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Transgenerational Effects of Social Environment on Variations in Maternal Care and Behavioral Response to Novelty

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Cross-fostering studies in the rat have illustrated the importance of the postnatal environment in mediating the transmission of maternal licking/grooming (LG) from mother to offspring. The authors addressed the question of how postweaning social conditions can alter the patterns of maternal behavior. Juvenile female offspring of high LG and low LG mothers were placed in either standard, enriched, or impoverished postweaning environments for 50 consecutive days and then mated and observed with their own litters. Analysis of LG behavior indicated that the effect of postweaning environment was dependent on the level of postnatal mother–infant interaction. Postweaning isolation reduced exploratory behavior, maternal LG, and oxytocin receptor binding in the offspring of high LG mothers, whereas social enrichment enhanced exploration, LG behavior, and oxytocin receptor binding of low LG offspring. These effects were also transmitted to the next generation of offspring. Thus, maternal LG and the neural mechanisms that regulate this behavior exhibited a high degree of plasticity in response to changes in environment both within and beyond the postnatal period, with implications for the transmission of behavioral response to novelty and maternal care across generations.

Keywords: maternal behavior, oxytocin, environment, transgenerational

Mother–infant interactions occurring early in development have a profound impact on offspring physiology and behavior. In primates and humans, reduced levels of maternal care, either in the form of complete maternal deprivation or of neglect, have been shown to increase stress responsivity, impair cognitive ability, and reduce social behavior (Arling & Harlow, 1967; Carlson & Earls, 1997; Glaser, 2000; Harlow, Dodsworth, & Harlow, 1965; Trickett & McBride-Chang, 1995). Likewise, in rodents, complete maternal deprivation or extended periods of mother–infant separation also affected response to stress, cognition, and juvenile and adult social interactions (Kalinichev, Easterling, Plotsky, & Holtzman, 2002; Lehmann, Pryce, Bettschen, & Feldon, 1999; Lovic, Gonzalez, & Fleming, 2001). Conversely, postnatal handling involving brief periods of maternal separation, which stimulate maternal care (Liu et al., 1997), decreased the stress response of offspring and enhanced learning and memory (Meaney, Aitken, Bhatnagar, & Sapolsky, 1991; Meaney, Aitken, Bodnoff, Iny, & Sapolsky, 1985). These studies have highlighted the importance of the postnatal period in shaping adult phenotype and the potential role of maternal care in mediating these long-term effects.

Direct evidence for the role of maternal care in mediating offspring phenotype has emerged from studies of the impact of natural variations in maternal behavior. In both primates and rodents, females have exhibited stable individual differences in maternal care that can be quantified and associated with developmental outcomes (Champagne, Francis, Mar, & Meaney, 2003; Fairbanks, 1989; Fairbanks & McGuire, 1988; Meaney, 2001). In rodents, natural variations in maternal licking/grooming (LG) of pups during the first week postpartum have been observed that alter gene expression, physiology, and behavior of both male and female offspring (Caldji et al., 1998; Francis, Diorio, Liu, & Meaney, 1999; Liu et al., 1997). Offspring of mothers who engaged in high levels of LG had increased levels of hippocampal glucocorticoid receptor expression, an attenuated hypothalamic–pituitary–adrenal response to stress, and enhanced performance on measures of cognitive ability (Caldji et al., 1998; Liu, Diorio, Day, Francis, & Meaney, 2000; Liu et al., 1997). Cross-fostering of the biological offspring of high LG mothers to low LG mothers and the offspring of low LG mothers to high LG mothers has illustrated that the adult phenotype of these offspring is shaped by the quality of postnatal care received (Francis et al., 1999). Moreover, these variations in maternal care are passed from one generation to the next such that the female offspring of high LG mothers exhibit high levels of LG toward their own offspring (Champagne, Francis, et al., 2003, Francis et al., 1999), providing a behavioral mechanism of inheritance of stress responsivity and social behavior (Champagne & Curley, 2005).

The transmission of maternal LG across generations is associated with the levels of neuropeptide receptors in the medial preoptic area of the hypothalamus (MPOA). Oxytocin receptor (OTR) binding is elevated in the MPOA of high LG compared to low LG females, and infusion of a highly selective OTR antagonist on postpartum Day 3 reduces levels of LG among high LG females and abolishes group differences in LG (Champagne, Diorio, Sharma, & Meaney, 2001; Francis, Champagne, & Meaney, 2000).

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Offspring of low LG mothers have reduced estrogen-sensitivity in the MPOA, associated with decreased levels of estrogen receptor alpha (ERα) expression in this region when compared to offspring of high LG mothers (Champagne et al., 2006; Champagne, Weaver, Diorio, Sharma, & Meaney, 2003). Estrogen and ERα exert potent regulatory effects on OTR expression, acting through genomic and nongenomic mechanisms to increase OTR levels (Bale, Davis, Auger, Dorsa, & McCarthy, 2001; Breton & Zingg, 1997; Zingg et al., 1998). Thus, differential levels of LG regulate offspring's levels of ERα mRNA in the MPOA and serve as a potential mechanism for the variations in OTR binding that have been observed in estrogen-primed female offspring of high compared to low LG mothers.

These studies have suggested that social interaction during the early postpartum period is critical in regulating the maternal behavior of offspring and thus in generating the transmission of maternal care across generations. The critical question addressed in the current study is whether social interactions beyond the postnatal period can alter patterns of maternal care and thus alter generational transmission. Few studies have examined the impact of postweaning social isolation and social enrichment (housed in same-sex, same-age groups) on maternal behavior in the rat (Genaro & Schmidek, 2002), however, the offspring of females reared in these environments "inherit" the phenotype characteristic of rats housed under these conditions (Dell & Rose, 1987; Kiyono, Seo, Shibagaki, & Inouye, 1985; McKim & Thompson, 1975). Both the biological and foster offspring of female rats raised in socially enriched environments spend more time exploring a novel environment and require fewer trials to learn to bar press for reinforcement when compared to females raised in impoverished environments (Dell & Rose, 1987). Maze learning is also enhanced in the male offspring of enriched females (Kiyono et al., 1985). These findings suggest the possibility that the female's behavior toward her offspring or her reproductive physiology is altered, thus resulting in a generational transmission of postweaning effects.

The present study is an investigation of the plasticity of maternal behavior in response to social cues experienced during both the postnatal and postweaning periods and how these experiences alter the phenotype of subsequent generations of offspring. To examine this issue, at weaning we placed the female offspring of high and low LG mothers in standard, enriched, and impoverished environments. We can reliably predict the maternal phenotype and hypothalamic OTR binding of these offspring on the basis of the quality of the postnatal care received, however the question is whether the quality of social interaction beyond this period can alter the predicted behavioral and neuroendocrine patterns. In addition, we assessed the effects of these conditions on behavior response to a novel environment as well as the impact of mothers' prenatal and postweaning environment on subsequent offspring maternal care and exploratory behavior.

Materials and Method

Subjects

The subjects were Long–Evans hooded rats born in our colony and housed in 46 cm × 18 cm × 30 cm Plexiglas cages with food and water provided ad libitum. The colony was maintained on a 12-hr light–dark cycle with lights on at 0800. Routine cage maintenance began on Day 7 of life, and rats 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. One week prior to parturition and throughout lactation, adult females were housed singly.

Procedure

Adult Long–Evans females ($n = 40$) from the Douglas Hospital Research Centre colony were singly housed, mated, and observed during the first 6 days postpartum. From these, nine low LG litters ($M = 8.8\%$, $SD = 0.11$) and seven high LG litters ($M = 13.8\%$, $SD = 0.21$) were selected for the study. At weaning on Day 21, 3 to 7 female pups were selected from each litter and then weighed and placed in standard, impoverished, or enriched housing ($n = 12$ low LG pups, $n = 12$ high LG pups per housing condition). Under *impoverished* housing conditions, pups were singly housed in 22 cm × 18 cm × 22 cm cages with wire mesh floors. Under *standard* housing conditions, pups were pair housed in same-sex, same-litter groups in 46 cm × 18 cm × 30 cm Plexiglas cages containing bedding material. Under *enriched* housing conditions, offspring were weaned into a multilevel, six-compartment steel cage with a wire mesh floor. Each 50 cm × 60 cm × 50 cm compartment was joined to adjacent compartments with tunnels through which rats could move freely between levels and compartments. Toys were placed in each of the compartments. These consisted of plastic and wooden blocks, tennis balls, plastic tubing, bells, and plastic cars. The location of the toys was altered each week. Though there are many stimulating aspects of this environment, previous studies have indicated that it is the social interaction made possible in this housing condition that is critical for inducing behavioral changes (Morgan, 1973). Due to restrictions on the number of rats that could be placed in this apparatus, only offspring of high and low LG dams were included in this study. After spending 50 days in the specified housing condition, females were removed, weighed, singly housed into standard Plexiglas cages, mated, and placed in the observation room. Following parturition and after 6 days of maternal observation, 5 females from each of the six conditions (low LG/standard housed, high LG/standard housed, low LG/impoverished, high LG/impoverished, low LG/enriched, high LG/enriched) were decapitated, and their brains were extracted for OTR binding assay. The remaining 7 females per group and their litters were left undisturbed, with the exception of routine cage maintenance, until weaning. At Day 21, 14 male and 14 female offspring from each condition were weaned into standard housing. This generation would thus be the grand-offspring of high and low LG dams whose mothers were housed under the three social conditions. Two weeks postweaning, dams were tested in the open-field and object-recognition tests. Male offspring of these dams were, as adults (Day 90), also tested in the open-field and object-recognition tests. Female offspring were not tested in the open-field and object-recognition tests to avoid the potential effects of the manipulation involved in testing on maternal behavior but were instead mated at Day 70 and observed with their litters for 6 days postpartum. Thus, none of the females included in this study were tested prior to mating. To address the potential influence of litter effects, we used litter as a covariant within all analyses of variance (ANOVAs) of the data.

Assessment of Postpartum Maternal Behavior

Maternal care was assessed during Days 1 to 6 postpartum through observations of home-cage mother–infant interactions as previously described (Champagne, Francis, et al., 2003). Dams were not disturbed for the duration of the 6-day observation period. Each day consisted of five observation periods (0600, 1000, 1300, 1700, 2100), 72 min in duration. Within each observation period, the behavior of each mother was scored every 3 min (25 observations/period × 5 periods/day = 125 observations/mother/day) for the following behaviors (also described in Myers, Brunelli, Squire, Shindeldecker, & Hofer, 1989): mother not in contact with pups, mother carrying pup, mother licking and grooming any pup (both body and anogenital licking were included), and mother nursing pups. Nursing posture was rated as either (a) an arched-back posture when the mother was arched over pups with legs extended, (b) a "blanket" posture in which the mother lays over the pups with no leg extension, or (c) a passive posture in which the mother is lying either on her back or side while the pups nurse. Observers were trained to a high level of interrater reliability (i.e., >0.90).

The selection of rats as high or low LG mothers was based on the mean and standard deviation for this measure for the maternal cohort. The characterization of individual mothers thus depended upon the reliability of the cohort data set. To provide more reliable estimates of individual differences in maternal behavior, we observed cohorts of approximately 40 mothers/litters. The size of this cohort was also determined by the observational procedure. One individual can accurately observe the behavior of as many as 40 rats with sequential observations within a 3-min time span. High LG mothers were defined as females whose mean frequency scores for LG over Days 1 to 6 postpartum were greater than 1.0 standard deviation above the mean. Low LG mothers were defined as females whose mean frequency scores for LG over Days 1 to 6 postpartum were greater than 1.0 standard deviation below the mean. LG data from multiple cohorts of females indicated that mean and standard deviation did not differ significantly between cohorts.

Receptor-Binding Assays

For the analysis of levels of postpartum OTR binding, lactating females were rapidly decapitated on postpartum Day 6. Brains were extracted, placed briefly in isopentane, and kept at −80 °C until processed. Brains were sectioned in the coronal plane at 16 μm, and sections thaw mounted onto poly-L-lysine coated slides that were stored at −80 °C until the assay was performed. We processed slide-mounted coronal brain sections for receptor autoradiography using ^{125}I-d(CH$_2$)$_5$[Tyr-Me)$_2$,Tyr-NH$_2^9$]OVT (^{125}I-OVT; New England Nuclear, Boston, MA) as previously described (Champagne et al., 2001; Francis et al., 2000). ^{125}I-OVT has a very high affinity for OTRs (K_d = 0.048 ± 0.008 nM) and a 10-fold greater affinity for OTRs than oxytocin (Elands et al., 1988). The affinity of this compound for OTRs is approximately 300-fold higher than it is for V1 and V2 vasopressin receptors (Elands et al., 1988). After a prewash in Tris-HCl (pH = 7.4), slides were exposed to a 75-min incubation (at room temperature) of 60 pM ^{125}I-OVT in Tris with MgCl (10 mM), bovine serum albumin (0.1%), and bacitracin (0.05%). Nonspecific binding was defined in adjacent sections by adding 50 nM Thr^{4}Gly^7-oxytocin (a concentration previously found to completely displace 60 pM ^{125}I-OVT; Elands et al., 1988) to the incubation buffer. The final 35-min wash was performed at room temperature in 50 mM Tris (pH = 7.4), 100 mM MgCl to reduce background. After air-drying, the slides were exposed to BioMax MR film (Kodak) for 48 hr. ^{125}I-OVT autoradiographic standards (Amersham) were included in the cassette for quantification.

Using an image-capture system (http://www.imagingresearch.com, Imaging Research, St. Catherines, Ontario), we analyzed autoradiograms with MCID software. Three sections were analyzed bilaterally at each level. For each rat, total and nonspecific binding was 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 rat by brain region according to the atlas of Paxinos and Watson (1986).

Open-Field Test

The open-field test is a standard tool for measuring behavioral aspects of anxiety in rodents (Archer, 1973; Belzung & Griebel, 2001; Crawley, 1985). The open field itself serves as a novel environment for the rat. Habituation to this environment is typically followed by exploratory behavior, operationally defined as increased entries into the inner area of the field. The open field used was a 120 cm × 120 cm wooden box. The behavior of the rat in this field was recorded with a video camera mounted on a tripod adjacent to the field. We completed the coding of these video recordings using a DOS-based program designed to give summaries of the amount of time spent in the inner and outer area of the field, as well as the overall activity of the rat. On the day of testing, the rat was removed from its home cage and placed directly into one corner of the open field. After a 10-min session, the rat was removed and returned to its home cage. All testing was conducted under standard lighting conditions. 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-min session.

Object-Recognition Test

The object-recognition test is a cognitive measure that has been used to detect memory impairments (Ennaceur & Aggleton, 1994; Ennaceur & Delacour, 1988). The test is designed to compare the amount of exploration of a novel object relative to that of an object identical to one that the animal has already been exposed to (discrimination ratio). The objects in this case differ in color and shape. The testing apparatus used was a black 75 cm × 75 cm wooden box containing bedding material (approximately 2-cm deep). The behavior of the rat in this box was recorded with a video camera mounted on a tripod adjacent to the box. Prior to the day of testing, rats were habituated to this testing apparatus and the process of being removed from their home cage. This aspect of the procedure was designed to reduce stress-related behaviors in this novel environment. There were 3 days of habituation trials. Each day, the rat was removed from the home cage and placed in the testing apparatus for 5 min. After the session, the rat was returned to the home cage. On the 4th day, the rat was placed in the testing

apparatus for 5 min with two identical objects. After a 15-min delay, the rat was returned to the apparatus. During this session, two new objects were placed in the apparatus; one identical to the two objects in the first session (the *old* object), and one *new* object. This session was 5 min in duration. To ensure that discrimination ratios were not affected by a preference for the physical characteristics of either the new or the old object, we used a counterbalanced design.

Results

Weaning weight and weight after 50 days in the specified housing condition did not differ as a function of maternal phenotype or housing condition. Day 50 weights for offspring of low LG dams were 321.8 ± 5.2 g for standard housed, 319.0 ± 4.3 g for impoverished, and 322.1 ± 4.6 g for enriched females and, for offspring of high LG dams, 317.9 ± 6.1 g for standard housed, 319.7 ± 4.9 g for impoverished, and 323.2 ± 5.1 g for enriched females. Under standard housing conditions, 91.7% (11/12) of both high and low LG offspring gave birth. Among females placed in impoverished housing conditions, 83.3% (10/12) of low and 75.0% (9/12) of high LG offspring gave birth. Enrichment housing resulted in a birth rate of 91.7% (11/12) for low LG offspring and a 100.0% (12/12) birth rate in high LG offspring. Only females who successfully gave birth were included in subsequent behavioral analyses.

Maternal Behavior of Standard Housed, Impoverished, and Enriched Females

Two-way ANOVA (Group × Housing Condition) of LG behavior of female offspring of high and low LG mothers indicated a significant main effect of housing, $F(2, 67) = 13.4$, $p < .001$, and a significant Group × Housing Condition interaction, $F(2, 67) = 4.0$, $p < .05$. Tukey post hoc analysis revealed a significant difference in LG behavior between offspring of high LG and low LG females housed under standard conditions ($p < .05$), however, no group differences were observed between females exposed to impoverished and enriched conditions (see Figure 1). Offspring of high LG females housed in impoverished conditions showed significant decreases in LG compared to high/standard housed females ($p < .05$). Conversely, offspring of low LG females housed in enriched conditions showed elevated levels of LG compared to low/standard housed females ($p < .001$). Impoverished housed offspring of low LG females and enriched housed offspring of high LG females showed no changes in LG relative to same-group females housed in standard conditions. No group or housing effects were observed on any other aspect of maternal behavior.

Exploration and Activity of Standard Housed, Impoverished, and Enriched Females

Following observation of maternal behavior 2 weeks postweaning, we observed dams for 10 min in the open-field test, and group comparisons were made of time spent in the inner-field area (exploration; see Figure 2) and total squares crossed during testing (activity). Two-way ANOVA of exploratory behavior indicated a main effect of group, $F(1, 30) = 12.9$, $p < .001$; a main effect of housing, $F(2, 30) = 12.1$, $p < .001$; and a significant Group × Housing Condition interaction, $F(2, 30) = 10.3$, $p < .001$. Post hoc analysis indicated a significant difference between standard housed offspring of high and low LG females ($p < .001$), with high LG females being more exploratory. Female offspring of high and low LG females placed in impoverished housing did not differ from each other in exploratory behavior, and their scores were significantly lower than standard housed offspring of high LG females ($p < .001$). Offspring of both high and low LG females placed in enriched housing exhibited high levels of exploratory behavior compared to the standard housed low LG females ($p < .01$) and impoverished housed high and low LG females ($p < .05$) but did not differ from each other or from standard housed offspring of high LG females. No group differences were found in total activity in the open field during the testing session.

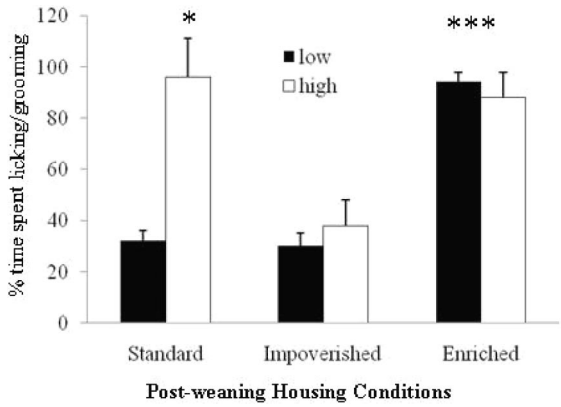

Figure 1. Mean percentage of time spent licking/grooming (LG) pups by the adult female offspring of high and low LG dams. Offspring were weaned into three housing conditions (standard, impoverished, or enriched) for 50 days, mated, and observed during the first 6 days postpartum. Analysis indicated that postweaning impoverishment and enrichment abolished group differences in maternal LG. High LG female offspring housed under impoverished conditions exhibited reduced levels of LG compared to standard housed high LG females ($p < .05$), whereas low LG female offspring housed under enriched conditions exhibited elevated levels of LG compared to standard housed low LG females ($p < .001$). Error bars represent standard error of the mean. $^{*}p < .05$. $^{***}p < .001$.

One week following open-field testing, females were assessed in the object-recognition test. Females were videotaped during a 5-min exposure to a new and old object. We conducted an analysis using a discrimination ratio, a measure of the ratio of time spent with the new versus the old object. Higher ratios indicate that relatively more time was spent with the new object. Two-way ANOVA indicated a main effect of group, $F(1, 34) = 8.4$, $p < .01$, and a main effect of housing, $F(2, 34) = 13.4$, $p < .001$, on the discrimination ratio. Offspring of high LG mothers exhibited higher discrimination ratios compared to offspring of low LG mothers ($p < .05$). Females housed in enriched conditions spent more time investigating the new object compared to standard ($p < .05$) and impoverished ($p < .001$) housed females.

OTR Binding of Differentially Housed Offspring of High and Low LG Mothers

Lactating females ($n = 5$ per group) were decapitated on Day 6 postpartum, and their brains were processed for OTR binding

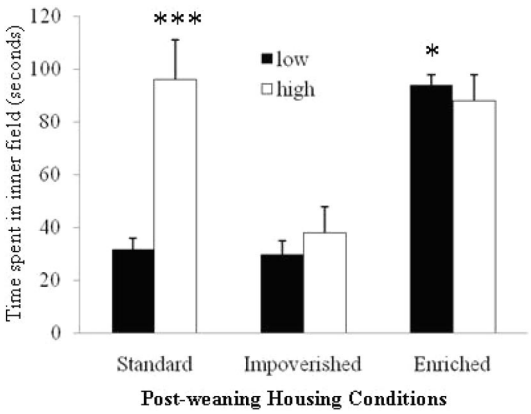

Figure 2. Mean open-field exploration of the adult female offspring of high and low licking/grooming (LG) dams. Under standard postweaning housing conditions, high LG offspring were more exploratory than low LG offspring. Analysis indicated that high LG female offspring housed under impoverished conditions exhibited reduced levels of exploration compared to standard housed high LG females ($p < .001$), whereas low LG female offspring housed under enriched conditions exhibited elevated levels of exploration compared to standard housed low LG females ($p < .01$). Error bars represent standard error of the mean. $^{*}p < .05$. $^{***}p < .001$.

assay. The six regions analyzed were the MPOA, bed nucleus of the stria terminalis (BNST), lateral septum, paraventricular nucleus (PVN), central nucleus of the amygdala, and ventral medial nucleus of the hypothalamus (VMH). Repeated-measures ANOVA indicated a main effect of group, $F(1, 29) = 45.3$, $p < .001$; a main effect of housing, $F(2, 29) = 65.8$, $p < .001$; a main effect of region, $F(5, 29) = 187.7$, $p < .001$; a significant Group × Housing Condition interaction, $F(2, 29) = 5.9$, $p < .01$; a Group × Region interaction, $F(5, 29) = 5.4$, $p < .001$; a Housing × Region interaction, $F(10, 29) = 8.4$, $p < .001$; and a significant three-way Group × Housing Condition × Region interaction, $F(10, 29) = 2.3$, $p < .05$.

Results and representative photomicrographs are presented in Table 1 and Figure 3. In the MPOA, Tukey post hoc analyses indicated elevated levels of OTR binding in the offspring of high LG females compared to low LG females weaned into standard housing conditions ($p < .001$). Under impoverished and enriched housing conditions, there were no significant group differences. OTR binding in the MPOA of offspring of high LG females housed in impoverished conditions was significantly lower than high LG/standard housed females ($p < .01$). OTR binding in the offspring of low LG females housed in enriched conditions was significantly higher than that of low LG/standard housed females ($p < .01$). In the BNST, standard housed offspring of high LG females had significantly elevated levels of OTR binding compared to low LG/standard housed females ($p < .05$). No group differences in binding were observed under impoverished conditions. Under enriched conditions, binding in the BNST of high LG offspring was significantly elevated compared to low LG females ($p < .05$). Binding in both high and low LG female offspring housed under enriched conditions was elevated compared to low LG/standard housed females ($p < .001$ and $p < .05$, respectively). In the lateral septum, standard housed offspring of high LG dams had higher OTR binding levels than low LG dams ($p < .05$). No group differences in binding were observed under impoverished or enriched housing conditions. In the PVN, high/low LG differences in OTR binding were detected under standard housing conditions ($p < .05$). Under impoverished and enriched housing conditions, no group differences were observed. Binding in high LG/impoverished females was not significantly different from binding in low LG/standard housed females. OTR binding in the PVN of low LG/enriched females was significantly elevated compared to that of low LG/standard housed females ($p < .05$). In the central nucleus of the amygdala, OTR binding was significantly elevated in the offspring of high LG females compared to low LG females housed in standard conditions ($p < .001$). Group differences were not observed under impoverished or enriched conditions. High LG/impoverished females had significantly lower levels of OTR binding than high LG/standard housed females in this region ($p < .01$). Higher levels of binding were detected in low LG/enriched housed females compared to low LG/standard housed females ($p < .01$). Finally, post hoc analyses of binding in the VMH indicated no significant differences as a function of group or housing condition.

Exploration and Activity of Male Offspring of Differentially Housed Females

From each of the six groups (high and low grand-maternal phenotypes by three housing conditions) of females, 15 adult male offspring were tested as adults in the open-field task. Two-way ANOVA of exploratory behavior (time spent in inner-field) indicated a main effect of group, $F(1, 59) = 45.2$, $p < .001$; a main

Table 1
Mean (SEM) Oxytocin Receptor Binding Levels in Offspring of Low and High LG Dams as a Function of Postweaning Housing Condition

Brain region and maternal LG level	Postweaning housing condition		
	Standard	Impoverished	Enriched
MPOA			
Low	16.8 (0.39)	16.9 (0.32)	20.0 (0.64)
High	20.5 (0.59)***	17.2 (0.38)	20.6 (0.22)
BNST			
Low	24.4 (1.47)	23.5 (0.27)	29.2 (1.21)
High	29.8 (1.61)*	24.9 (0.43)	34.8 (0.71)*
LS			
Low	21.4 (0.48)	21.5 (0.79)	23.0 (0.92)
High	24.5 (0.30)*	22.4 (0.20)	25.2 (0.34)
PVN			
Low	17.1 (0.35)	18.6 (0.44)	19.5 (0.77)
High	19.8 (0.30)*	17.8 (0.12)	20.4 (0.82)
Amygdala			
Low	21.4 (0.48)	21.5 (0.79)	23.0 (0.92)
High	24.5 (0.30)***	22.4 (0.20)	25.2 (0.34)
VMH			
Low	21.0 (0.63)	19.3 (0.67)	20.3 (0.49)
High	19.8 (0.15)	19.4 (0.64)	20.5 (1.20)

Note. LG = licking/grooming; MPOA = medial preoptic area of the hypothalamus; BNST = bed nucleus of the stria terminalis; LS = lateral septum; PVN = paraventricular nucleus; VMH = ventral medial nucleus of the hypothalamus.
$^{*}p < .05$. $^{***}p < .001$.

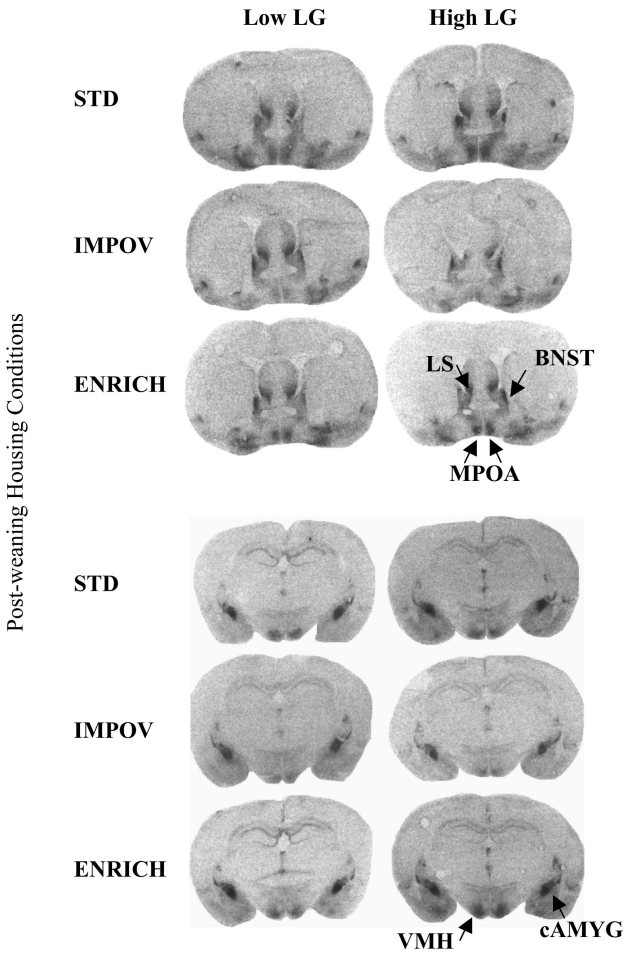

Figure 3. Representative photomicrographs of hypothalamic-oxytocin receptor binding in Day 6 postpartum lactating female offspring of high and low licking/grooming (LG) dams. Females were placed at weaning into standard (STD), impoverished (IMPOV), or enriched (ENRICH) housing conditions. LS = lateral septum; BNST = bed nucleus of the stria terminalis; MPOA = medial preoptic area of the hypothalamus; VMH = ventral medial nucleus of the hypothalamus; cAMYG = central nucleus of the amygdala.

effect of housing, $F(2, 59) = 30.7$, $p < .001$; and a significant Group × Housing Condition interaction, $F(2, 59) = 23.0$, $p < .001$. Post hoc analysis indicated that the male offspring of high LG/standard housed females exhibited higher levels of exploration than the male offspring of low LG/standard housed females ($p < .001$; see Figure 4). The male offspring of females housed in impoverished and enriched conditions showed no group differences in exploration. The male offspring of both high LG/impoverished and low LG/impoverished females showed reduced exploration compared to the male offspring of high LG/standard housed females ($p < .001$). Male offspring of low LG/enriched females were significantly more exploratory than offspring of low LG/standard housed females ($p < .001$). No significant differences were found in overall activity of the male offspring in the open field as a function of mother's group or housing condition.

From each of the six conditions, 8 male offspring were tested in the object-recognition task (see Figure 5). Two-way ANOVA of

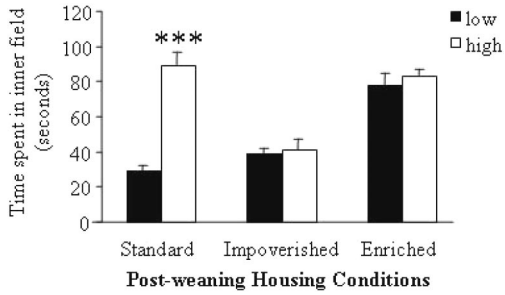

Figure 4. Mean open-field exploration of the adult male offspring of standard, impoverished, or enriched housed females (who were themselves the offspring of standard housed high and low licking/grooming mothers). Error bars represent standard error of the mean. $^{***}p < .001$.

the discrimination ratio (time spent exploring new vs. old object) indicated a main effect of group, $F(1, 34) = 12.5$, $p < .001$; a main effect of housing, $F(2, 34) = 13.3$, $p < .001$; and a significant Group × Housing Condition interaction, $F(2, 34) = 9.8$, $p < .001$. Post hoc analyses indicated significantly higher discrimination ratios in the male offspring of high LG/standard housed females compared to the offspring of low LG/standard housed females ($p < .001$). No group differences were observed in the offspring of impoverished or enriched females. The offspring of high LG/impoverished females were significantly less exploratory than offspring of high LG/standard housed females ($p < .001$). No group differences were observed in overall total time spent exploring objects during the testing session.

Postweaning Housing Effects on the Transgenerational Inheritance of Maternal Behavior

Female offspring of differentially housed mothers ($n = 14$ per group) were mated and observed with their litters for 6 days postpartum. Two-way ANOVA of LG scores indicated a main

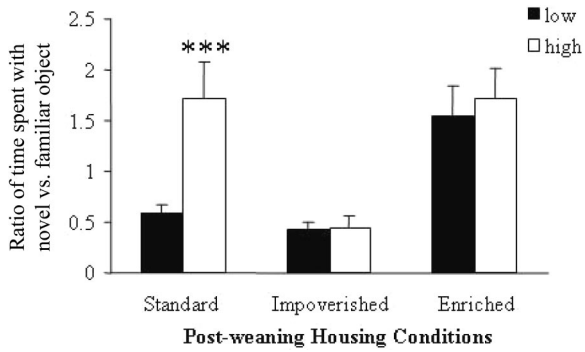

Figure 5. Mean ratio of time spent exploring a novel versus familiar object in a 5-min session observed in the adult male offspring of standard, impoverished, or enriched housed females, who were themselves the offspring of high and low licking/grooming (LG) mothers. Discrimination ratios of male offspring of high LG/standard housed females were elevated compared to the offspring of low LG females ($p < .001$). The male offspring of high LG/impoverished housed females were significantly less exploratory than offspring of high LG/standard housed females ($p < .001$). Error bars represent standard error of the mean. $^{***}p < .001$.

effect of group, $F(1, 42) = 5.8$, $p < .05$; a main effect of housing, $F(2, 42) = 29.0$, $p < .001$; and a significant Group × Housing Condition interaction, $F(2, 42) = 4.0$, $p < .05$ (see Figure 6). Post hoc analysis indicated that female offspring of high LG/standard housed females engaged in higher levels of LG than offspring of low LG/standard housed females ($p < .01$). Offspring of high LG/impoverished females exhibited significantly lower levels of LG compared to offspring of high LG/standard housed females ($p < .001$). Offspring of low LG/enriched females exhibited higher levels of LG than low LG/standard housed females ($p < .01$).

Discussion

These results provide evidence for the role of social conditions beyond the postnatal period in altering patterns of maternal care and thus offspring phenotype and illustrate the interaction between the effects of postnatal and postweaning environments. Thus, the female offspring of dams that provided low levels of LG stimulation toward pups during the 1st week postpartum period, if placed into socially enriched postweaning conditions, exhibited high levels of LG toward their own pups, and the offspring of high LG dams placed into social isolation during the postweaning period exhibited low levels of LG toward their offspring. However, amongst low LG females placed in social isolation and high LG females placed in social enrichment there was no change in phenotype. Moreover, these altered patterns of maternal care were also observed in subsequent offspring without manipulation of postweaning housing conditions (see Figure 6). Thus there was an environment-environment interaction through which postnatal and postweaning social interactions mediated variations in adult maternal care and shaped the transmission of these variations across generations (see Figure 7).

The patterns of behavior of these females in response to social environment are associated with altered levels of OTR binding in

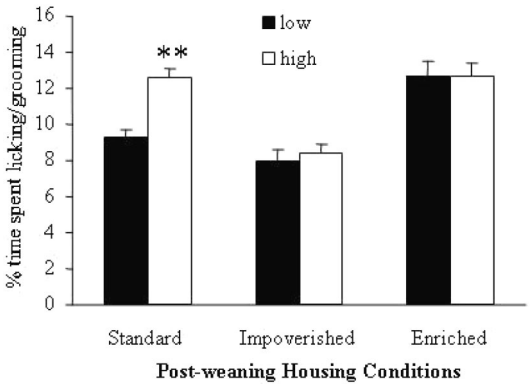

Figure 6. Mean percentage of time spent licking/grooming (LG) pups by the adult female offspring of standard, impoverished, or enriched housed females (who were themselves the offspring of high and low LG dams). Female offspring of high LG/standard housed females were higher in LG than offspring of low LG/standard housed females ($p < .01$). Offspring of high LG/impoverished females exhibited significantly lower levels of LG compared to offspring of high LG/standard housed females ($p < .001$). Offspring of low LG/enriched females exhibited higher levels of LG than low LG/standard housed females ($p < .01$). Error bars represent standard error of the mean. $^{**}p < .01$.

brain regions critical to maternal care (Numan, 1988; Stack, Balakrishnan, Numan, & Numan, 2002), such as the MPOA, and in those regions associated with indices of anxiety (Davis & Whalen, 2001), such as the PVN and amygdala. Previous studies examining the reversibility of postnatally induced changes in the neuroendocrine system through postweaning social enrichment have indicated that the underlying neural substrates are unaffected despite the behavioral changes observed (Francis, Diorio, Plotsky, & Meaney, 2002). However, both the peripheral and central oxytocin system display an incredible degree of plasticity as a normal process of the reproductive cycle in response to elevated hormone levels (Bale & Dorsa, 1997; Breton & Zingg, 1997; De Kloet, Voorhuis, & Elands, 1985; Montagnese, Poulain, Vincent, & Theodosis, 1988; Theodosis & Poulain, 1992). Estrogen is a potent up-regulator of OTR levels, and we have shown that the offspring of high and low LG mothers differ in estrogen sensitivity, particularly in the MPOA (Champagne et al., 2001; Champagne, Weaver, et al., 2003). Thus, in the offspring of low LG dams there is no dose response increase in OTR binding in the MPOA in response to increasing doses of estradiol. This lack of response is thought to be mediated by decreased levels of ERα expression in the MPOA of the offspring of low compared to high LG dams (Champagne, Weaver, et al., 2003). It is the decrease in ERα expression associated with decreased LG received in infancy that may generate stable individual differences in maternal care and mediate the transmission of these natural variations in behavior across generations. These transgenerational effects are associated with epigenetic regulation of the expression of ERα through differential DNA methylation of the promoter region of this gene during the postnatal period (Champagne et al., 2006). Though it is possible that epigenetic regulation of steroid receptors may account for postweaning effects on OTR binding, there are other mechanisms through which these changes could be achieved (Bale et al., 2001; Zingg et al., 1998). Previous studies have indicated that social isolation during the postweaning period results in decreased biosynthesis of estradiol and estrone in female rats (Fulgheri, Di Prisco, & Verdarelli, 1975). This suppression in estradiol may reduce increases in OTR binding in the hypothalamus that occur as females reach sexual maturity (Tribollet, Charpak, Schmidt, Dubois-Dauphin, & Dreifuss, 1989; Tribollet, Goumaz, Raggenbass, & Dreifuss, 1991) and prevent estrogen-induced up-regulation of OTR binding observed in high LG females during lactation, resulting in a phenotype that resembles that of a low LG dam. It is perhaps this plasticity in oxytocin neurons and receptors in response to environmental and hormonal cues that allows for the dynamic regulation of maternal care by the environment.

The effects of postnatal social isolation on maternal behavior that we have observed are consistent with those previously reported in both primates (Harlow et al., 1965; Ruppenthal, Arling, Harlow, Sackett, & Suomi, 1976) and rodents (Brunelli, Shindledecker, & Hofer, 1989; Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001; Lovic et al., 2001). In Harlow's artificial rearing studies, female infant rhesus macaques displayed impairments in maternal behavior as adults, engaging in high levels of abuse and neglect (Arling & Harlow, 1967; Harlow & Suomi, 1971; Seay, Alexander, & Harlow, 1964). Females exposed to social restriction rather than total deprivation spent less time in contact with their infants and were observed to nurse less frequently (Schapiro, Bloomsmith, Suarez, & Porter, 1995). In rodents, artificially reared

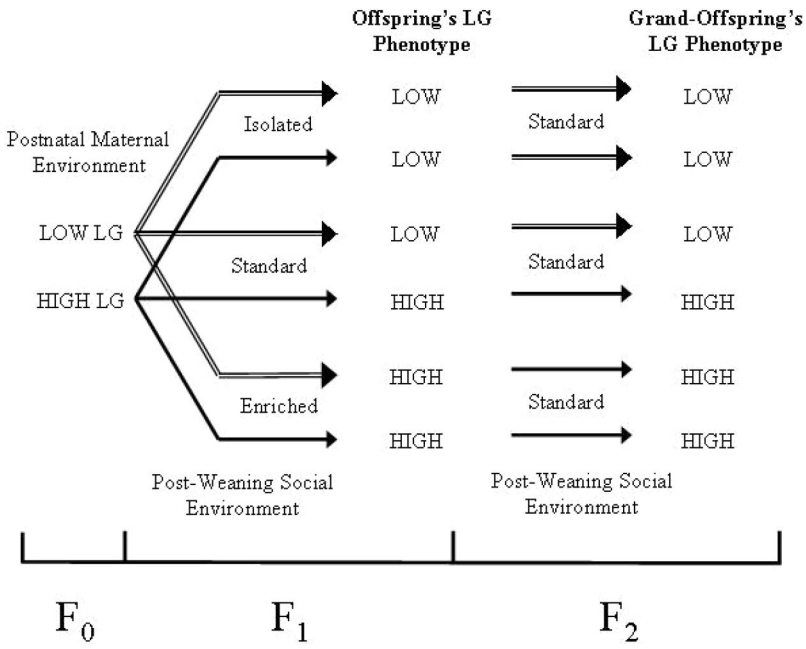

Figure 7. Summary of research design and results indicating the effects of postweaning social conditions on the transmission of maternal behavior across generations. In the grand-maternal generation (F_0), all females are housed under standard postweaning conditions. Offspring of these high and low licking/grooming (LG) females (F_1) are then weaned into three housing conditions that differ in the levels of social interaction provided. All F_1 females placed into social isolation were observed to be low LG, and all F_1 females placed into enriched social housing were observed to be high LG, regardless of the characteristics of the maternal phenotype of F_0. Under standard housing conditions, F_1 maternal phenotype is the same as F_0 maternal phenotype. Grand-offspring (F_2) are all housed under standard postweaning conditions and share the same LG phenotype as F_1.

females displayed deficits in maternal LG and contact toward their own pups (Fleming et al., 2002). Artificial rearing under social conditions (in which pups are raised with one peer) can ameliorate the deficits normally observed and produce females who provide adequate levels of maternal care (Gonzalez et al., 2001). The partial isolation of pups, using the maternal separation paradigm, has also been found to exert long-term consequences for maternal behavior (Boccia & Pedersen, 2001; Fleming et al., 2002; Ladd et al., 2000; Liu et al., 2000; Plotsky & Meaney, 1993; Pryce, Bettschen, & Feldon, 2001), with females showing deficits in maternal LG toward their own offspring (Fleming et al., 2002).

Taken together, these studies have illustrated the developmental impact of social contact, particularly between mother and infant. In humans, these effects are also evident. Infants raised in institutional settings have elevated cortisol levels and are at high risk for developmental disorders (Carlson & Earls, 1997; MacLean, 2003). Children who are raised in environments characterized by neglect show impairments in learning and memory, a heightened response to stress, and an increased risk of psychopathology (Briere & Runtz, 1988; Egeland, Sroufe, & Erickson, 1983; Trickett & McBride-Chang, 1995). Social contact appears to play an important role in regulating stress reactivity. In rats, examples of *socially facilitated behavior* (Clayton, 1978) include reduction in fearfulness (Davitz & Mason, 1955; Epley, 1974) and increased exploratory behavior (Simmel, 1962). In preadolescent rats, presence of familiar conspecifics reduces novelty-induced corticosterone levels (Terranova, Cirulli, & Laviola, 1999). Our findings of increased exploratory behavior and reduced behavioral indices of anxiety in females exposed to social enrichment during the juvenile period are consistent with this earlier work.

One of the issues addressed by the current study and previous work with postweaning environmental manipulation (Bredy, Humpartzoomian, Cain, & Meaney, 2003; Francis et al., 2002) is that of reversibility, i.e., can the effects of early environment be altered later in the course of development? Despite the severity of the impairments resulting from maternal separation and social isolation described in primates and rodents, there is evidence that reversal is possible through social stimulation. Socially isolated rhesus monkeys, placed into continuous contact with peers, began to show normal social interaction (Harlow et al., 1965). Likewise, females who were isolation reared and showed deficits in maternal behavior began to show more species-typical mother–infant interactions following prolonged contact with infants (Harlow & Suomi, 1971). Females could thus "recover," showing low levels of abuse and neglect toward subsequent offspring. The deficits in maternal behavior shown by socially restricted rhesus monkeys could also be overcome, either by allowing for social interaction with peers or, to some degree, by allowing females to observe mother–infant interactions of socially housed rhesus females (Schapiro et al., 1995). Thus, behavioral patterns that can be predicted from experiences occurring early in development can be altered through subsequent environmental conditions.

Plasticity in both maternal care and central OTRs in response to postnatal and postweaning environmental cues provides a potential

mechanism through which offspring phenotype can be adapted to the environmental conditions into which they will be reared. Under stressful conditions, such as those experienced during social isolation, decreases in maternal LG result in reduced exploratory behavior and decreased LG of offspring, consistent with previous studies in which gestational stress decreased maternal LG and central levels of OTR, with similar effects on subsequent generations (Champagne & Meaney, 2006). From an evolutionary perspective, these disruptive conditions would predict a highly variable environment in which a heightened behavioral response to stressful situations would allow an individual to respond more rapidly to environmental change and increase likelihood of survival. By altering the quality of mother–infant interactions, these adaptive behavioral responses can then be passed on to subsequent generations

The social environment experienced during development has a significant role in regulating adult maternal care and exploratory behavior. Our findings suggest that though mother–infant interactions during the postnatal period typically predict the adult phenotype of offspring, social environment experienced beyond this period can alter the predicted phenotype. Thus, offspring that have received reduced levels of care during the postnatal period and are thus predicted to be more inhibited of novelty and provide less care to their own offspring can "recover" in response to juvenile social enrichment. Likewise, juvenile social isolation can reduce exploration of a novel environment and decrease maternal care in those females who would be predicted to be exploratory and show high levels of maternal care toward their offspring based on the high level of care they received during the postnatal period. Our results suggest that environmental change occurring later in development can induce phenotypic changes in an organism, which interact with effects mediated by cues obtained from earlier periods of development, in this case providing an example of an environment-environment interaction. This developmental plasticity may produce an adult phenotype that is optimally suited to the environmental conditions of adulthood. The long-term effects of social experiences during the postnatal and postweaning period on maternal behavior create a mechanism through which traits can be "inherited" by subsequent generations (Gluckman, Hanson, Spencer, & Bateson, 2005). This transmission has implications not just for maternal behavior but for all aspects of phenotype that can be altered through mother–infant interactions.

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Correction to St. Andre and Reilly (2007)

In the article "Effects of Central and Basolateral Amygdala Lesions on Conditioned Taste Aversion and Latent Inhibition," by Justin St. Andre and Steve Reilly (*Behavioral Neuroscience,* 2007, Vol. 121, No. 1, pp. 90–99), Figure 4 on p. 96 (Results and Discussion, *Experiment 2: Behavioral* section) was incorrect. The correct figure is printed below.

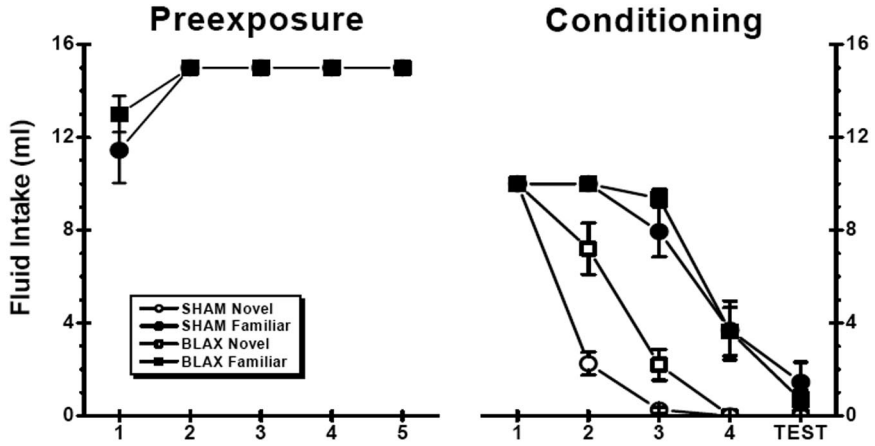

Figure 4. Mean (± *SE*) saccharin intake for the nonlesion (SHAM) control rats and for rats with bilateral *N*-methyl-D-aspartate lesions of the basolateral amygdala (BLAX) during the preexposure and conditioning phases of Experiment 2.

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Correction to Champagne and Meaney (2007)

In the article "Transgenerational Effects of Social Environment on Variations in Maternal Care and Behavioral Response to Novelty," by Frances A. Champagne and Michael J. Meaney (*Behavioral Neuroscience*, 2007, Vol. 121, No. 6, pp. 1353–1363), Figure 1 on Page 1356 was incorrect. The correct figure is printed below.

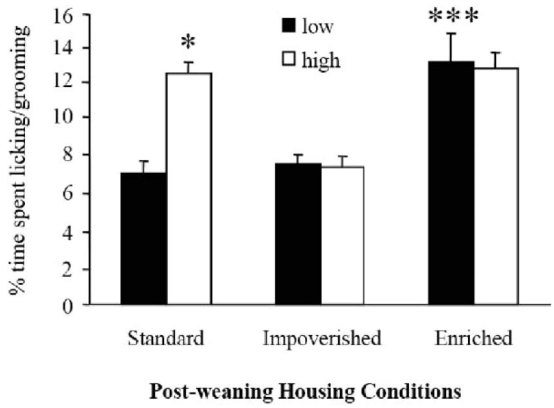

Figure 1. Mean percentage of time spent licking/grooming (LG) pups by the adult female offspring of high and low LG dams. Offspring were weaned into three housing conditions (standard, impoverished, or enriched) for 50 days, mated, and observed during the first 6 days postpartum. Analysis indicated that posweaning impoverishment and enrichment abolished group differences in maternal LG. High LG female offspring housed under impoverished conditions exhibited reduced levels of LG compared to standard housed high LG females ($p < .05$), whereas low LG female offspring housed under enriched conditions exhibited elevated levels of LG compared to standard housed low LG females ($p < .001$). Error bars represent standard error of the mean $^{*}p < .05$. $^{***}p < .001$.