and corticosterone levels were correlated when all time points were combined. All correlations were analyzed using a Pearson's coefficient.

2.6. Experiment one

Effects of early deprivation and maternal separation on levels of ACTH and corticosterone and behavior in an open field arena in the juvenile female rat.

2.6.1. Results

2.6.1.1. Levels of ACTH and corticosterone. There was a time by early separation interaction on corticosterone levels ($F(6,103) = 2.51$, $p < .026$), with ED rats having lower levels of corticosterone at 5 min post-stress than both MS and AFR rats but not at other time points (Tukey's, $p < .05$) (see Fig. 1b). There was no effect of separation on ACTH levels (see Fig. 1a). There was also a main effect of time on levels of ACTH ($F(3, 103) = 10.61$, $p < .000$) and corticosterone ($F(3, 103) = 65.07$, $p < .000$) (see Fig. 1a and b). Levels of both hormones rose to a peak at 20 min with a return to baseline by 60 min (see Fig. 1).

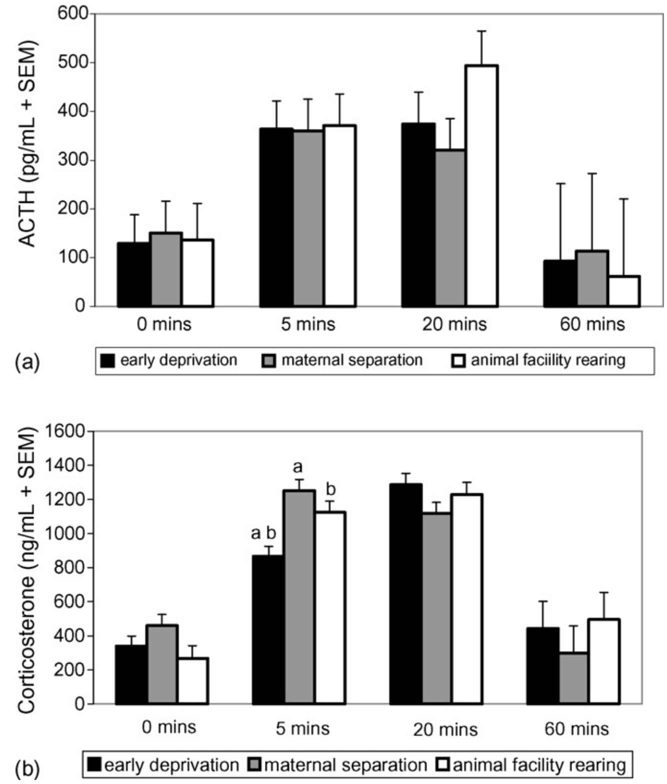

Fig. 1. (a) Effects of early deprivation and maternal separation on ACTH levels in juvenile female rats before and after exposure to novelty (mean + S.E.M.). (b) Effects of early deprivation and maternal separation on corticosterone levels in juvenile female rats before and after exposure to novelty (mean + S.E.M.; shared letters indicate significant differences, $p < .05$).

2.6.1.2. Open field behavior. For juvenile rats, there were no effects of early experience on measures taken in the open field (see Fig. 2).

2.6.1.3. Correlations between ACTH, corticosterone, and open field measures. There was a significant positive correlation between corticosterone at 5 min post-stress and defecation in the open field ($r = .406$, $p < .036$). There were no other significant correlations between hormone levels and open

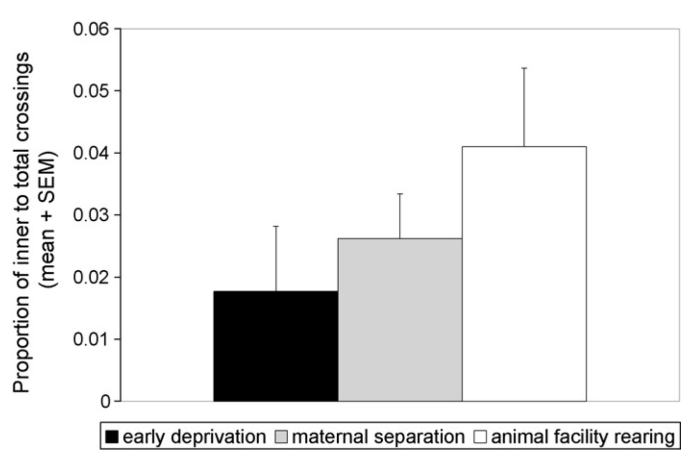

Fig. 2. Effects of early deprivation and maternal separation on the proportion of inner to total crossings in the open field in juvenile female rats (mean + S.E.M.).

field measures. Overall, there was a positive correlation between corticosterone and ACTH levels when all time points were combined ($r = .615$, $p < .000$).

2.7. Experiment two

Effects of early deprivation and maternal separation on levels of ACTH and corticosterone and behavior in an open field arena in the adult female rat.

2.7.1. Results

2.7.1.1. Levels of ACTH and corticosterone. There was a main effect of separation on ACTH levels ($F(2, 67) = 4.42$, $p < .016$) and on corticosterone levels ($F(2, 66) = 3.81$, $p < .027$). MS rats had higher ACTH levels than both ED and AFR rats and higher corticosterone levels than ED rats (Tukey's, $p < .05$) (see Fig. 3). Also, at baseline (0 min), ED rats had lower ACTH than MS rats and higher corticosterone levels than AFR rats (see Fig. 3).

There was also a time by early separation effect on corticosterone levels ($F(6, 66) = 2.46$, $p < .033$) with ED rats showing peak corticosterone levels at 5 min post-stress. MS and AFR rats showed peak corticosterone levels at 20 min post-stress (see Fig. 3a).

There were main effects of time for both ACTH ($F(3, 67) = 9.59$, $p < .000$) and corticosterone ($F(3, 66) = 32.89$, $p < .000$) with the lowest levels of both hormones seen at baseline (Tukey's, $p < .05$). Also, corticosterone levels significantly increased between 5 and 20 min post-stress, but then significantly declined by 60 min post-stress (Tukey's, $p < .05$). Corticosterone levels at 60 min post-stress, however, were still significantly higher than baseline levels (Tukey's, $p < .05$). ACTH levels did not differ among the 5, 20, and 60 min post-stress points (see Fig. 3b).

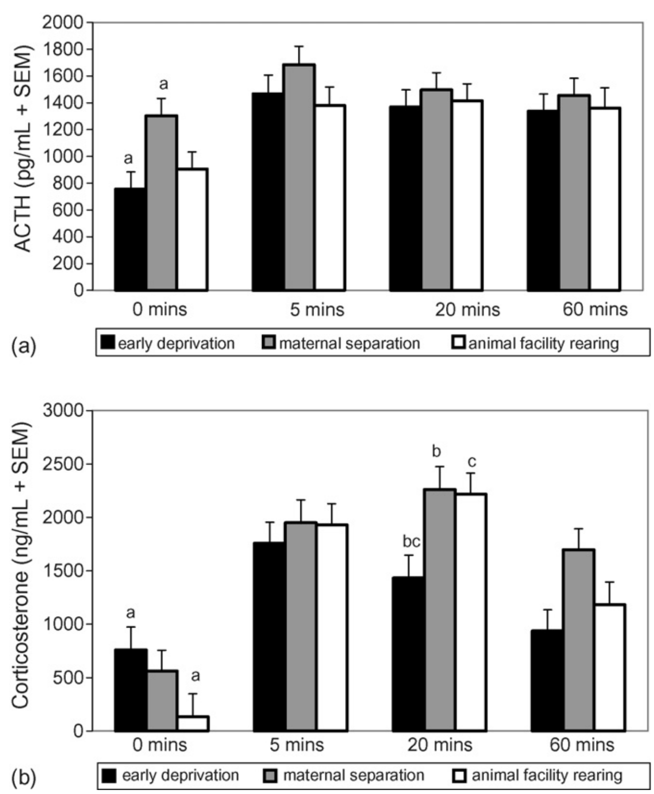

Fig. 3. (a) Effects of early deprivation and maternal separation on ACTH levels in adult female rats before and after exposure to novelty (mean + S.E.M.; shared letters indicate significant differences, $p < .05$). (b) Effects of early deprivation and maternal separation on corticosterone levels in adult female rats before and after exposure to novelty (mean + S.E.M.; shared letters indicate significant differences, $p < .05$).

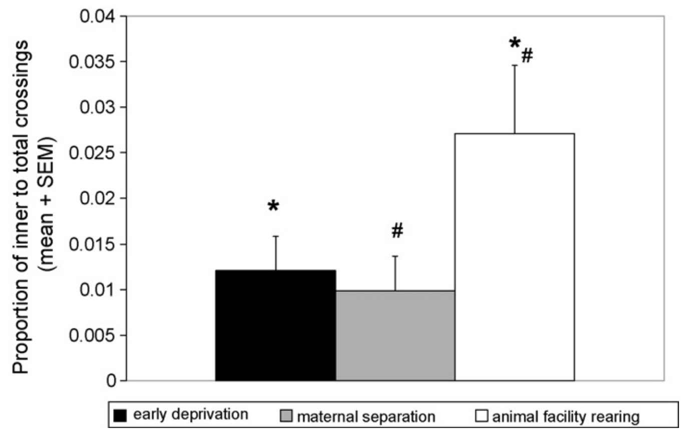

Fig. 4. Effects of early deprivation and maternal separation on the proportion of inner to total crossings in the open field in adult female rats (mean + S.E.M.; shared symbols indicate differences, $p < .100$).

2.7.1.2. Levels of estrogen. There were no effects of separation or stress on estrogen levels.

2.7.1.3. Open field behavior. There was little effect of separation on any open field measure except for a trend toward significance for the proportion of inner crossings to total crossings ($F(2, 60) = 3.09$, $p = .053$) (see Fig. 4). ED and MS rats tended to have a lower proportion of inner grid crossings to total crossing than AFR rats.

2.7.1.4. Correlations between ACTH, corticosterone, estrogen, and open field measures. There was a significant negative correlation between ACTH levels at 5 min post-stress and outer grid crossing ($r = -.472$, $p < .048$). Overall, there was a positive correlation between corticosterone and ACTH levels when all post-stress time points were combined ($r = .502$, $p < .000$). There were no significant correlations among any of the hormones or between each hormone and the open field measures.

3. Discussion

In summary, separation had effects on both HPA axis measures and open field behavior. However, these effects were age- and manipulation-specific. Specifically, in comparison to both AFR and MS rats, ED female rats had lower corticosterone levels both during the juvenile period at 5 min post-stress and in adulthood at 20 min post-stress. Also during adulthood, ED female rats had higher basal (no stress) corticosterone levels than did AFR female rats. There was little effect of separation on ACTH levels. In adulthood, ED female rats showed decreased basal levels of ACTH when compared to MS female rats. This may reflect the attenuated corticosterone response of ED adult female rats seen after stress. ACTH levels in adult female rats, regardless of group, did not return to baseline after stress despite the fact that corticosterone levels decreased compared to other post-stress points (although they were still higher than baseline levels of corticosterone). It remains unclear why ACTH levels did not reflect corticosterone levels in adult female rats, but it is probable that 60 min post-stress was not a sufficient amount of time for corticosterone levels to return to baseline.

During the juvenile period, deprivation/separation had no effect on ACTH levels despite deprivation attenuating corticosterone levels after stress. This may be due to a deprivationinduced effect on adrenal sensitivity during the juvenile period. Juvenile female rats show an extended corticosterone response to stress when compared to adult female rats, yet show no effect of stress on ACTH levels [60]. Romeo et al. [60] suggest that this may be due to a decrease in adrenal sensitivity to ACTH with age. In the present experiment, this suggests that ED may accelerate development as juvenile ED rats show no difference in ACTH levels at any point before or after stress despite an attenuated corticosterone response to stress. However, ED also attenuated corticosterone levels in adulthood suggesting that deprivation affects adrenal sensitivity regardless of age and development.

These effects of separation on the HPA axis response to stress are very similar to previous research on adult female rats (no effect of MS group: 68; ED attenuating corticosterone response: 52) despite differences among separation procedures. On the whole, for female rats being deprived (ED), there is an attenuation of corticosterone response after exposure regardless of age (although there were age-dependent patterns in corticosterone response to stress) and the presence of pups with mother during the separation period. However, general age differences may be more evident if adult female rats were sampled at a different period of the estrus cycle since corticosterone levels fluctuate over the estrus cycle in the female rat and only differ from male rats during proestrus [2].

Like the effects of separation on male rats, it is possible that the effects of separation on the HPA response to stress in female rats are due to changes in other aspects of the HPA axis such as CRH levels and mineralocorticoid/glucocorticoid receptors (MR/GR). In male rats, separation increased levels of CRH mRNA and content in the hypothalamus and increased CRH depletion after stress [47]. These effects may be due specifically to changes in both CRH-receptors and MR/GR receptors, suggesting a change in negative feedback mechanisms. For example, separation had no effect on corticosterone levels in CRH-R1 deficient male mice [64], suggesting that CRH-R1 are necessary for the effects of separation on corticosterone levels. As well, CRH-R1, but not CRH-R2, densities are changed by separation in an area-specific manner [25,48]. In terms of MR/GR, separation decreases the ratio of GR to MR in the hippocampus and GR levels in the prefrontal cortex of adult male rats in comparison to brief separation/handling or non-handling [41]. Separation used in the above cases was a form similar to the MS group in the present experiments, so it remains unclear what the effects of being deprived (ED) are on these mechanisms in either male or female rats.

Another possible mediating mechanism that may underlie manipulation-specific effects of separation on HPA responses to stress is maternal behavior received upon reunion and during non-separation periods. Meaney and colleagues (e.g. [5,6,16,40,42]) have shown that maternal behavior received during the neonatal period can have profound and long-lasting effects on the HPA axis response to stress. However, whether maternal behavior received is the mediating mechanism for the effects of separation remains unclear and controversial [33]. Upon reunion, some studies show that there is an increase in maternal behavior [33,34,51] and this increase may create or contribute to the effects of separation. Sex differences exist in the effects of separation [53] and in maternal behavior received [44]. While the effects of separation on this sex-bias in maternal behavior received have not been investigated, it has been found that prenatal stress decreases maternal behavior received during the postnatal period by decreasing maternal behavior toward male, but not female, pups [49]. As prenatal stress also has sex-specific effects [38], a similar effect may occur with separation and may be relevant to how female pups are treated upon reunion after separation. This may be especially interesting in relation to the present study as male pups were left with the mother rat while only the female rats were removed during the separation period.

Unlike the effects of separation on the HPA axis, deprivation and separation had little effect on open field measures, although being deprived or separated tended to decrease to proportion of inner to total grid crossings made in adult female rats. This suggests an increase in anxiety in deprived and separated rats and is in contrast to Eklund and Arborelius [13] who found that maternal separation (twice a day) decreased anxiety in female but not male rats in adulthood. Maternal separation increases activity and rearing in an open field test and anxiety in the elevated plus maze in juvenile male rats without changing these measures in adult male rats ([1,8,14,24,27,58,65,73–75], although see [20]). This suggests a gender difference in the effects of separation in pre-pubertal rats as female rats in the present experiment were unaffected by separation for open field measures. In experiment two, adult female rats were tested during estrus (high levels of estrogen) and tended to be affected by separation for open field measures, again suggesting a gender difference. Anxiety-related behavior in the open field does not change over the estrous cycle, despite changes in general activity [4,12,66], although behavior in other anxiety-related tasks does change over the estrous cycle [18].

In contrast to the present experiments, McIntosh et al. [39] found that deprivation (ED) decreased anxiety in female rats, but only during the juvenile period. Why this discrepancy in comparison to the present experiment occurred remains unclear. However, McIntosh et al. [39] used slightly older juvenile rats (PND [35–42]) than the present experiment (PND [30]) and also used the elevated plus maze to measure anxiety. Research also shows that early experience affects other behaviors in female rats, including maternal behavior [6,15,32,55]. Whether these effects are due to changes to the HPA axis remains unclear, however, the fact that corticosterone affects adult maternal behavior [19,56,57] and that separation affects the corticosterone response in female rats (present experiments) suggests that the HPA axis may be involved in the modification of maternal behavior by separation.

Although separation affected HPA measures, there was little effect of separation on open field measures. Deprivation and separation tended to increase anxiety in the open field in adult female rats while early deprivation lowered the corticosterone response to novelty, suggesting decreased anxiety. There was also no correlation between grid crossing and HPA measures, suggesting no relation between HPA measures and open field behavior. However, in male rats, when separation (similar to MS groups) affected both corticosterone levels and activity in response to novelty, results suggested a decrease in anxiety in both measures [35]. Wigger and Neumann [74] found that although maternal separation increased anxiety in both male and female adult rats in the elevated plus maze, only separated male rats showed increased HPA axis activation relative to controls after exposure to the plus maze. Perhaps this dissociation is sex-specific such that separation affects anxiety-related behavior and the HPA axis in a similar fashion in male rats, but in a divergent fashion in female rats. Alternatively, separation-induced changes in behavior of female rats may not be due to changes in the HPA axis.

In conclusion, being deprived (ED) decreases corticosterone release after exposure to a novel environment in both juvenile and adult female rats, similar to what has been found by Pryce et al. [47]. In contrast, however, both being deprived (ED) and being separated (MS) tended to increase anxiety (proportion of inner to total grid crossings) in the open field, but only in adulthood. These findings are very relevant to the study of the influence of early experience on the female-specific behaviors, such as maternal behavior. The present experiments are also a preliminary step in characterizing the effects of early maternal separation on the development of the physiology and behavior of the female rat.

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