Stress During Gestation Alters Postpartum Maternal Care and the Development of the Offspring in a Rodent Model

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Background: *Epidemiological studies suggest that environmental adversity can alter parental care and thus influence child development. We addressed the question of whether stressors can directly affect parental behavior using a rodent model of stable, individual differences in maternal behavior.*

Methods: *Lactating rat mothers were characterized as high or low in pup-directed licking/grooming (LG) behavior, rebred, and subjected to 7 days of intermittent stress or control conditions during gestation. Female rats were mated a third time without any subsequent intervention. Maternal behavior, oxytocin receptor (OTR) binding, and offspring behavior were examined.*

Results: *Stress reduced OTR levels and pup LG of high LG mothers to levels comparable with those of low LG mothers. The adult offspring of the gestational stress/high LG mothers resembled those of low LG mothers on behavioral measures of anxiety and maternal behavior, as well as OTR levels. The results of the third mating revealed an enduring effect of gestational stress on both mother and offspring maternal LG.*

Conclusions: *These findings suggest that stress can directly alter maternal care through the neuroendocrine systems that normally regulate this behavior. Thus, the effects of environmental adversity can be transmitted across generations through a nongenomic mechanism involving maternal care.*

Key Words: Maternal, medial preoptic area, gestational stress, oxytocin receptor

Environmental adversity, such as poverty, is associated with patterns of parental care that involve abuse, neglect, and harsh, inconsistent discipline (Conger et al 1994; McLoyd 1990, 1998). Such forms of parenting are, in turn, associated with impaired cognitive development, as well as an increased risk for mood disorders, substance abuse, and posttraumatic stress disorders in the offspring (Ammerman et al 1986; Brown and Anderson 1993; Feehan et al 1991; Holmes and Robins 1988; Trickett and McBride-Chang 1995). A number of authors have suggested that the stressors associated with poverty increase parental anxiety and irritability and thus directly influence parent-child interactions (McLoyd 1990; McLoyd and Wilson 1990). Hence, parental care is viewed as a mediator for the effects of environmental adversity on child development. In support of this idea are findings that indicate that the effects of poverty on cognitive development are eliminated if parental care factors are statistically factored out of the equation (Conger et al 1994; Eisenberg and Earls 1975; McLoyd 1998). In general, parental abuse and neglect are associated with increased anxiety and stress reactivity in the offspring (Heim et al 2000; De Bellis 2002), which, in turn, renders individuals at greater risk for a wide range of stress-related illnesses.

Parental care may not only mediate the effects of stressors on child development but may also serve as the basis for the intergenerational transmission of variations in parental behavior (Champagne and Meaney 2001; Meaney 2001). Evidence from both human and nonhuman primates suggest that individual differences in infant-directed behaviors are transmitted from mother to daughter (Berman 1990; Fairbanks 1989; Ijzendoorn 1991; Miller et al 1997). Certainly, this has been long suspected in the area of abuse and neglect (Bifulco et al 2002; Maxfield and Widom 1996); child abuse is more likely to occur among parents who were abused as children. These and other findings suggest that the mental health of the parent is associated with the quality of parent-child interactions, which then influences the emotional developmental and parental care of the offspring. Fleming and Corter (1988) found that more anxious mothers are generally less responsive to their infants, underscoring the relationship between anxiety and parental care. Thus, environmental adversity alters parental care, which, in turn, influences the mental health and parental care of the offspring, serving as a basis for the nongenomic, intergenerational transmission of vulnerability for disease.

While the results of existing studies are generally consistent with the proposed role for parental care, such findings in human populations are inevitably correlational and thus do not provide direct evidence for an effect of environmental adversity on parent-child interactions. Studies with nonhuman primates suggest that environmental adversity can directly influence the quality of mother-infant interactions (Lyons et al 1998; Rosenblum and Andrews 1994). In these studies, increased foraging demand (requiring greater time on the part of the mother in the search for food, with a less reliable success rate) increases mother-infant conflict and produces long-term changes in the activity of neural systems that underlie the expression of behavioral and emotional responses to stress (Coplan et al 1996, 1998). While such studies link environmental adversity to maternal behavior, the ensuing association with development in the offspring is only by implication. Moreover, we have little understanding of potential mechanisms for either the effects of environmental stressors on parental behavior or the intergenerational transmission of individual differences in parental care.

In the studies reported here, we have addressed these issues using a rodent model of naturally occurring variations in maternal behavior. The model is based on individual differences

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Received June 3, 2005; revised September 13, 2005; accepted October 6, 2005.

0006-3223/06/$32.00
doi:10.1016/j.biopsych.2005.10.016

among lactating rats in the frequency of maternal licking/grooming (LG) of pups. Nursing bouts form the nexus of mother-infant interactions in the rat and involve multiple periods of pup licking/grooming, particularly at the outset of the bout. Licking/grooming, in turn, forms the basis for tactile stimulation, which is critical for pup physiology and central nervous system (CNS) development (Kuhn and Schanberg 1998; Levine 2001). The adult offspring of mothers that naturally exhibit increased rates of pup licking/grooming show decreased behavioral and endocrine responses to stress by comparison with the offspring of low LG mothers (Caldji et al 1998; Liu et al 1997). Cross-fostering the biological offspring of high or low LG mothers to mothers of the opposite phenotype completely reverses the expected pattern of development (Francis et al 1999). These findings suggest a direct link between maternal behavior and the development of individual differences in stress reactivity. Interestingly, individual differences in maternal behavior in the rat are also transmitted from mother to female offspring, and the pattern of development is reversed with cross-fostering (Francis et al 1999): female offspring born to low LG mothers but reared by high LG dams are, as adults, high LG mothers (and vice versa).

Individual differences in maternal behavior in the rat are associated with oxytocin receptor (OTR) levels in brain regions that are known to mediate the expression of maternal care. Thus, high LG mothers exhibit increased oxytocin receptor levels in the medial preoptic area of the hypothalamus (MPOA) by comparison with low LG mothers. Central infusion of an oxytocin receptor antagonist significantly reduces pup licking/grooming in high LG mothers, thus completely eliminating the group differences in maternal behavior (Champagne et al 2001). Not surprisingly, the female offspring of high LG mothers show increased oxytocin receptor binding in the MPOA (Champagne et al 2001; Francis et al 2000). The virtue of rodent studies is the ability to better define casual relationships between environmental events and both maternal behavior and pup development and to identify underlying neurobiological mechanisms. In the current studies, we examined the effects of a chronic, intermittent stressor imposed during the last week of gestation on the maternal behavior and oxytocin receptor binding of high and low LG mothers. Adult female animals were bred and maternal behavior of lactating female animals was observed to identify high and low LG mothers. The same female animals were then bred for a second time (Figure 1). Importantly, individual differences in maternal behavior in the rat are very stable across multiple litters (Champagne et al 2003). The critical questions here were whether gestational stress would affect pup licking/grooming and whether such effects would involve changes in oxytocin receptor binding. The same cohort of mothers was then bred for a third time, with no further experimental manipulations. We then examined the same measures in the adult, female offspring. The results demonstrate that environmental stress decreases pup licking/grooming in high LG mothers, eliminating the group difference in maternal behavior in the second as well as the third litter. Oxytocin receptor binding in the MPOA revealed the same pattern of effects. The effects of gestational stress were also apparent in both the maternal behavior and oxytocin receptor binding in the offspring, including those derived from the third litter where no gestational (i.e., prenatal) stressor was imposed. The studies provide support for the idea that, at least in a rodent species, environmental stressors can directly alter maternal behavior and that these effects can be transmitted across generations.

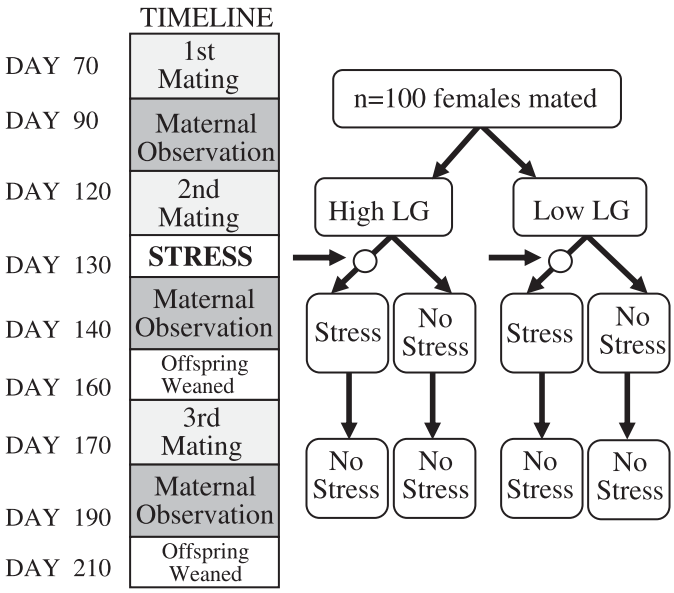

Figure 1. Outline of methodology used in this study. Note that female animals are mated three times but only exposed to stress during the second pregnancy. Offspring of the second and third matings are compared on measures of behavior and oxytocin receptor binding.

Methods and Materials

Subjects

The animals used were outbred Long-Evans, hooded rats born in our colony and housed in 46 cm × 18 cm × 30 cm Plexiglas cages that permitted a clear view of all activity within the cage. Food and water were provided ad libitum. The colony was maintained on a 12:12 light:dark schedule with lights on at 8:00 AM. The animals underwent routine cage maintenance beginning on day 8 of life but were otherwise unmanipulated. All procedures were performed according to guidelines developed by the Canadian Council on Animal Care and protocols approved by the McGill University Animal Care Committee. At the time of weaning on day 22 of life, the offspring were housed in same-sex, same-litter groups of two animals per cage. Following mating and throughout lactation, adult female animals were housed singly.

Maternal Behavior. Maternal behavior was observed for five, 75-minute observation periods daily for the first 6 days postpartum by individuals trained to a high level of interrater reliability (i.e., >.90) (Champagne et al 2003; Caldji et al 1998; Francis et al 1999; Liu et al 1997). Observations occurred at three periods during the light (9:00 AM, 1:00 PM, 5:00 PM) and two periods during the dark (6:00 AM and 9:00 PM) phases of the L:D cycle. Within each observation period, the behavior of each mother was scored every 3 minutes (25 observations/period × 5 periods per day = 125 observations/mother/day) for the following behaviors: mother off pups, mother carrying pup, mother licking and grooming any pup, mother nursing pups in either an arched-back posture, a "blanket" posture in which the mother lays over the pups, or a passive posture in which the mother is lying either on her back or side while the pups nurse. A detailed description of these behaviors is provided in Myers et al (1989) (also see Champagne et al 2003).

Open-Field Exploration. The open field serves as a novel environment, and decreased fear of this novelty is typically followed by exploratory behavior, operationally defined as in-

creased time spent in the inner area of the field (Belzung and Griebel 2001; Crawley 1985). On the day of testing, the rat was removed from its home cage and placed directly into one corner of the open field (a 120 cm × 120 cm enclosed area). The behavior of the animal was recorded for 10 minutes under standard lighting conditions (overhead fluorescent lights) with a video camera, and recordings were coded using a disk operating system (DOS)-based program. During analysis of the recordings, the field was divided into a grid of 8 × 8 squares. *Exploration* was defined as the time spent in the inner 6 × 6 squares, whereas *overall activity* was defined as the number of squares crossed during the 10-minute session.

Plasma Corticosterone Assay. Blood samples were placed in tubes containing ethylenediaminetetraacetic acid (EDTA) and aprotinin (Trasylol) and centrifuged at 3000 rpm for 10 minutes at 4°C. Plasma was collected and stored at −80°C. Plasma corticosterone was measured (Krey et al 1975) using a highly specific CORT antiserum (B3-163; Endocrine Sciences, Tarzana, California) with [^{3}H]corticosterone (32,782 Bq/mmol) (New England Nuclear, Boston, Massachusetts) as tracer.

Oxytocin Receptor Autoradiography. Slide-mounted coronal brain sections were processed for receptor autoradiography using ^{125}I-d(CH$_2$)$_5$[Tyr-Me)$_2$,Tyr-NH$_2^9$] ornithine vasotocin (^{125}I-OVT) (New England Nuclear), as previously described (Champagne et al 2001). After a prewash in Tris-HCl (pH 7.4; Sigma, Oakville, Ontario, Canada), slides were exposed to a 75-minute incubation (at room temperature) of 60 pM ^{125}I-OVT in Tris with magnesium chloride (MgCl) (10 mmol/L), bovine serum albumin (BSA; .1%), and bacitracin (.05%). Nonspecific binding was defined in adjacent sections by adding 50 nmol/L Thr^{4}Gly^7-oxytocin to the incubation buffer. The final 35-minute wash was performed at room temperature in 50 mmol/L Tris, 100 mmol/L MgCl (pH 7.4) to reduce background. After air drying, the slides were exposed to BioMax MR film (Kodak) for 48 hours. Iodine-125 autoradiographic standards (Amersham, Princeton, New Jersey) were included in the cassette for quantification. The autoradiograms were analyzed using an image-analysis system (MC1D-4, Imaging Research, St. Catherines, Ontario, Canada). Three sections were analyzed bilaterally at each level. For each rat, total and nonspecific binding were measured for each region and the difference taken to yield specific binding. Specific binding was greater than 90% of total binding. The statistical analysis was performed on the mean of these values for each animal by brain region according to the atlas of Swanson (1992). Comparison was performed by applying three-way analysis of variance (ANOVA) (maternal phenotype × stress × brain region).

Procedure

The frequency of maternal licking/grooming across a large number of mothers is normally distributed (Champagne et al 2003). Hence, high and low LG mothers represent two ends of a continuum, rather than distinct populations. High LG mothers were defined as female dams whose frequency scores for licking/grooming were greater than 1 SD above the cohort mean. Low licking/grooming mothers were defined as female dams whose frequency scores for licking/grooming were greater than 1 SD below the cohort mean.

High and low LG dams (n = 20 per group) were selected; bred for a second time, 10 days postweaning; and assigned to either the stress or control conditions (Figure 1). In the stress condition, pregnant female dams were removed from their home cage and placed in Plexiglas restrainers for three sessions per day (30 minutes each) during the last 7 days of pregnancy (days 15 to 21), a procedure previously shown to alter offspring phenotype in rodent studies of prenatal stress (Weinstock et al 1988). The timing of the sessions was distributed randomly throughout the 24-hour period to render the procedure less predictable (Fride and Weinstock 1984; Quirce et al 1981). Control female dams were left undisturbed. Following birth, female dams were observed for 6 consecutive days. Thus, the effects of gestational stress are examined in multiparous rather than primiparous dams. Offspring were weaned on postnatal day 22. One week postweaning, all dams were remated for a third time and observed with their pups on postnatal days 1 to 6 with weaning on postnatal day 22.

At 70 days of age, male offspring were assessed in the open-field exploration task. Female offspring were mated and observed with their pups to assess maternal behavior. Female offspring were sacrificed on postnatal day 6 and brains prepared for receptor autoradiography.

To evaluate the effect of the stress, an additional sample of 100 female animals were mated and characterized on measures of maternal behavior. From this sample, 20 high and 20 low LG female animals were selected and assigned, as in the first study, to either the stress or control conditions. However, these female animals were sacrificed on postnatal day 6. Prior to sacrifice, dams were removed from their litters in the morning and assessed in the open-field apparatus. Immediately following this assessment, brains were obtained and blood samples were taken for corticosterone assay.

Results

In the first sample of 20 high and 20 low LG dams, 12 from each group were randomly assigned to the stress condition and 8 to the control condition. There were no group differences in the litter size of animals that successfully gave birth. However, only 58% (7/12) of the female dams in the stress condition gave birth compared with the 88% (7/8) in the control condition. Though the exact cause (i.e., reabsorption, stillbirth) of the differential rates of parturition were not investigated, effects on parturition have previously been observed in animals exposed to stress (Chapillon et al 2002). The resulting sample size was seven dams per group. Analysis of the plasma corticosterone taken from the second sample of female dams indicated an effect of stress on corticosterone levels 1 week poststress [stress: mean = 16.8 ± .6 μg/dL, control: mean = 12.7 ± .7 μg/dL; $t(26) = 4.54$, $p < .001$]. These dams also showed behavioral indications of increased level of anxiety. Gestationally stressed dams exhibited increased overall activity in the open-field test [stress: mean = 426.7 ± 23.4 total squares crossed, control: mean = 335.9 ± 12.4 total squares crossed; $t(26) = 3.4$, $p < .01$] and decreased inner field exploration relative to no-stress dams [stress: mean = 37.3 ± 5.4 minutes, control: mean = 71.31 ± 1.3 minutes; $t(26) = 2.7$, $p < .01$].

Maternal Behavior

Across all litters, the frequency of pup LG was significantly elevated in control high compared with control low LG mothers, reflecting the stability of this maternal phenotype. The maternal behavior of these dams toward their second litter (Figure 2A) indicated a main effect of stress on maternal LG [$F(1,24) = 11.7$, $p < .01$], a main effect of maternal phenotype [high or low; $F(1,24) = 22.67$, $p < .001$], and a significant stress by maternal phenotype interaction [$F(1,24) = 5.83$, $p < .05$]. Post hoc (Tukey) analysis revealed that low/no-stress, low/stress, and high/stress

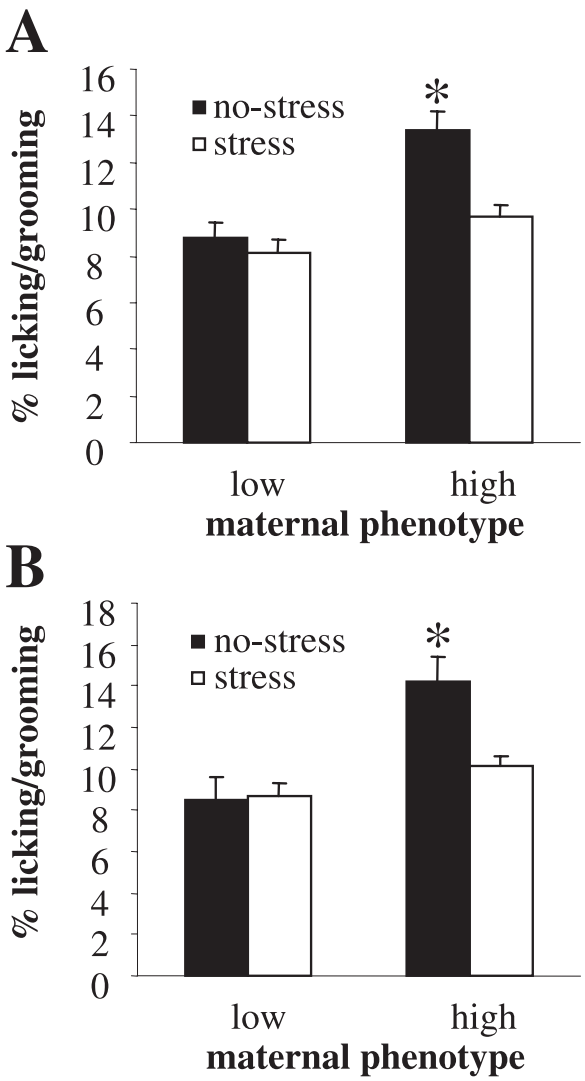

Figure 2. Mean ± SEM licking/grooming of female animals toward their **(A)** second litter and **(B)** third litter offspring. Gestational stress resulted in a significant decrease in licking/grooming exclusively in high LG mothers. Mothers that displayed decreased maternal care toward their second litter as a result of gestational stress continued to show decreased licking/grooming. *$p < .05$. LG, licking/grooming.

dams exhibited reduced levels of LG relative to the high/no-stress dams ($p < .01$, $p < .01$, $p < .05$, respectively). The effect of stress was not observed in the low LG dams. These effects were replicated in the second sample of female animals, with a main effect of stress [$F(1,24) = 11.33$, $p < .01$] and a main effect of maternal phenotype [$F(1,24) = 12.68$, $p < .01$]. The amount of time dams spent in contact with pups or the amount of time spent in arched-back, passive, or blanket nursing postures did not differ as a function of maternal phenotype or stress.

All female animals were remated and observed with their pups to determine the long-term effects of gestational stress (Figure 2B). It is important to note that gestational stress was not administered during the third pregnancy. As observed with the second litter, two-way ANOVA indicated a main effect stress on maternal LG [$F(1,24) = 5.02$, $p < .05$], a main effect of maternal phenotype [$F(1,24) = 17.48$, $p < .001$], and a significant stress by maternal phenotype interaction [$F(1,24) = 6.14$, $p < .05$]. Post hoc analysis revealed that control low/no-stress, low/stress, and high/stress dams exhibited reduced levels of LG relative to the high/no-stress dams ($p < .05$).

Oxytocin Receptor Binding

Oxytocin receptor binding was analyzed in the bed nucleus of the stria terminalis (BNST), medial preoptic area, lateral septum (LS), central nucleus of the amygdala (cAMYG), and ventral medial hypothalamus (VMH) of postpartum day 6 lactating dams. Three-way ANOVA (stress × maternal phenotype × region) indicated a main effect of stress [$F(1,75) = 21.58$, $p < .001$], a main effect of maternal phenotype [$F(1,75) = 18.58$, $p < .001$], a main effect of region [$F(4,75) = 17.53$, $p < .001$], a maternal phenotype by stress interaction [$F(1,75) = 7.58$, $p < .01$], and a three-way stress by maternal phenotype by region interaction [$F(4,75) = 3.96$, $p < .01$]. Oxytocin receptor binding (Figure 3A and 3B) was elevated in the high/no-stress dams relative to low/no-stress, low/stress, and high/stress female dams in the BNST, MPOA, and cAMYG. No group differences were observed in the VMH.

Behavioral Assessment of Offspring

Adult male offspring from the second and third litters of the dams were tested in the open-field task. Though no differences were observed in overall activity (Figure 4B), group differences were observed in inner-field exploration (Figure 4A). For offspring of the second litter, ANOVA results indicated a main effect of stress [$F(1,47) = 10.44$, $p < .01$], a main effect of maternal phenotype [$F(1,47) = 13.86$, $p < .001$], and a stress by maternal phenotype interaction effect [$F(1,47) = 12.96$, $df(1,47)$, $p < .001$]. The male offspring of gestationally stressed high LG mothers showed significantly less inner-field exploration than the offspring of nonstressed high LG dams ($p < .001$). Inner-field exploration did not differ between the offspring of low/no-stress, low/stress, and high/stress dams. For offspring of the third litter, ANOVA results indicated a main effect of stress [$F(1,47) = 11.6$, $p < .001$], a main effect of maternal phenotype [$F(1,47) = 31.18$, $p < .001$], and a stress by maternal phenotype interaction effect [$F(1,47) = 17.7$, $p < .001$]. As was the case with the offspring of the second litter, the male offspring of gestationally stressed high LG mothers showed significantly less inner-field exploration than the offspring of nonstressed high LG dams ($p < .001$).

Maternal Behavior in Female Offspring

Adult female offspring from the second and third litters of the dams were mated and observed during the first week postpartum to assess maternal behavior (Figure 5). For the offspring of the second litter, two-way ANOVA (stress × maternal phenotype) of maternal LG indicated a main effect of stress [$F(1,26) = 7.2$, $p < .05$] and a main effect of maternal phenotype [$F(1,26) = 7.2$, $p < .05$]. For the offspring of the third litter, ANOVA of maternal LG indicated a main effect of stress [$F(1,26) = 6.8$, $p < .05$], a main effect of maternal phenotype [$F(1,26) = 5.6$, $p < .05$], and a stress by maternal phenotype interaction [$F(1,26) = 10.5$, $p < .01$]. The offspring of high/stress dams from both cohorts exhibited reduced levels of LG toward their own offspring relative to the offspring of high/no-stress dams ($p < .05$). No differences in LG were observed between the low/no-stress, low/stress, and high/stress dams.

Oxytocin Receptor Binding in Offspring

On day 6 postpartum, lactating female offspring were sacrificed and oxytocin receptor binding was analyzed in the BNST, MPOA, LS, cAMYG, and VMH. Significantly reduced levels of oxytocin receptor binding were observed in the BNST ($p <$

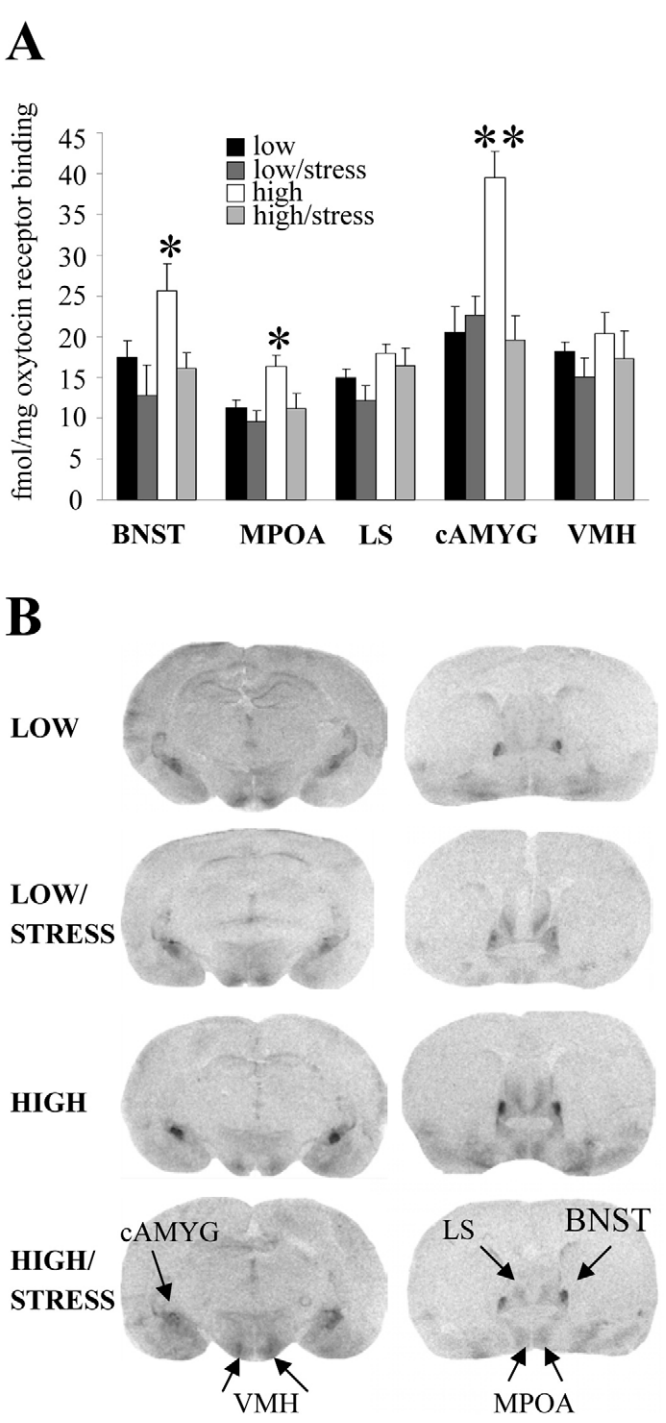

Figure 3. (A) Mean ± SEM OTR binding in the BNST, MPOA, LS, cAMYG, and VMH of lactating (day 6 postpartum) gestationally stressed/nonstressed high and low LG female animals. Stress resulted in significantly lower levels of OTR binding in the BNST, MPOA, and cAMYG of high LG dams. *$p < .05$; **$p < .01$. **(B)** Representative photomicrographs of OTR binding in day 6 postpartum lactating high/no-stress, high/stress, low/no-stress, and low/stress dams. OTR, oxytocin receptor; BNST, bed nucleus of the stria terminalis; MPOA, medial preoptic area; LS, lateral septum; cAMYG, central nucleus of the amygdala; VMH, ventral medial hypothalamus; LG, licking/grooming.

.05), MPOA ($p < .05$), and cAMYG ($p < .01$) of the offspring of gestationally stressed, high LG mothers relative to the lactating offspring of high/no-stress dams (Figure 6A). This pattern of oxytocin receptor levels was observed in both the offspring of the second and third litters (Figure 6B). Analysis of variance of oxytocin receptor binding indicated a main effect of stress [$F(1,80) = 13.63$, $p < .001$], a main effect of maternal phenotype [$F(1,80) = 19.26$, $p < .001$], a main effect of region [$F(4,80) = 49.45$, $p < .001$], a significant stress by maternal phenotype interaction [$F(1,80) = 48.39$, $p < .001$], a maternal phenotype by region interaction [$F(4,80) = 3.99$, $p < .01$], and a three-way stress by maternal phenotype by region interaction [$F(4,80) = 15.01$, $p < .001$] for the offspring of the second litter and a main effect of stress [$F(1,80) = 5.78$, $p < .05$], a main effect of maternal phenotype [$F(1,80) = 31.55$, $p < .001$], a main effect of region [$F(4,80) = 24.69$, $p < .001$], a stress by maternal phenotype

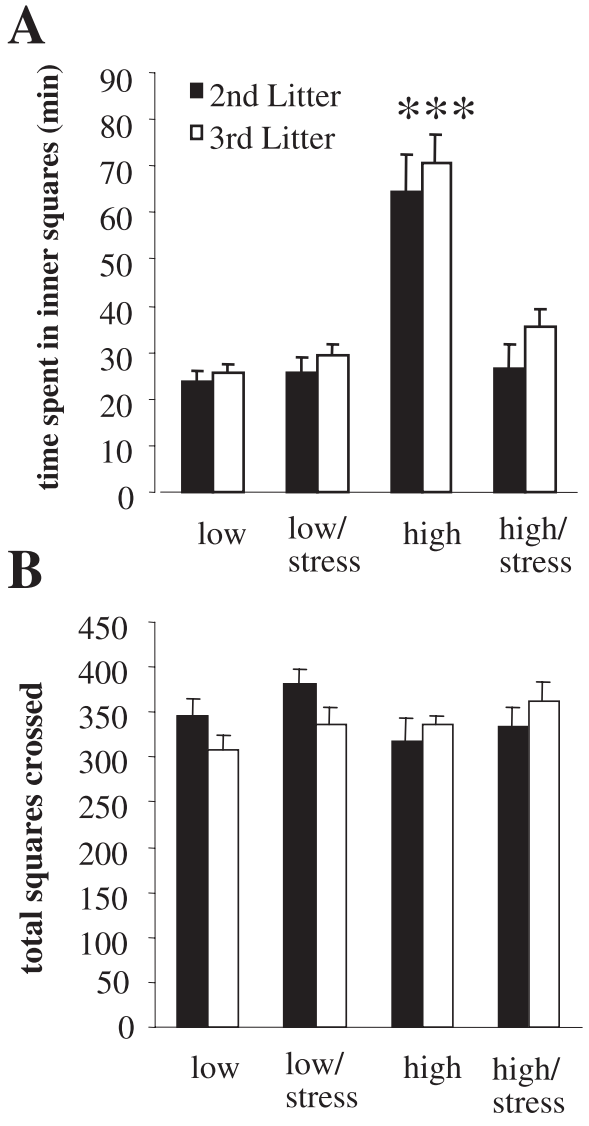

Figure 4. (A) Mean ± SEM open-field exploration (seconds) of male offspring from the second and third matings of the gestationally stressed/nonstressed high and low LG female animals. Male offspring of gestationally stressed/high LG female animals were significantly less exploratory than offspring of nonstressed/high LG female animals. $p < .001$. The offspring of the second and third matings of these female animals displayed the same pattern of behavior. ***$p < .001$. **(B)** Mean ± SEM open-field activity of male offspring from the second and third matings of the gestationally stressed/nonstressed high and low LG female animals. No differences in total activity were observed as a function of group. LG, licking/grooming.

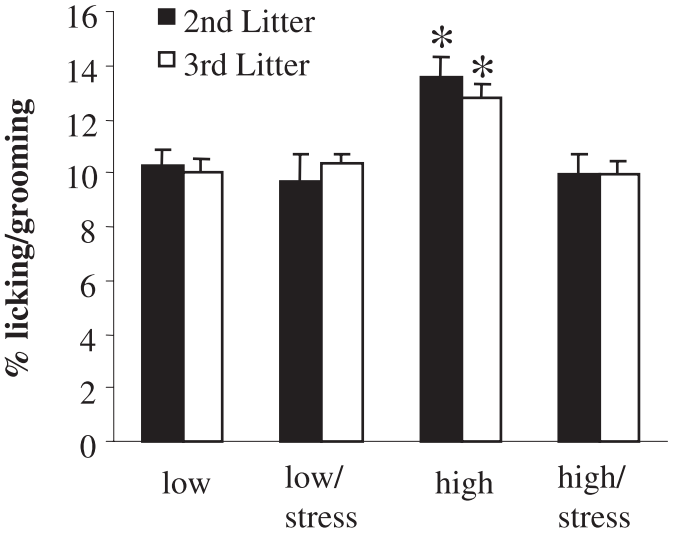

Figure 5. Mean ± SEM licking/grooming of the female offspring of gestationally stressed/nonstressed high and low LG mothers. Offspring of gestationally stressed high LG mothers displayed lower levels of maternal LG than did offspring of nonstressed high LG mothers. $p < .05$. This pattern of maternal behavior was observed in the offspring of both the second and third matings of these mothers. *$p < .05$. LG, licking/grooming.

interaction [$F(1,80) = 17.94$, $p < .001$], a maternal phenotype by region interaction [$F(4,80) = 6.14$, $p < .001$], and a three-way stress by maternal phenotype by region interaction [$F(4,80) = 3.96$, $p < .01$] for the offspring of the third litter.

Discussion

The major findings presented here are that 1) gestational stress can directly alter maternal behavior in the rat, 2) these effects involve neural substrates that are normally associated with the expression of maternal behavior (i.e., the oxytocin receptor), and 3) the stress-induced alterations in maternal behavior observed in gestationally stressed mothers can be transmitted across generations and this includes effects on the neural substrates of maternal behavior in female progeny. Taken together, these findings provide support for the idea that parental care can mediate the effects of environmental adversity on child development.

Effect of Gestational Stress on Oxytocin Receptor Binding and Maternal Behavior

Gestational stress resulted in a decreased frequency of pup licking/grooming in mothers that were previously characterized as high LG mothers. Note that previous studies have found that individual differences in maternal behavior are stable across multiple litters, and in the current study, we indeed found that among control groups, the frequency of pup licking/grooming in high LG mothers was significantly greater than that of low LG dams for both the second and third litters (Figure 2A and 2B). There was no effect of gestational stress on the pup licking/grooming of the low LG mothers. Moreover, the effects of gestational stress were selective: stress had no effect on total time spent with pups for either high or low LG mothers. It is interesting to note that these long-term effects are observed despite the administration of stress during a stress hyporesponsive period (Neumann 2001), and the importance of the timing of the stress, either prior to or during gestation, may be particularly relevant to changes in anxiety phenotype of the dam (Darnaudery et al 2004).

High and low LG mothers differ in oxytocin receptor levels in the MPOA (Champagne et al 2001; Francis et al 2000), a region critical to the expression of maternal care in rodents (Numan and Callahan 1980). The importance of oxytocin receptors in expression in parental behavior has been characterized in a number of species (Insel and Shapiro 1992; Perdersen and Boccia 2002; Rosenblatt 1994; Unvas-Moberg et al 2001), and the differences in receptor binding appear to serve as a mechanism for the differences in pup licking/grooming between high and low LG mothers. Thus, an infusion of an oxytocin receptor antagonist directly into the brain on day 3 postpartum reduces levels of maternal licking/grooming to those that are comparable with low LG mothers, thus eliminating the group differences in maternal behavior (Champagne et al 2001). Interestingly, as with the effect of gestational stress, there is no effect of the oxytocin receptor antagonist on time spent with pups (Champagne et al 2001; Fahrbach et al 1985). In the current studies, the effects of gestational stress on oxytocin receptor binding in the MPOA

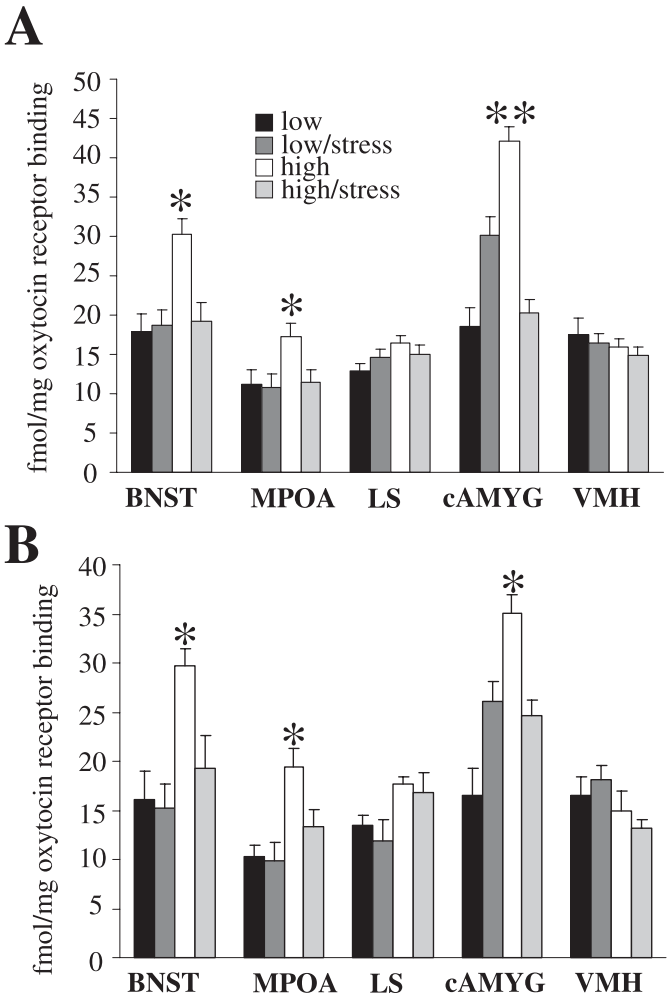

Figure 6. (A) Mean ± SEM OTR binding in the BNST, MPOA, LS, cAMYG, and VMH of lactating (day 6 postpartum) offspring of the second mating and **(B)** third mating of gestationally stressed/nonstressed high and low LG female animals. Levels of OTR binding were significantly decreased in the BNST, MPOA, and cAMYG of the offspring of gestationally stressed high LG mothers (from both the second and third matings). *$p < .05$; **$p < .01$. OTR, oxytocin receptor; BNST, bed nucleus of the stria terminalis; MPOA, medial preoptic area; LS, lateral septum; cAMYG, central nucleus of the amygdala; VHM, ventral medial hypothalamus; LG, licking/grooming.

parallel those on maternal behavior, suggesting that the effect of stress on the maternal behavior of high LG mothers is mediated by changes in oxytocin receptor levels. The mechanism mediating these decreases in oxytocin receptors is not clear; however, there is evidence to suggest that glucocorticoids exert regulatory effects on central oxytocin receptor expression (Liberzon and Young 1997; Patchev et al 1993).

Transmission of the Effects of Gestational Stress to the Offspring

Gestational stress reduced maternal licking/grooming in high LG mothers not only toward the second litter, which was associated with the imposition of the stressor during gestation, but also for the third litter, for which the gestational status was unperturbed. This effect allowed us to examine the effects of stress-induced alterations in maternal behavior independent of any direct application of stress during pregnancy (i.e., in the absence of prenatal stress of the offspring). The results reveal evidence for the transmission of the stress effect to the offspring both in terms of maternal behavior and neural oxytocin receptors. Thus, the offspring of stressed, high LG mothers from the third litter exhibited patterns of maternal behavior and oxytocin receptor binding that were indistinguishable from those of the offspring of either control or stressed, low LG mothers. These findings are consistent with the results of cross-fostering studies which show that, as adults, the biological offspring of high LG mothers reared by low LG dams are indistinguishable from the normal offspring of low mothers on measures of stress reactivity and maternal behavior (Francis et al 1999; Liu et al 1997).

Low LG mothers are behaviorally more fearful in response to threat than are high LG dams (Francis et al 2000). Likewise, the offspring of low LG dams are more fearful than those of high LG mothers (Caldji et al 1998; Liu et al 1997). The results of cross-fostering studies suggest that such individual differences in fearfulness can be transmitted from mother to offspring through a behavioral mode of transmission (Francis et al 1999). The behavioral transmission hypothesis suggests that the offspring of stressed, high LG dams would resemble those of low LG mothers on measures of fear and this was precisely what we observed. The offspring of gestationally stressed, high LG mothers showed increased levels of behavioral anxiety by comparison with the offspring of control high LG dams and did not differ from the offspring of low LG mothers. This same finding was apparent in the offspring of both the second and third litters, suggesting that in these studies, the effects were mediated by maternal behavior and not simply to prenatal stress on the fetus. To further disentangle the effects of prenatal and postnatal determinants of offspring phenotype would require the use of cross-fostering, as demonstrated by Moore and Power (1986); however, on the measures examined in the current study, offspring exposed in utero to stress were not significantly different from the subsequent nonstressed offspring of previously stressed dams. Taken together, these findings suggest that environmental stress alters the maternal behavior of the normally less fearful high LG mothers, resulting in decreased pup licking/grooming. The decreased frequency of pup licking/grooming then results in offspring that, as adults, are low LG mothers with increased levels of fearfulness. Hence, the effects of gestational stress are transmitted to the offspring through stress-induced alterations in maternal behavior.

Conclusions

Taken together, these findings suggest that environmental adversity can alter the emotional well-being of some mothers, decreasing maternal responsivity. In humans, Fleming and Corter (1988) reported that while many factors contribute to the quality of the mother's attitude toward her newborn, none were correlated more highly than the woman's level of anxiety. Mothers who felt depressed and anxious were, not surprisingly, less positive toward their baby (Field 1998; Fleming and Corter 1988). Moreover, there is evidence for the behavioral transmission of anxiety. Highly anxious mothers are more likely to have children who are shy and timid, and the behavior of the mother predicts the level of such behavioral inhibition in the child (Hirshfeld et al 1997a, 1997b). More extreme variations in parental care have predictable results. Studies of human mothers indicate that the experience of extreme levels of stress are associated with abuse and neglect of infants (Trickett and McBride-Chang 1995; Whipple and Webster-Stratton 1991). Heim et al (2000) recently reported that women with a history of abuse showed increased endocrine and autonomic responses to stress, and this finding is consistent with the results of the current work suggesting a maternally mediated transmission of fearfulness to offspring.

We do not suggest that the transmission of individual differences in fearfulness could not also be transmitted through a genetic form of inheritance and under normal conditions both genetic and behavioral influences would converge in determining the level of fearfulness in the offspring. However, we believe that intergenerational transmission via maternal behavior represents an adaptive approach to development. Children inherit not only genes from their parents but also an environment (West and King 1987). Under conditions of increased environmental demand, it is commonly in the animal's interest to enhance its behavioral (e.g., vigilance, fearfulness) and endocrine (hypothalamic-pituitary-adrenal [HPA] and metabolic/cardiovascular) responses to stress. These responses promote detection of potential threat, avoidance learning, and metabolic/cardiovascular responses that are essential under the increased demands of the stressor. Since the offspring usually inhabit a niche that is similar to their parents, the transmission of these traits from parent to offspring could serve to be adaptive. A metaphor for this argument exists in the physiology of the thrifty phenotype in rodents (Hales and Barker 1992; Neel 1999). In response to the deprivation of energy substrates in fetal life, rodents show a pattern of development that favors energy conservation and an increased capacity for both gluconeogenesis and lipolysis in adulthood. Both effects appear to reflect "anticipatory" patterns of development that would be adaptive under repeated periods of food shortages. Interestingly, these effects are sustained by changes in the expression of genes in hepatic tissues that mediate glucose and fat metabolism (Bauer et al 1998; Seckl et al 1999). We believe that the effects of maternal care of the expression of genes involved in the regulation of behavioral and endocrine responses to stress reflect a comparable anticipatory developmental strategy.

The key issue here is that of the potential adaptive advantage of the increased level of stress reactivity apparent in the offspring of low LG mothers. In the present context, the research of Farrington et al (1988) and Haapasalo and Tremblay (1994) on young males growing up in impoverished, high-crime, urban environments provides an excellent illustration of the potential advantages of increased stress reactivity. In this environment, the males that were most successful in avoiding the pitfalls associ-

ated with such a "criminogenic" environment were those that were shy and somewhat timid. Under such conditions, a parental rearing style that favored the development of a greater level of stress reactivity to threat would be adaptive. It is thus perhaps understandable that parents occupying a highly demanding environment would transmit to their young an enhanced level of stress reactivity in anticipation of a high level of environmental adversity. The quality of the environment influences the behavior of the parent that, in turn, is the critical factor in determining the pattern of development. The obvious conclusion is that there is no single ideal form of parenting: various levels of environmental demand require different traits in the offspring. Nature would seem to favor such plasticity in phenotypic development, allowing the young to anticipate a certain level of demand. The degree to which such phenotypes are or are not adaptive depends on the nature of the environment of the offspring. Through most of the natural history of humans and that of other species, the environmental conditions of the childhood are reasonable predictors of those prevailing in adulthood.

Finally, for such phenotpyic plasticity to be truly adaptive, it must operate in both directions. If adversity promotes reduced behavioral investment in the offspring, then more favorable environments should have the opposite effect. Interestingly, we have recently found that the maternal behavior and MPOA oxytocin receptor binding of low LG mothers is shifted in the direction of high LG females with an extensive period of peripubertal environmental enrichment. While these data emerge from rodent studies, the social implications would seem to be of obvious importance for human intervention programs.

FAC and MJM were supported by grants from the Canadian Institutes of Health Research (CIHR).

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