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The Effects of Early Isolation on Sexual Behavior and c-fos Expression in Naïve Male Long-Evans Rats

ABSTRACT: *Previous findings have demonstrated that the maternal environment is important for the development of male sexual behavior. The present study examined the effects of complete early life isolation and replacement 'stroking' stimulation on male sexual behavior and neural activation as seen by Fos immunoreactivity (Fos-IR). Animals were either artificially reared (AR) with minimal (AR-MIN) or maximal (AR-MAX) body simulation, or maternally reared (MR). In adulthood, animals were either given an exposure to an estrous female (EXP) or left undisturbed (NoEXP). No significant effects of early development were found in sexual behavior; however differences in activation in response to this exposure were observed. AR-MIN animals showed lower Fos-IR in the medial preoptic area and the ventromedial hypothalamus compared to MR animals. AR-MAX animals were not significantly different from either condition. These findings demonstrate that although there are no differences in the quality of the first copulatory exposure between AR and MR animals, the brain's response to this exposure differs in sites within the brain that subserve sexual behavior.*
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Keywords: *early experience; artificial rearing; pup licking; reproductive behavior; Long-Evans*

INTRODUCTION

Early environmental stimuli, especially those provided by the mother, play a crucial role in the normal emotional (Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001), behavioral (Moore, 1984) and endocrine (Moore, 1982) development of the offspring. In the rat, the neonate depends on its mother for all aspects of survival and for physiological homeostasis. The mother provides nutrition and warmth when she nurses her young, retrieves them when they wander from the nest, and licks her pups especially in the anogenital region in order to stimulate both urination and defecation (Rosenblatt & Lehrman, 1963) and to restore salt and water balance in the dam (Friedman, Bruno, & Alberts, 1981). However, maternal anogenital licking is not equally distributed to females and males within a litter (Moore & Morelli, 1979). Male pups in a litter are licked and handled more frequently by the dam and for a longer duration. This increased licking has been attributed to the differential hormonal state of the pups (Moore, 1982). Injection of testosterone into female pups on the day of birth results in an equivalent amount of anogenital licking as male pups (Moore, 1982), suggesting a role for androgens in this behavior.

This variation in maternal stimulation between males and females led to the theory that it may be important in the development of sexually differentiated behaviors. Anogenital stimulation has been shown to contribute to the development of a number of sexually dimorphic behaviors such as play behavior during the juvenile period (Birke & Sadler, 1987) and sexual behavior in adulthood (Moore, 1984). Male pups that received less maternal anogenital licking; by way of olfactory disruption in the dam; demonstrated a change in the temporal pattern of sexual behavior in adulthood, evidenced by increases

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in inter-mount interval and increased postejaculatory intervals (PEIs). This manipulation did not disrupt other elements of sexual behavior or sexual interest in the female (Moore, 1984). Furthermore, male offspring of strains in which the dam spends more time anogenital licking her pups, show a more robust pattern of sexual behavior (Moore, Wong, Daum, & Leclair, 1997) as well as better sperm competition (Dewsbury, 1984).

Social stimulation including that provided by the mother has been shown to be important in masculine copulatory behavior (Gerall, Ward, & Gerall, 1967; Moore, 1992). Removing this source of stimulation by separation of rat pups from their mother results in a multitude of physiological (Plotsky & Meaney, 1993), behavioral (Mathews, Wilkinson, & Robbins, 1996) and neurological (Colorado, Shumake, Conejo, Gonzalez-Pardo, Gonzalez-Lima, 2006) changes in adulthood that are dependent on both the duration of the separation (Plotsky & Meaney, 1993) and the environmental conditions that surround it (Rees, Steiner, & Fleming, 2006). Maternal deprivation through repeated separations results in changes in emotional behavior (Gonzalez et al., 2001), as evidenced by increases in novelty induced locomotion, the hypothalamic–pituitary–adrenal (HPA) system, and deficits in both maternal care in females (Lovic, Gonzalez, & Fleming, 2001) and copulatory rate in males (Rhees, Lephart, & Eliason, 2001). Complete deprivation by way of artificial rearing (AR) also produces deficits in maternal behavior (Gonzalez et al., 2001) and in the ability to acquire a maternal memory through interaction with pups (Fleming et al., 2002), with these deficits emerging around the third postpartum day (PPD; Gonzalez et al., 2001). Furthermore, AR results in a reduction in attention and increases in activity in adulthood (Lovic & Fleming, 2004). Many of these deficits produced by AR may be reversed by the addition of licking-like stimulation during the early developmental period (Gonzalez et al., 2001). This important somatosensory experience has been shown to be crucial in both brain and behavior development during this critical period (Fleming et al., 2002).

In light of these findings, the present study aimed to investigate the effects of complete early isolation and replacement stimulation on male sexual behavior and the neuronal activation associated with it. When exposed to sexually receptive females, male rats typically display high levels of sexual activity that is accompanied by the expression of protein products of immediate early genes such as *fos* (Pfaus & Heeb, 1997) in a number of brain regions implicated in male sexual behavior such as the medial preoptic area (mPOA), the medial nucleus of the amygdala (MeA), the bed nucleus of the stria terminalis (BNST) and the nucleus accumbens (NAC; for review see Pfaus and Heeb, 1997). Because early maternal deprivation has been shown to result in alterations in male sexual behavior, the present study explored whether such effects may be accompanied by a decrease in neuronal activation in areas that subserve sexual behavior in the male. Furthermore, although the importance of anogenital stimulation during the postnatal period in adult male copulatory behavior has been well documented (Moore, 1984), the role of other aspects of the maternal environment, such as body licking, on male sexual behavior development has not yet been established.

METHODS

Subjects

The experimental animals were the offspring of primiparous 60–90 day old Long-Evans female rats. Forty-Eight Long-Evans male rats derived from a stock originally obtained from Charles River Farms (St. Constant, Quebec, Canada) were utilized for the present study. Animals were bred and raised at Erindale Vivarium at the University of Toronto at Mississauga. Animals were maintained on a 12 hr light/dark cycle with lights on at 8 am to 8 pm until PND 21. Food and water were made available ad lib.

Groups and Design

The three early rearing conditions consisted of two groups of artificially reared (AR) animals (AR-MIN and AR-MAX) and a mother-reared control sham operated group (MR-CONTRL). Within each rearing condition, animals were randomly assigned to two sexual exposure conditions: a sexual exposure (Exp) group or a control group which received no sexual exposure (NoExp). Hence, six groups of animals were assessed.

Procedure

Artificial Rearing. Female virgin rats were mated by being placed with a proven stud male for a period of 7 days and left alone until parturition. On PND 1, the day after birth, litters were culled to four females and six males. On PND 4, three male pups from each litter were randomly assigned to one of three experimental conditions; an artificially reared minimum stimulation group (AR-MIN), artificially reared maximum stimulation group (AR-MAX), and a mother-reared surgical control group (CONTRL). All animals were weighed on PND 4 and the AR-MIN and AR-MAX groups received a cheek cannulation. Lidocaine (EMLA), a topical anesthetic, was applied on the right cheek of the pup for 5 min. A leader wire (stainless steal, 0.25 mm in diameter, VWR) sheathed with the cannula (polyethylene tubing, PE 10; flared at one end with a flat washer to hold the tubing in place; VWR) was then dipped into reagent grade mineral oil (Sigma, Oakville, Canada), to lubricate the tubing. The leader was then led over the tongue and penetrated through the translucent cheek muscle and skin and gently pulled until the flared end contacted the inside wall of the cheek. The leader wire was then removed. Polysporin antibacterial cream was applied topically at the site of penetration. A flat washer, followed by a T-washer (flared PE 50 tubing), was slid on the PE

10 tubing and placed firmly against the outside wall of the pups' cheek. The washers were held in place with Superglue (Gonzalez et al., 2001). The PE 10 tubing was flushed with sterile water to prevent blocking. As a surgical control, a similar surgery was performed on the CONTRL group, however; the tubing was removed, polysporin was applied to the surgical site, and the animal was tail marked and returned to the mother until the time of weaning. From PND 4 to PND 21, the tails of the rats in the CONTRL group were remarked every 2 days.

Rearing and Weaning. AR pups (AR-MIN and AR-MAX) were individually placed in plastic cups (11 cm diameter × 20 cm deep) with approximately 1 cm of corncob bedding (Renseed) covering the bottom. The cups were then placed in a second weighted cup, which were submerged one inch into a heated, temperature controlled aquarium tank in a room maintained at 25°C and a humidity level of 48%. Cup temperatures were taken five times daily to ensure proper temperature. Cup temperatures were maintained at nest temperatures according to PND. The top of each cup remained open to allow each individual pups' tubing (PE 10) to be attached to a PE 50 tubing (60 cm length) that was in turn connected to a nearby 10 cm^3 syringe with a 26G1 needle. Each syringe was filled with milk formula diet (Messer diet, from the University of Iowa) and placed on a timer-controlled infusion pump (Harvard Apparatus). The pumps were programmed to infuse the formula for 10 min every hour, 24 hr daily.

Every morning pups were removed from the cups, weighed, their lines flushed with sterile water and a new infusion rate was calculated. The infusion rate was calculated based on a specified fraction of the mean pup weight. The average weight of all the pups on a pump was multiplied by a constant (commencing with .33 on PND 4, and increasing by .02 each day), and the product was divided by 240. The resulting quotient was the new infusion rate for the day. Every morning the syringes were replaced with new sterile syringes containing fresh formula.

To initiate and facilitate urination and defecation, pups in both AR conditions received two anogenital stimulations (AGS; 30 s each) per day, once in the morning and once in the evening. AGS were administered using a wet paintbrush. The AR-MAX group additionally received five dorsal stimulations (DS; 90 s each) per day, to mimic mothers licking, administered with a dry paintbrush on the dorsal surface of the pup. DS were conducted between 10:00 and 20:00 hr. The first and last DS were done following the AGS and the three DS were done throughout the day and never within 2 hr of each other. These manipulations were carried out from the day the pups were placed on the pumps (PND 4) until the day of weaning. On PND 21, all AR and MR animals were ear marked and weaned. Animals were placed in larger cages (18″ × 10″ × 6″) with a maternally-reared same-sex social conspecific and placed on a 12 hr light/dark reverse cycle with lights on at 8 pm to 8 am in a colony room maintained at 21°C and a humidity level between 40% and 50%. Food and water were made available ad lib.

Sexual Exposure

Females. Female Long-Evans rats (Charles River Canada, St Constant, QC) weighing between 220 and 270 g were ovariectomized via bilateral lumbar incisions under Xylazine Hydrocholoride (10 mg/kg) and Ketamine Hydrochloride (75 mg/kg) anesthetic. Females were housed in pairs and were sexually experienced at the start of the experiment. Sexual receptivity was brought on with subcutaneous administration of estrodial benzoate (10 μg; Sigma) and progesterone (500 μg; Sigma) administered 48 and 4 hr prior to testing, respectively.

Apparatus. Bilevel chambers (Mendelson & Gorzalka, 1987; Mendelson & Pfaus, 1989) were constructed of melamine (back wall) and plexiglas (the outside dimensions of approximately 68.5 cm × 60 cm × 15 cm). A platform 39 cm in length centered and set 27.5 cm above the bottom of the apparatus divided the chamber into two levels. Ramps on either side of the interior with a landing (13.75 cm × 7.5 cm; 13 cm above the floor) allowed the animals to freely move from one level to the other. The ramps were set at a 120° angle and had thin strips of plexiglass placed at regular intervals to provide foot holding. The floor of each level contained an inset of woven stainless steel wire mesh (Ferrier, Toronto, Canada) to provide animals with footholds during sexual behavior. Woodchip bedding was placed under the wire mesh. The front of the chamber had a sliding clear plexiglas door to allow for easy placement and removal of the animals.

Copulation Test. Males in the Exp condition were handled for 5 min and were habituated to the bilevel chambers for 30 min each for 4 consecutive days prior to the copulation test. On PND 75, males were placed in the bilevel chamber for a 5 min acclimation period prior to the introduction of the female. Sexually receptive females were placed in the bilevel chambers with the males for 40 min and their behavior was video recorded. After the termination of the test, females were removed first followed by the males. Two hours following the introduction of the female, males were injected intraperotoneally with a lethal dose of sodium pentobarbital and transcardially perfused with .9% saline and 4% paraformaldehyde at which time blood was taken for testosterone and corticosterone assays. Brains were extracted, postfixed in the same fixative for 4 hr and transferred into 30% sucrose for 48 hr. Brains were sliced at 30 μm and processed for immunocytochemistry. Males in the no exposure condition were also handled for 5 min for 4 consecutive days and were left in their home cage in the animal colony until they were sacrificed.

Immunocytochemistry. Sections were incubated in 30% (w/w) hydrogen peroxide in Trizma Buffered Saline (TBS) for 30 min at 4°C. Slices were washed 3 × 5 min in TBS. Slices were then preblocked in 3% normal goat serum (NGS, Vector Laboratories, Mississauga, ON, Canada) in .05% Triton TBS for 90 min at 4°C. Slices were then incubated for 72 hr in rabbit polyclonal anti-Fos (Oncogene Science, Boston, MA; diluted at 1:75,000) in .05% Triton TBS with 3% NGS at 4°C. Slices were washed and incubated in biotinylated goat anti-rabbit IgG (Vector Laboratories; 1:200) in .05% Triton TBS with 3% NGS for 1 hr at 4°C. Slices were washed and placed in avidin-biotinylate-peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories; 1:50) for 2 hr at 4°C. The peroxidase complex was visualized by sequential incubation at room temperature with 50 mM Tris for 10 min, 3,3′-diaminobenzidine (DAB) in 50 mM Tris (pH 7.8) for 10 min on an agitator, and DAB/3% hydrogen

peroxide/8% Nickel Choride in 50 mM Tris. Sections were then mounted on gel-coated slides and coverslipped.

Histological Analysis. Tissue sections were examined under a 10× objective lens on an Olympus BX60 light microscope and images were captured using a Coolsnap-Pro Monochrome camera (Media Cybernetics, Bethesda, MD) on a computerized imaging program (Image Proplus v. 5.0). Four sections per rat per area were counted unilaterally. The following regions were analyzed and defined using the borders in the Paxinos and Watson (1998): mPOA (plates 18–20), medial amygdala (plates 29–32), nucleus accumbens shell (plates 11–14), mBNST (plates 18–20), ventromedial hypothalamus (plates 29–32), MePd (plates 30–32) and the cingulate cortex as a control site (plates 11–13). The mean count was calculated for each area for each rat.

Data Analyses. An experimenter, blind to the animals' group condition, coded the videotapes using the BEST analyses software program. During the 5 min acclimation period, appetitive level changes (LC) were scored when the male moved completely (indicated by all four paws) from one level of the bilevel chamber to the other (Mendelson & Pfaus, 1989). The appetitive behaviors during the copulation test consisted of body sniffing the female (BDSF), and anogenital investigation of the female (AGI). Latencies to BDSF and AGI were also calculated. The copulatory behaviors that were recorded consisted of mounts, intromissions (IL) and ejaculations (EL). Latencies were calculated to the first mount (ML), IL and EL of the first ejaculatory series. Four secondary measures were calculated from the primary copulatory measures: the inter-mount interval (IMI; calculated as the EL divided by the number of mounts), the inter-intromission interval (III; calculated as the EL divided by the number of intromissions), the PEI (calculated as the time from the first EL to the next intromission) and the intromission ratio (IR; calculated as the number of intromissions divided by the total frequency of both mounts and intromissions). For Fos counts, univariate analysis of variance (ANOVA) was used to assess the individual dependent variables. For each significant ANOVA, posthoc comparisons of the developmental groups were made using Tukey's honestly significant difference (HSD) test. Differences were considered significant at $p < .05$. Because there were unequal variances between groups for many of the behaviors (as established by the Levine's Test for Equality of Variance), nonparametric analysis was used.

RESULTS

No significant differences were found on any of the consummatory parameters of sexual behavior between the developmental groups. Means and standard error of the mean (SEM) of several key behavioral parameters are presented in Table 1. Furthermore, there was no significant difference in the percent of animals to initiate copulation or reach EL (see Table 2). Based on these data, it was assumed that the quality of the sexual behavior obtained during the copulation test was not different across all developmental conditions. However, there was a significant main effect of development ($\chi^2 = 6.65$, $p < .036$) on LC during the copulation trial. Animals in the CONTRL condition showed more LCs than either AR-MIN or AR-MAX animals (see Fig. 1). Significant differences in Fos immunoreactivity (Fos-IR) were found in several regions in the sexual circuit. A significant main effect of Exp, $F(1, 38) = 9.984$; $p < .003$), was found in the MPOA, with significantly more Fos-IR found in males that received a Exp. Furthermore, a significant main effect of development, $F(2, 38) = 4.528$; $p < .019$) was also found in the MPOA (for photopictographs see Fig. 2). Posthoc analysis revealed that animals in the AR-MIN condition had significantly less Fos-IR when compared to CONTRL animals. AR-MAX was not significantly different from any other condition (see Fig. 3). There was no significant interaction between Exp and development in the MPOA.

In the VMH, specifically the ventrolateral portion (vlVMH), there was a significant main effect of development; $F(2, 38) = 4.131$; $p < .025$; in Fos-IR. Posthoc analysis revealed that animals in the AR-MIN condition had significantly less Fos-IR when compared to CONTRL animals. AR-MAX was not significantly different from any other condition (see Fig. 4). There was no significant main effect of Exp or significant interaction between Exp and development in the vlVMH.

Furthermore, as expected, there were significant main effects of Exp in both the MeA, $F(1, 47) = 10.722$; $p < .0002$, and the BNST, $F(1, 47) = 6.164$, $p < .017$.

Table 1. Mean (+/- SEM) Scores for Sexual Behavior Parameters of Males in the Different Developmental Conditions

	Developmental Condition					
	MIN		MAX		CONTRL	
Behavioral Parameters	Mean	SEM	Mean	SEM	Mean	SEM
Anogenital investigation	47.01	±17.02	78.49	±29.29	48.64	±11.35
Mount frequency	13.78	±4.21	12.64	±4.61	19.75	±4.92
Intromission frequency	6.67	±2.94	4.78	±1.76	11.75	±2.76
Mount latency	715.92	±371.50	934.29	±469.07	384.83	±253.06
Intromission latency	999.59	±413.98	1249.29	±518.78	820.14	±332.42
Ejaculation latency	1699.34	±324.15	1498.66	±424.89	1413.15	±282.01

Table 2. Percent Males in Each Developmental Condition Showing Ejaculation, Mounting or no Sexual Behavior

	Developmental Condition		
Behaviors	MIN	MAX	CONTRL
Percent ejaculated	44.4%	55.5%	66.6%
Percent mounted	77.7%	77.7%	83.3%
Percent showing no sexual behavior	22.2%	22.2%	16.6%

Males that had received a Exp had significantly greater Fos-IR in both the MeA (see Fig. 5) and the BNST (see Fig. 6). There were no significant main effects of development or significant interactions between Exp and development in the MeA or the BNST. Furthermore, there were no significant differences in the control site (cingulate cortex).

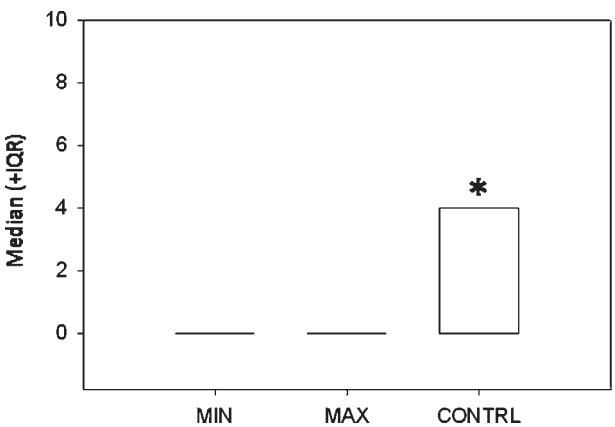

FIGURE 1 Median (+IQR) for number of level changes during the copulation test. AR-MIN and AR-MAX animals show reduced level changing in the bilevel chambers compared to CONTRL animals. The asterisk indicates that CONTRL animals have a significantly higher mean number of level changes than either MIN or MAX animals.

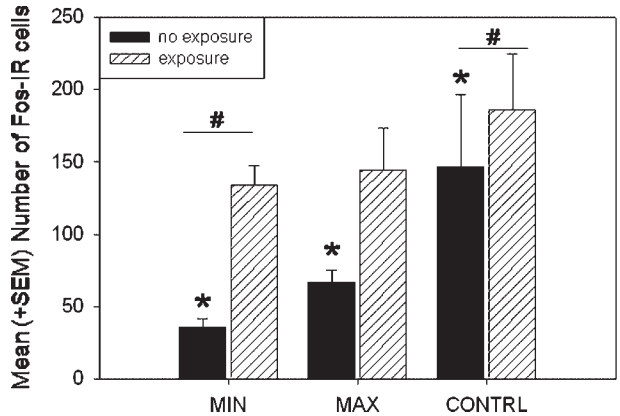

FIGURE 3 Mean (+SEM) number of Fos-IR cells in the MPOA. There was a main effect of development ($p < .01$). Animals in the MIN condition had less Fos-IR than animals in the CONTRL condition. There was a main effect of sexual exposure (Exp; $p < .001$). Animals with exposure had increased Fos-IR compared to animals with no exposure. The asterisk indicates animals in the no exposure condition had lower Fos-IR compared to animals in the exposure condition. The (#) indicates that animals in the CONTRL condition had increased Fos-IR compared to MIN animals.

A significant main effect of Exp was found in testosterone levels; $F(1, 40) = 8.318$; $p < .007$. Males that had received a Exp had higher testosterone levels than those that did not (see Fig. 7). There was no significant main effect of development or significant interaction. Furthermore, a significant main effect of Exp was found in corticosterone levels, $F(1, 47) = 4.503$; $p < .04$ (see Fig. 8). No significant main effect of development or significant interaction was found.

DISCUSSION

The present study demonstrates that although there are no differences in any of the consummatory components of sexual behavior during the first copulatory exposure

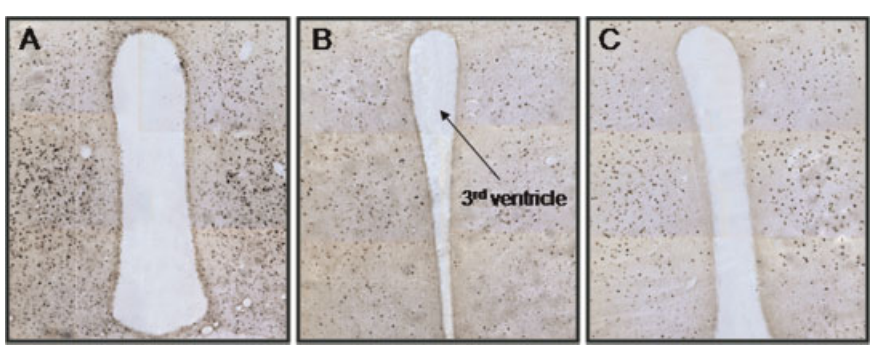

FIGURE 2 FOS-IR following copulatory stimulation in: (A) MR-CONTRL; (B) AR-MAX; and (C) AR-MIN animals in the mPOA.

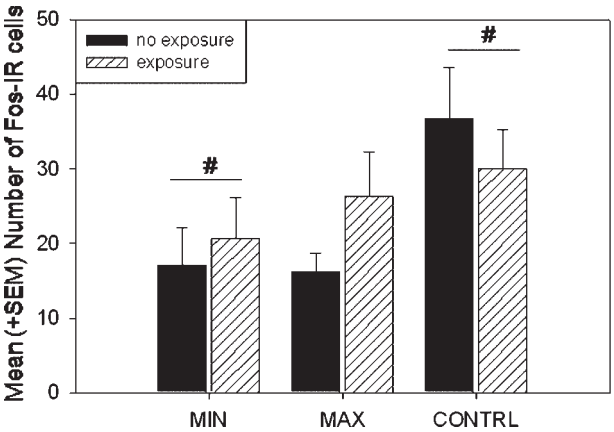

FIGURE 4 Mean (+SEM) number of Fos-IR cells in the vlVMH. There was a main effect of development ($p < .025$). Animals in the MIN condition had less Fos-IR than CONTRL animals. The (#) indicates that animals in the CONTRL condition had increased Fos-IR compared to MIN animals.

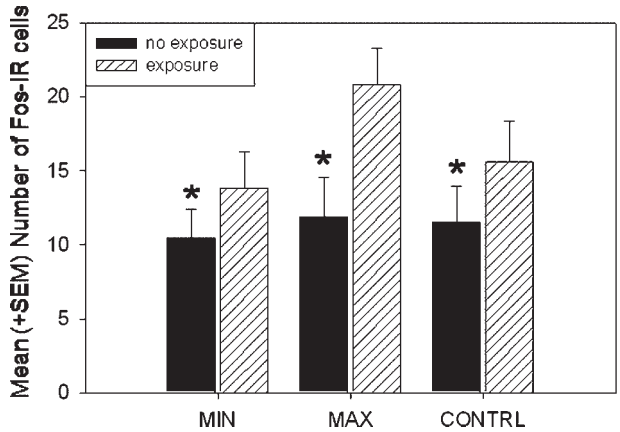

FIGURE 6 Mean (+SEM) number of Fos-IR cells in the BNST. There was a main effect of Exp ($p < .02$). Animals with exposure showed increased Fos-IR compared to no exposure animals. The asterisk indicates animals in the no exposure condition had lower Fos-IR compared to animals in the exposure condition.

between maternally and AR animals, there are differences in the activation patterns of brain sites that subserve this behavior. However, differences in level changing behavior; an appetitive aspect of sexual behavior; were revealed. This is of particular interest because, given that AR animals have been shown to have increased activity (Gonzalez et al., 2001), we expected to find increased level changing behavior in AR animals, possibly as a function of increased locomotor behavior. However, the fact that the opposite was found may strengthen the findings that this early developmental manipulation alters appetitive aspects of sexual behavior.

We found that following a copulatory exposure in naïve animals, AR-MIN males show a lower Fos induction in the mPOA when compared to MR animals. AR-MAX animals were not significantly different from either developmental condition. The mPOA has been shown to be a critical area critical to the initiation of male sexual behavior (Chen & Bliss, 1974; Ginton & Merari, 1977; Heimer & Larsson, 1966), and lesions to this hypothalamic nucleus result in deficits in male sexual behavior (Ginton & Merari, 1977; Heimer and Larsson, 1966, 1967). Furthermore, we found that independent of copulatory exposure, a differential pattern of Fos activation was found in the vlVMH between AR and MR animals. AR-MIN animals had a lower Fos-IR in this region when compared to controls. AR-MAX animals were not different from either developmental

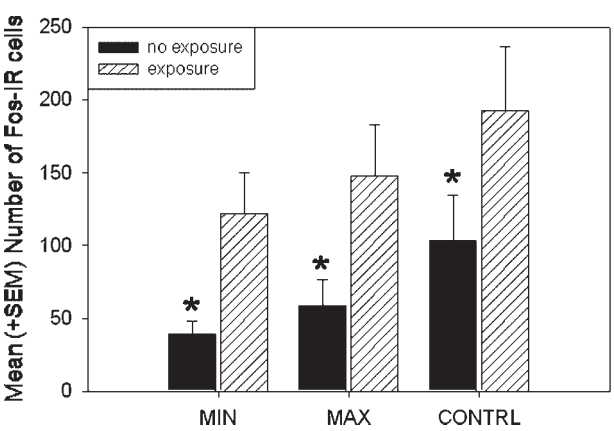

FIGURE 5 Mean (+SEM) number of Fos-IR cells in the MeA. There was a main effect of Exp ($p < .01$). Animals with exposure showed increased Fos-IR compared to no exposure animals. The asterisk indicates animals in the no exposure condition had lower Fos-IR compared to animals in the exposure condition.

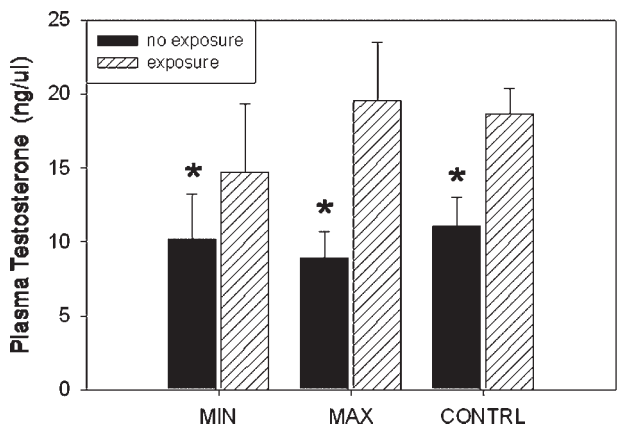

FIGURE 7 Mean (+SEM) testosterone levels. There was a significant main effect of Exp ($p < .01$). Animals with exposure showed higher levels of testosterone compared to no exposure animals. The asterisk indicates animals in the no exposure condition had lower testosterone levels compared to animals in the exposure condition.

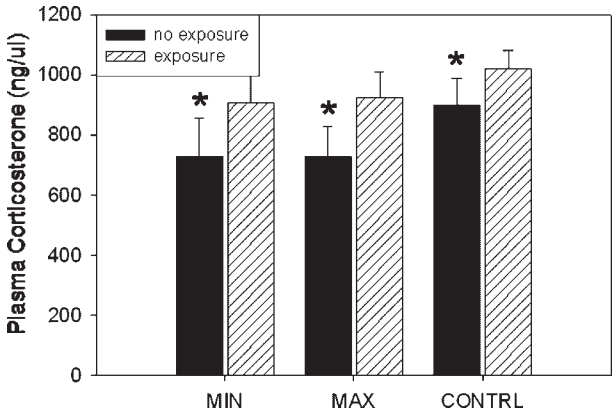

FIGURE 8 Mean (+SEM) corticosterone levels. There was a significant effect of Exp ($p < .04$). Animals with exposure showed higher levels of corticosterone compared to no exposure animals. The asterisk indicates animals in the no exposure condition had lower corticosterone levels compared to animals in the exposure condition.

condition. It is not clear as to the effects of early life experience on the plasticity of the VMH. This area has been shown to be critical to female sexual behavior (for review, see Pfaff et al., 1994). However, several recent studies have examined its role in the expression of male copulatory behavior. This area is rich in both androgen and estrogen receptors (Simerly, Chang, Muramatsu, & Swanson, 1990), and has been found to be critical in the restoration of copulation in male rats (McGinnis, Williams, & Lumia, 1996). However, the present findings did not find a significant effect of Exp in Fos-IR within the VMH. Our findings in the MeA and the BNST corroborate previous studies that these areas show increases in Fos induction following copulation. However, no developmental effects were found in these sites, demonstrating that decreases in Fos-IR seen in AR-MIN animals are not global. An explanation for why there are developmental effects in the mPOA and the VMH and not in other brain regions investigated may relate to the clear developmental changes in these regions during the early PND weeks. For example, within the mPOA, little cytological changes are seen between birth and PND 5. However, from PND 5 to 10, neuronal maturation is accelerated, so that by PND 10 most cells within this region exhibit features suggestive of elevated metabolic activity (Reier, Cullen, Froelick, & Rothchild, 1977). Similarly, although much of the basic electrophysiological characteristics are established prenatally in the VMH, further developmental changes in the neurophysiological properties of this region are seen between PND 1 and 25 (Almli & Fisher, 1985). In the present study, isolation began on PND 4, therefore encompassing the time period of postnatal plasticity and structural change in the mPOA and the VMH, possibly making these areas more susceptible to developmental manipulations. What is of particular interest is that although there is a lower absolute Fos induction in AR-MIN animals in the mPOA and vlVMH, there is an equivalent activation of Fos-IR following Exp when compared to CONTRL animals. Whether these findings relate to functional performance in subsequent trials, remains to be seen.

An interesting and unexpected finding was that there was an increase in testosterone levels following a first copulatory exposure. Although elevations in testosterone have been reliably found in sexually experienced males (Bonilla-Jaime, Vazquez-Palacios, Arteaga-Silva, & Retana-Marquez, 2006; Kamel, Mock, Wright, & Frankel, 1975), to our knowledge no such elevations have been reported in naïve animals (Bonilla-Jaime et al., 2006; Kamel et al., 1975). The methodological difference between the present study and previous ones is the time at which blood samples were taken. Previous studies (Bonilla-Jaime et al., 2006) sampled blood immediately following a 30 min copulatory bout in both naïve and experienced males, whereas we sampled blood 90 min following a 40 min copulatory exposure. Therefore, this data indicates that elevations in testosterone do occur following copulation in naïve males; however this increase seems to be delayed in comparison to experienced males. However, a comparison of testosterone levels at different time points following copulation, and between experienced and naïve males must be made in order to clarify these findings.

Maternal behavior in mammals presents an important source of stimulation during early development (Moore, 1984). In the rat, the presence of the mother provides her offspring with a complex set of chemical, somatosensory and thermal stimuli. This complex set of behaviors includes licking that is primarily directed at the anogenital region to stimulate urination and defecation in the offspring. Although there has been a substantial focus on the importance of anogenital licking that the mother provides on the development of male sexual behavior (Moore, 1984), little is known about the role of the somatosensory experience of body licking on the development of male copulatory behavior. Although no consummatory behavioral differences on the first copulatory test were found, the activational patterns of sites within the sexual circuit differed between AR-MIN and MR-CONTRL animals in response to the exposure. Therefore, although the quality of the somatosensory experience was no different, the response to that experience was blunted in the AR-MIN animals. This was partially reversed in the AR-MAX animals, suggesting that this early somatosensory stimulation is important in brain plasticity. The appetitive differences in level changing behavior between AR and MR animals may suggest that with increased sexual experience, consummatory behavioral deficits may

emerge in the AR-MIN animals following such changes in brain activational patterns in areas that subserve sexual behavior. Preliminary observations indicate that this may in fact be the case. With increased sexual experience, AR-MIN animals show a differential temporal pattern of sexual behavior. This may reflect the fact that the mPOA is differentially activated as early as the first copulatory exposure as demonstrated by the present study. Studies examining the effects of early maternal separation have yielded mixed results. Rhees et al. (2001) reported increased MLs and ILs and a decrease in the number of males that reached EL in animals that were maternally separated for 6 hr daily from PND 2 to 10. Conversely, Greisen et al. (2005) reported enhanced sexual performance as evidenced by decreased MLs, ILs and PEIs in animals maternally separated daily for 180 min from PND 2 to 14. This dissimilarity may be attributed to methodological differences in the length and duration of the separation.

In summary, the present study demonstrates that complete maternal isolation during early development may alter appetitive aspects of sexual behavior as early as the first copulatory exposure but does not affect consummatory behavior during this first trial. However it does decrease the activation of Fos-IR to that exposure in areas that have been shown to be important in male sexual behavior. This decrease may be partially reversed by the addition of licking-like stimulation during the early postnatal period.

NOTES

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