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Maternal and Littermate Deprivation Disrupts Maternal Behavior and Social-Learning of Food Preference in Adulthood: Tactile Stimulation, Nest Odor, and Social Rearing Prevent These Effects

ABSTRACT: Maternal and littermate (social) separation, through artificial rearing (AR), disrupts the development of subsequent maternal behavior and social learning in rats. The addition of maternal-licking-like stimulation during AR, partially reverses some of these effects. However, little is known about the role of social stimuli from littermates and nest odors during the preweaning period, in the development of the adult maternal behavior and social learning. The purpose of this study was to examine the effects of peer- and peer-and-odor rearing on the development of maternal behavior and social learning in rats. Female pups were reared with mothers (mother reared—MR) or without mothers (AR) from postnatal day (PND) 3. AR rats received three different treatments: (1) AR-CONTROL group received minimal tactile stimulation, (2) AR-ODOR females received exposure to maternal nest material inside the AR-isolation-cup environment, (3) AR-SOCIAL group was reared in the cup with maternal nest material and a conspecific of the same-age and same-sex and received additional tactile stimulation. MR females were reared by their mothers in the nest and with conspecifics. In adulthood, rats were tested for maternal behavior towards their own pups and in a social learning task. Results confirm our previous report that AR impairs performance of maternal behavior and the development of a social food preference. Furthermore, social cues from a littermate, in combination with tactile stimulation and the nest odor, reversed the negative effects of complete isolation (AR-CONTROL) on some of the above behaviors. Exposure to the odor alone also had effects on some of these olfactory-mediated behaviors. These studies indicate that social stimulation from littermates during the preweaning period, in combination with odor from the nest and tactile stimulation, contributes to the development of affiliative behaviors.

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INTRODUCTION

For many mammals, the nest constitutes the complete environment of the newborn and is necessary for optimal growth and development. Early experiences acquired in the nest are critical for the normal development of almost all physiological, emotional, social, cognitive,

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neuroendocrine, and behavioral systems that underlie later responsiveness to stimuli and behavior (Barnett & Burn, 1967; Beach & Jaynes, 1954; Fairbanks, 1996; Fleming, O'Day, & Kraemer, 1999; Hofer, 1994; Lehmann & Feldon, 2000; Levine, Haltmeyer, Karas, & Denenberg, 1967). In rats, once the offspring (pups) emerge from the birth canal, the mother retrieves them to the nest, licks their bodies and anogenital region, and develops a nursing posture over them (Rosenblatt & Lehrman, 1963; Wiesner & Sheard, 1933). In this way, the pups receive warmth, nutrients, protection, and sensory stimulation in the form of social contact from the mother. On the other hand, the mother is exposed to and affected by cues coming from her offspring. Disruption of this interaction between the mother and pups, produces long lasting changes in the offspring's behavior and physiology (Hall, 1998; Hofer, 1994; Kuhn & Schanberg, 1998).

For example, rat pups reared in isolation, that is, without mother and littermates, using artificial rearing (AR) (maternal, litter, and nest deprivation), show deficits in maternal behavior toward their own offspring (Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001) and show reduced attentional abilities (Lovic & Fleming, 2004). Providing AR pups with stroking stimulation (tactile stimulation of their bodies and genitals with a paintbrush) during AR prevents these effects, thus indicating that early life stimulation plays an important role in the development of adult behaviors. Furthermore, AR rats display abnormal ongoing social behavior and social learning (Lévy, Melo, Galef, Madden, & Fleming, 2003). In a social recognition task, in comparison to mother-reared (MR) rats, AR rats do not show a distinction between a new and a previously presented juvenile. In a social food preference task, in contrast to MR rats, AR rats do not develop the usual preference for a diet previously associated with a conspecific (Lévy et al., 2003).

AR females also displayed deficits in a maternal memory task: 15 days after an interaction with their own pups at parturition AR females responded to test pups as though they had never experienced pups before, whereas MR rats responded to pups more rapidly (Lévy et al., 2003). While tactile stimulation ameliorates the negative effects of AR conditions on maternal behavior (Gonzalez et al., 2001), it has no effect on other forms of nonpup-related social learning (Lévy et al., 2003), indicating that other types of stimulation (e.g., other social and odor based) might be important for the development of adult social behaviors.

Indeed, for altricial animals, like rats, olfaction is the primary window to a new world and that influences much of the neonate's early behavior. Olfaction strongly shapes the expression of three vital social behaviors of the newborn: orientation, huddling, and suckling (Blass,

1986). The expression of these behaviors in turn, promotes a reorganization of the neonate's olfactory circuitry, which is responsive to odors from the mother, littermates, and the nest (Polan & Hofer, 1998; Wilson & Sullivan, 1994). This early olfactory learning within the nest is critical for aspects of social recognition, including age, sex, status, and reproductive condition of a conspecific and is the foundation on which all social relationships are built (e.g., Fillion & Blass, 1986; Shah, Oxley, Lovic, & Fleming, 2002; Wilson & Sullivan, 1994). For example, rat pups acquire a preference for olfactory stimuli experienced while they are suckling (Brake, Shair, & Hofer, 1981; Shah et al., 2002), while they are stimulated by stroking (Sullivan & Hall, 1988) or while they spend time in a warm (33°C) environment (Pedersen & Blass, 1981). These olfactory-based memories are retained over a long time (Shah et al., 2002).

The early preweaning experiences include not only experiences with the maternal and nest odors and with mothers' licking stimulation, but they also involve experiences acquired when pups are together and interacting contingently (Sokoloff & Blumberg, 2001). Within the nest, littermates huddle together and eventually groom one another and engage in play (Alberts, 1978, 1979; Alberts & May, 1984; Pankseep, 1981). Although the contact among the littermates during the preweaning period cannot be properly called social interaction, there are data that suggest that this social experience is important. Thus, before postnatal day (PND) 10, pups huddle together to keep warm and after huddling, they remain quiescent and lay close together, skin-to-skin (some researchers have referred to this phenomenon as quiescent behavior; Schank & Alberts, 2000). This behavior, also expressed after suckling, induces relaxation and sedation, and provides the pups a comfortable warm environment, which favors anabolic metabolism (Uvnäs-Moberg, 1997). Thus, manipulations that disrupt the contact of the pups with the littermates, like maternal separation, induce high levels of ultrasonic vocalization and agitate searching behavior (Hofer, 1994; Hofer & Shair, 1978; Hofer, Brunelli, & Shair, 1993).

Between PND 7 and 10, pups start to exhibit a "coupled activity" or synchronized activity (Schank & Alberts, 2000). Therefore, as development ensues, an individual's behavior becomes increasingly influenced by the behavior of its littermates, revealing what may be a basic component in the development of cooperative behavior and, indirectly, social behaviors (Calhoun, 1962). This hypothesis suggests that stimuli from conspecifics during the preweaning period, have an important role for the development of social behaviors.

The above results strongly suggest that social stimuli during early development play an important role in the development of different kinds of behavioral and

physiological systems, including social behaviors. In our previous studies, the addition of tactile stimulation, during isolation rearing, did not completely reverse the effects of isolation. Based on previous research, we have identified nest odors, maternal behaviors (hovering, licking etc.), and social interactions as significant nest stimuli. In the present study, we investigated the role of these factors (nest odor and social-tactile stimulation) during early life on the development of social behaviors. These included adult maternal behavior and social learning of food preferences.

In the first experiment, we investigated the role of nest odors, social stimulation, and tactile stimulation, associated with particular rearing conditions (see below) on maternal responsiveness to pups. In the second experiment, using a different cohort of rats, we tested the hypothesis that preweaning exposure to conspecifics and nest odors, or nest odors alone, during AR, would alter social food preference learning. These AR groups were compared to MR siblings.

METHODS

Subjects

Subjects were female offspring derived from primiparous 60–90-day old Sprague–Dawley female rats. Female rats were bred at the University of Toronto at Mississauga, from a stock originally from Charles Rivers Farm in St. Constant, Quebec. After mating, the dams were individually housed in Plexiglas cages (22 × 44 × 30 cm). The dams were provided with woodshavings and had ad libitum access to Purina Rat Chow and water. Room temperature was maintained at 24°C and humidity at 40–50%. Lights were off 2000–0800 hr. All the procedures described in this report conformed to the guidelines set by the Canadian Council on Animal Care and were approved by the Local Animal Care Committee, which supervises ethics protocols at the University of Toronto.

General Procedures

On the day of parturition (PND 0), litters were culled to seven females and four males. On PND 3, five female pups were removed from the nest and four of them were implanted with gastric cannulae (gastrostomy, see below) and a fifth was marked on its back with coloring and returned to the nest to be reared by its mother (mother reared-control; MR-CONTROL). Three of the four pups that were implanted with gastric cannulae were raised artificially (AR) from PND 3 to 18. On PND 18 to 22, AR rats were placed individually into small cages (15 × 22 × 10 cm) and given a diet consisting of mashed Purina Rat Chow and their rearing milk diet. The fourth pup, MR-SHAM, underwent gastrostomy, but the tube cut off just outside the skin and the pup was returned to the nest to be reared by its mother. MR-SHAM pups were marked with a color different from MR-CONTROL. All of the MR and AR groups were weaned on PND 22, by pairing subjects with same sex conspecifics (nonexperimental,

MR partners). Three female siblings that received gastric cannulae were randomly assigned to one of three conditions (see below; Treatments and Groups).

Pup Surgery

Detailed description of gastric cannulae implants and AR can be found elsewhere (Diaz, Moore, Petraccia, Schacher, & Stamper, 1981; Gonzalez et al., 2001; Hall, 1975). All pups were weighed and anesthetized in a bell jar with approximately 1–2 mL of methoxyflurane (Metofane, CDMV, Inc., Saint-Hyacinthe, Quebec). The surgery involved inserting a leader wire (stainless steel, .25 mm in diameter) sheathed in Silastic tubing (Down Corning) and PE-10 (Clay Adams) tube into the pup's mouth and down the esophagus. When the end of the leader was visible, through the translucent skin of the pup, the pup was held firmly and the leader was pushed from within the stomach through the lateral wall of the stomach. The rest of the gastrostomy tube was lubricated with oil and was pulled gently through the pup until the flanged end contacted the inside wall of the stomach. A washer was placed over the gastrostomy tube against the skin of the abdomen of the pup and held in place with a small amount of Superglue. Antibacterial cream (Neosporin) was applied topically at the sites of penetration. The implantation usually took no longer than 90 s and the pups awakened within 3–4 min.

Artificial Rearing

Following the gastrostomy, the pups were housed individually in plastic cups (11 cm in diameter × 20 cm deep), which fitted into a second cup floating in temperature-controlled water bath (maintained at 37°–39°C, depending on the age of pups). The cups were filled with corncob bedding (Bed O'Cobs) and the lids of the cups remained open to allow the gastrostomy tubing to emerge and to connect to nearby syringes containing milk formula. The pups were fed with an artificial milk; formula (Messer diet; Messer, Thoman, Galofre, Dallman, & Dallman, 1969; Smart, Stephens, & Katz, 1983) delivered from syringes mounted on programmable infusion pumps (Harvard Apparatus Syringe Pumps, PHD 2000). The pumps were programmed to infuse the diet for 10 min every hour, 24 hr daily. The amount of diet the pumps delivered was based on a specific fraction of the mean pup weight for the pumps. For the first day (PND 3), the amount was 33% of the mean body weight and was increased 1% per day, up to a maximum of 45% of mean body weight. Each day (morning), the pups were removed from the cups, weighed, and their tubing were flushed with distilled water. The syringes were replaced with new ones containing fresh diet. The pumps were recalibrated according to the new mean pup weight per pump.

Treatments and Groups

During AR, pups in the different AR groups received one of three following treatments: (1) AR-CONTROL, pups were stimulated twice a day, for 45 s, with a warm, wet paintbrush swiping their anogenital regions to stimulate urination, (2) AR-ODOR, pups were like AR-CONTROL pups, with an addition of 10 g of nest

material taken from their mother's cage, placed into the pups' cups (nest material was exchanged at 12-hr intervals), (3) AR-SOCIAL, pups were like AR-ODOR pups, with an addition of conspecific (same-age-sex from a different mother changed every 12 hr) to the AR cup; in addition, these pups received anogenital stimulation plus eight daily body stimulations with a paintbrush. The rationale for this was based on the findings that Lévy et al. (2003), partially reversed the effects of isolation by just giving the tactile stimulation. Hence, we sought to reverse completely the isolation effects by providing additional stimuli normally encountered in the nest, (4) MR-SHAM, and (5) MR-CONTROL, pups were reared by their own mothers. Two different cohorts of rats were raised and used in two, separate experiments (see below).

Statistical Analyses

In different studies, different sets of comparisons were undertaken. AR-CONTROL, AR-ODOR, AR-SOCIAL, and MR groups were compared (SHAM and CONTROL were combined as they were not different from each other). All comparisons were done using nonparametric statistics; Mann–Whitney *U*-tests and χ^2 tests for two group analyses and Kruskal–Wallis and χ^2 tests for four group analyses; *p*-values of $<.05$ were accepted as significant. Because the data did not always show homogeneity of variance and sample sizes were small, analyses were done using only nonparametric statistics, which were appropriate to these data.

STUDY 1: THE EFFECTS OF REARING EXPERIENCE ON ADULT MATERNAL BEHAVIOR

In this study, we explored the effects of maternal and littermate deprivation (AR), AR plus maternal nest odor cues, and AR plus conspecific cues (social)/tactile/maternal nest odor (see above) on maternal responsiveness to pups.

Groups and Procedures

All AR [AR-CONTROL group ($n = 4$), AR-ODOR group ($n = 4$), AR-SOCIAL group ($n = 5$)] and MR [MR-CONTROL group ($n = 9$), MR-SHAM group ($n = 7$)], rats were raised as described under General Procedures. When they were 60–100 days old, they were mated and those that became pregnant were housed singly in a 22 cm \times 44 cm \times 30 cm Plexiglas cage with nesting material. After parturition (Day 0), the litters were culled to 10 pups, 5 females, and 5 males. Females were tested for maternal behaviors during five tests (postpartum Days 2, 4, 6, 8, and 10) between 0900 hr and 1300 hr. On each test day, pups were removed from the nest for 2–4 min and weighed. Next as the pups were returned to the diagonally opposite corner to the nest, the 10-min test commenced.

During testing, the frequency and duration of the different components of maternal behavior (pup retrieval, pup genital and body licking, pup body sniffing, crouching (low and high) over the pups, mouthing the pups, nest-building, approaching towards the pups) and nonmaternal behaviors (e.g., grooming, eating, and sniffing air) were recorded, using the Best software (Educational Software, Inc., Las Vegas, NV) installed onto a laptop computer.

Results

AR-CONTROL, AR-ODOR, AR-SOCIAL Versus MR.

Maternal behaviors. To determine whether providing AR rats with additional odor or social stimulation would ameliorate the negative effects of isolation on maternal behavior, the four groups were compared using Kruskal–Wallis tests, followed by Mann–Whitney *U*-test to compare independent groups. Overall, the data suggest that the replacement of odor alone does not reverse significantly the effects of isolation from mother and the nest, whereas for some behaviors, replacement with a conspecific and associated stimulation, does.

There were group differences for time spent inside the nest ($\chi^2 = 7.7$, $df = 3$, $p < .05$), high crouching ($\chi^2 = 10.7$, $df = 3$, $p < .01$), and pup licking ($\chi^2 = 6.3$, $df = 3$, $p = .10$). Post hoc comparisons showed that compared to MR rats, AR-CONTROL rats spent less time engaging in these behaviors: inside the nest ($p < .04$), high crouching over pups ($p < .02$), and pup body licking ($p = .06$). MR rats were also different from the AR-ODOR group: time spent in nest ($p < .039$), body licking ($p < .04$), and nest-building ($p < .05$) (see Fig. 1). There were no differences between AR-ODOR and AR-CONTROL rats. In contrast,

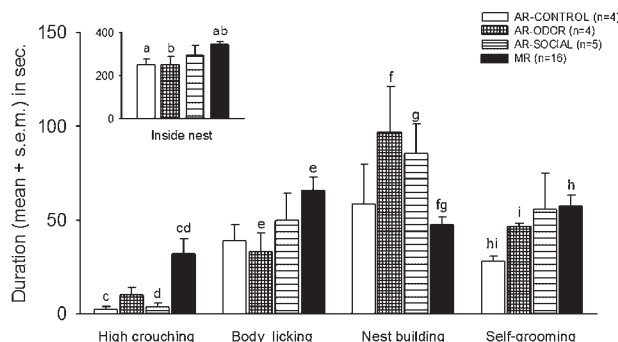


FIGURE 1 Effects of complete maternal and littermate deprivation on the duration of maternal behavior in AR rats, AR-CONTROL rats ($n = 4$), AR-ODOR rats ($n = 4$), AR-SOCIAL rats ($n = 5$), and MR animals ($n = 16$). Groups sharing the same superscript letter are significantly different from one another, $p < .05$.

the effects of social/tactile experience was reflected in the fact that AR-SOCIAL rats did not differ from MR rats in time in nest, body licking, genital licking, or crouching, although, they did show lower levels of high crouch ($p < .01$) and higher levels of nest-building ($p < .03$) than MR rats. AR-SOCIAL rats' behavior durations were intermediate between AR-CONTROL and MR, and hence also did not differ from other AR groups

Nonmaternal behaviors. AR-CONTROL group spent less time self-grooming than MR group ($p < .01$), and than the AR-ODOR group ($p < .02$; Fig. 1).

Discussion

These data suggest that social experiences in combination with tactile stimulation and nest odor during early rearing contribute to naturally occurring ongoing social behavior in adulthood, specifically maternal behavior. However, exposure to maternal nest odor alone was not adequate to reverse the isolation-induced deficits. Since all rats became maternal and showed quite adequate maternal behavior after parturition, the onset of maternal behavior does not seem to be affected by isolation rearing. Instead, both isolation rearing and the combination of social and tactile "replacement" seems to affect the intensity of maternal behaviors. Whether these effects are due to a disruption of mechanisms mediating maternal behavior per se, or to processes underlying other aspects of the behavioral repertoire, like attention, emotion, etc., which affects the expression of maternal behavior, is the focus of intensive study. We know, for instance, that AR affects the function and structure of cells within the medial preoptic area (MPOA), the final common path of maternal behavior (Gonzalez & Fleming, 2002). AR also produces deficits in attention (Lovic & Fleming, 2004), increases impulsivity (Lovic & Fleming, in prep) and activity levels (Gonzalez et al., 2001; Lovic, Fleming, & Fletcher, under review). It is possible that these behavioral changes could also contribute to the expression of maternal behavior. Which of these systems is most affected by the influences of early exposure to a conspecifics and/or extra tactile stimulation is not clear at this point, and awaits further study.

Unfortunately, in the present study, we did not include a tactile alone AR group. However, we have explored the effects of tactile stimulation alone in AR rats in previous studies (Gonzalez et al., 2001; Lovic & Fleming, 2004; Llinas, Gonzalez, & Fleming, in prep) and can, therefore, discuss the differences between early sensory experiences. The combination of social and tactile stimulation in the present study resulted in a level of

maternal licking that was greater than has been found for tactile stimulation alone in our previous studies. This suggests that the social stimulation component of the social/tactile (AR-Social) manipulation likely has effects over and above the effects of tactile stimulation alone. The fact that social stimuli/touch stimuli during isolation did not completely reverse the negative effects of isolation on maternal behavior, suggests either that social stimuli from conspecifics during the preweaning period plays only a small role in the development of maternal behavior, or that the intensity (or quantity) of the social stimuli received from a single conspecific was not sufficient and did not adequately simulate the effects of a full litter. The latter possibility is supported by evidence from preweaning social-housing studies that showed that the effect of social stimuli on development was greater when the experimental rat was reared with three or more conspecific than when reared with only one (Shishelova, 2000).

The next question was whether the effects of early social experience influences the expression of a social behavior, other than maternal behavior, with emphasis on the, encoding of social information. This question was addressed in the next study.

STUDY 2: THE EFFECTS OF REARING EXPERIENCE ON SOCIAL LEARNING ABOUT NEW FOODS

In this study, we explored the effects of maternal and littermate deprivation (AR), AR plus maternal nest odor cues, and AR plus conspecific cues (social)/tactile/maternal nest odor, on the ability of rats to learn about a new food associated with social conspecifics in adulthood (60–100 days old). Rats were tested on a social food preference task in which rats were exposed first to a novel food associated with a familiar conspecific on one day and then tested for food preferences between the "familiar" food previously consumed by the conspecific and a new food.

Groups and Procedures

AR-CONTROL ($n = 6$), AR-ODOR ($n = 8$), AR-SOCIAL ($n = 6$), and MR-CONTROL ($n = 10$), MR-SHAM ($n = 10$) subjects were administered a task to assess social learning of a food preference (Galef Social Food Learning Task; Galef & Wigmore, 1983). AR and MR rats were designated Observers, and conspecifics with whom the new foods were associated were designated Demonstrators.

Diets consisted of powdered Purina chow mixed with either 2% cocoa (COC, Hershey's unsweetened) or 1%

cinnamon (CIN, McCormicks). The diets were mixed 1 day before they were used. Demonstrator females were unrelated to subjects but had resided with the Observer rats for 2 days in a test cage ($40 \times 20 \times 18$ cm). Demonstrators were food deprived for 23 hr, removed to a room away from their respective Observer and fed either COC or CIN for 60 min. Observer rats were food deprived as well. Then, each Demonstrator was placed back into the test cage with the associated Observer where it remained for 30 min. Social interactions between Demonstrator and Observer rats were observed and recorded using the Best software installed onto a laptop computer. The social behaviors displayed by the Observer towards the Demonstrator that were recorded were as follows: partner face sniffing, partner body sniffing and playfighting. After removal of the Demonstrator, two food cups were placed into the front left and right corners of the test cage and counterbalanced across rats. Each dish contained 50 g of a scented chow diet, one of which was identical with the diet eaten by the Demonstrator. Observer rats were allowed to eat the two diets undisturbed for 2 hr at which time the two diets were weighed. The proportion of the total diet intake that was the same as the Demonstrator diet was computed for each rat (proportional intake of Demonstrator diet = Demonstrator diet intake/total food intake). Mann–Whitney *U*-tests comparing the MR and AR groups in their proportional Demonstrator intake were computed.

Results

Demonstrator Food Preference AR-CONTROL, AR-ODOR, AR-SOCIAL Versus MR. As can be seen in Figure 2, there was a significant difference in the percent of rats, across groups, showing a preference for the Demonstrator diet ($\chi^2 = 5.3$, $df = 3$, $p < .022$), with the

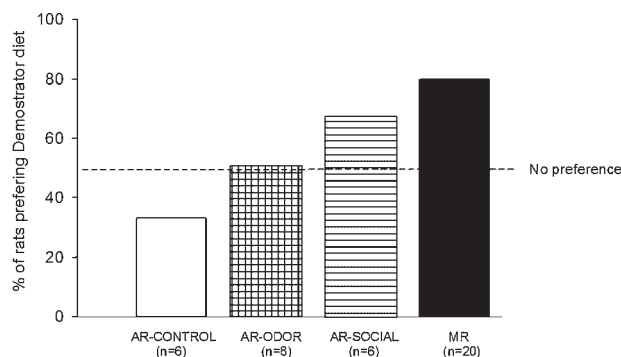


FIGURE 2 Effects of complete maternal and litter deprivation on the percent of rats in different groups preferring Demonstrator diet. AR-CONTROL rats ($n = 6$), AR-ODOR rats ($n = 8$), AR-SOCIAL rats ($n = 6$), and MR ($n = 20$). Overall $\chi^2 = 5.3$, $df = 3$, $p < .022$.

MR group showing the highest percent (80%), the AR-CONTROL the lowest (33%), and the AR-ODOR (50%) and AR-SOCIAL (67%) groups between the two. With respect to proportional intake of Demonstrator diet, overall group comparisons were in the predicted direction, but showed only a marginal effect ($p = .12$) (proportional intake of demonstrator/total: AR-CONTROL = .36; AR-ODOR = .57; AR-SOCIAL = .52; MR = .71).

Social Behaviors during Exposure Test. In order to determine whether there were any differences in social behavior among the groups during the period of exposure to the Demonstrator rat, the time that the Observer rat spent engaged in sniffing the Demonstrator rat's face or body, or in agonistic-like "playfight" with the Demonstrator rat was recorded. In order to test the effect of early social experience on social behavior/learning, MR group was compared to AR groups that received early social contact with a conspecific (AR-SOCIAL) and to those that had not (AR-CONTROL, AR-ODOR). Significant main effects of group were found for face sniffing ($F_{(3,32)} = 2.96$, $p < .047$; Fig. 3a) and playfighting ($F_{(3,32)} = 6.64$, $p < .001$; Fig. 3c). Post-hoc Mann–Whitney *U*-tests showed that AR-CONTROL rats spent significantly less time sniffing the face and the body of the Demonstrator in comparison to MR groups ($p < .01$, $p < .03$, respectively) and in comparison to the AR-SOCIAL group ($p < .03$, $p < .02$, respectively; Fig. 3a and b). Furthermore, the AR-CONTROL group spent less time sniffing the Demonstrator's rat body than did the AR-ODOR group ($p < .01$; Fig. 3b). In contrast, the AR-SOCIAL group and the AR-ODOR group spent significantly more time engaged in playfighting than did the MR group ($p < .005$; Fig. 3c).

Discussion

These data replicate previous results reported by Lévy et al. (2003) showing that AR early in life disrupts the normal development of processes involved in social learning food preference. These data extend the Lévy et al. (2003) study by showing that social cues from littermates, over and above, the condition of additional tactile stimulation, during the preweaning period participate in the development of this type of social learning. As we expected, the addition of social/odor/tactile cues reversed the negative effects of isolation: AR-SOCIAL rats behaved similarly to MR rats in their intake of Demonstrator diet and in their behavior toward the Demonstrator rats (face and body sniffing). The Lévy et al. (2003) study found that additional tactile stimulation, in the absence of social or odor cues, given to AR animals was not able to significantly reverse deficits in this type of social learning. This study also showed that being

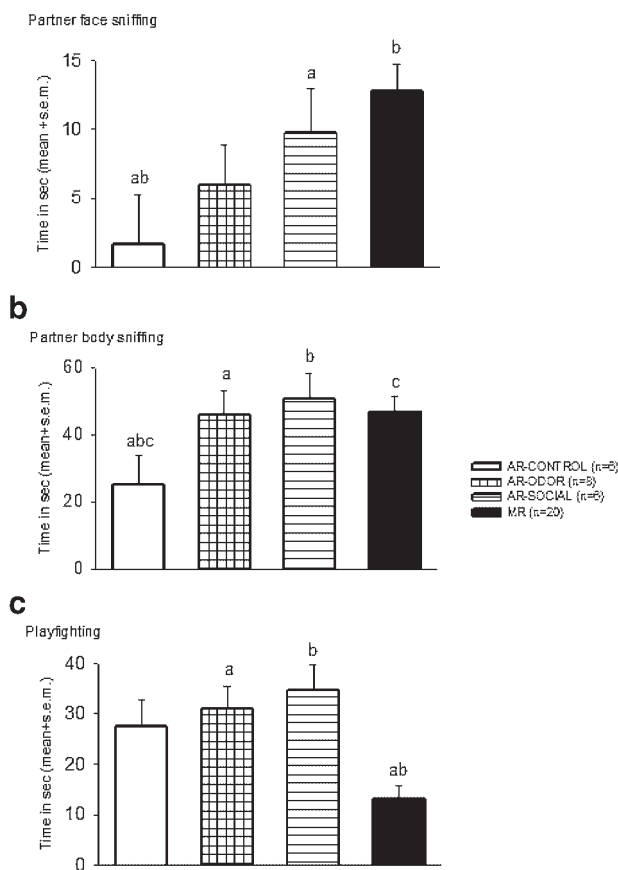
a Social Behaviors During Exposure Phase

FIGURE 3 Effects of complete maternal and litter deprivation on social interactions during exposure test in AR-CONTROL rats ($n = 6$), AR-ODOR rats ($n = 8$), AR-SOCIAL rats ($n = 6$), and maternally reared animals (MR; $n = 20$). (a) Time spent engaged sniffing the partner's face, (b) time spent sniffing the partner's body, and (c) time spent engaged playfighting against the Demonstrator female rat. Groups sharing the same super-script letter are significantly different from one another, $p < .05$.

exposed to the maternal and nest odors in the AR context also makes a contribution to social learning and partially enhances olfactory-based social learning in adulthood. In the odor-food learning task, the AR-ODOR rats spent more time sniffing the Demonstrator than did the AR-CONTROL group. Although they also sniffed less than AR-SOCIAL and MR, they did not differ from these groups on this measure. Moreover, in the overall analyses of proportion of animals showing a preference for the demonstrator associated foods, there was a clear overall difference among groups, and the effect seemed to be graded, with AR-CONTROL showing a tendency towards an "aversion," the AR-ODOR group having no specific preference, and the AR-SOCIAL, and MR groups showing increased numbers showing a preference.

Why would AR, isolated animals spend less time investigating the Demonstrator's face, than do MR rats? And why would the combination of social/ tactile and odor cues added into the inside of the cup reverse these negative effects on social investigation? Previously, we have found that AR produces an increase in locomotor activity (Gonzalez et al., 2001) and a reduction in prepulse inhibition, a measure of sensorimotor gating (an automatic aspect of attentional processes) (Lovic & Fleming, 2004). These deficits were reversed with additional tactile stimulation provided to AR rats. Deficits in sensorimotor gating can be interpreted as attentional deficits due to poor filtering of irrelevant stimuli, which in turn causes insufficient processing of relevant and salient stimuli. Hence, it is possible that AR rats, due to increased locomotor activity and poor filtering abilities, have poor attentional abilities that result in less attentional resources being directed towards the relevant stimuli, that is, the Demonstrator rat. In fact, less attention was paid to the conspecific. It is possible that this reduction in attention produced, as a result, deficits in learning about relevant stimuli, the odor of a novel food. The addition of a conspecific and tactile stimulation during the isolation period ameliorated the negative effects on these attention-based processes. However, we cannot speculate what the mechanism of effects of early life stimuli on adult attentional processes.

GENERAL DISCUSSION

The present results confirm the previously reported findings that isolation rearing in the AR condition, in the absence of additional stimulation, results in changes in adult maternal behavior (Gonzalez et al., 2001) and in adult social learning (Lévy et al., 2003). This study extends these earlier findings by showing that if isolated rats are reared in the presence of a same-sex and same-age pups, in combination with additional odor and tactile stimulation, they show fewer deficits in both their maternal licking and in their ability to learn about conspecific-associated cues. Whereas in previous studies, additional tactile stimulation to isolated animals (AR-MAX) partially reversed isolation-induced deficits in maternal behavior (Gonzalez et al., 2001), it did not reverse the deficit in social learning (Lévy et al., 2003). In this study, the addition of a peer experience added to the tactile stimulation and nest odor, clearly reversed the deficits in social learning and AR-SOCIAL animals were for the most part indistinguishable from the MR controls. Although maternal behavior (inside nest and body licking) of AR-SOCIAL females were not significantly different among AR-CONTROL and MR, the social rearing showed a tendency to be closer to MR

than AR-CONTROL. As well, for social learning, but not for the expression of maternal behavior, prior exposure to the maternal nest odor in AR condition (AR-ODOR) had long-term effects and enhanced aspects of the olfactory processing that precedes social learning. The odor-exposed AR animals sniffed the unfamiliar conspecific more than did the non-odor-exposed AR-CONTROL group. Furthermore, AR-ODOR females displayed an intermediate response in the social learning task (Fig. 2).

Although these studies clearly show that stimuli from the mother and from the littermates, during the postnatal period (PND 3–18), have an important role on the development of maternal behavior and social learning, we do not know what sensitive period within the postnatal timeframe is most relevant to these effects.

Reasons for the effectiveness of early social experience on later social learning may be explained at both a behavioral and psychological level as well as by alterations in physiology. At a behavioral level, pups reared with conspecific experience displayed a variety of behaviors not seen in socially isolated pups. While in the cup, pups huddle together and move in relation to one another. During the first 10 or so days, huddling, which is a prominent behavior normally seen in the litter situation (Alberts, 1978, 1979), provides a means of regulating body temperature as well as providing additional somatosensory stimulation as pups move across, and burrow into, one others' bodies. Although we have not done microanalysis of the actual interactions between pups in the cup (AR-SOCIAL group), we had the impression that they tended to move more than did isolated pups (AR-CONTROL group) and were less often stationary. After Day 10, once body hair appeared and eyes and ears had opened, pups were more likely to explore the conspecific and to even try to grasp and suckle on them. Unfortunately, we have not yet established the critical or sensitive period for the effects of AR on later behavior.

What do these experiences do to pups to enhance their later cognitive and social behavior? While we do not know the extent to which isolated and socially reared AR pups differ during the preweaning period, we know that they are quite different already during the early juvenile period, immediately after weaning. In a recently completed study, we have found that in comparison to AR-isolated rats (AR-CONTROL rats in these studies), AR-SOCIAL rats are more interactive and engage in less nonsocial behaviors, while with younger pups (preliminary data). These data suggest that the benefits of social rearing are already seen during the juvenile period and extend into adulthood (Fleming et al., 2002). Although we have yet to test the hypothesis directly, we predict that preweaning social rearing in rats has the effect of reducing rats' neophobia. It has been proposed that this occurs under

normal mother-reared conditions (Levine, 2001). Also, it is possible that social rearing may familiarize rats with their species-characteristic olfactory, visual, auditory, and somatosensory cues (Gheusi, Goodall, & Dantzer, 1997; Wilson & Sullivan, 1994), needed for normal development of social behavior.

There are several studies relating to the importance of social cues during early postnatal period on the development of the pups. Some of these studies have shown that "passive behaviors" between littermates have short-term effects during the preweaning period: partial maternal separation during 3 to 14 PNDs increases vocalization (Carden & Hofer, 1992; Hofer & Shair, 1978; Hofer, Brunelli, & Shair, 1993) and decreases milk intake (Johanson & Hall, 1981). Social interactions with littermates ameliorates the above effects. In addition, rearing in the presence of littermates reduces isolation-induced heightened activity in the 15-day-old pup (Randall & Campbell, 1976) and facilitates the learning of the passive avoidance (Smith & Spear, 1978). These data, and the results of the present studies, suggest that preweaning socialization reverses the negative effects of partial and total isolation on behavioral and cognitive systems. However, not all studies have shown that the presence of littermates can ameliorate the effects of maternal deprivation. For example, Cirulli, Gottlieb, Rosenfeld, and Levine (1992) experiments have shown that nutrition is more important than the presence of littermates, especially later on the preweaning period, with respect to regulation of stress responsiveness. Hence, the presence of littermates, during maternal deprivation, probably has selective effects that depend on a number of factors.

Alternatively, reductions in tactile, thermal, auditory, and other sensory stimuli, as a result of maternal and littermate deprivation, could disturb the normal development of sensory systems that process social cues; hence, the abnormalities in maternal behaviors and social learning in AR-CONTROL rats may be the result of deficits in the sensory processing of socially relevant cues. Experimental stimuli, in pups, such as brushing of a leg or stroking, lowers blood pressure, decreases electrical activity in the sympathoadrenal nerve, decreases levels of catecholamines in blood, and increases activity in the vagal nerve, reflected by the release of gastrointestinal hormones, thus promoting anabolic metabolism and growth (Araki, Ito, Kurosawa, & Sato, 1984; Kurosawa, Suzuki, Utsugi, & Araki, 1982; Uvnäs-Moberg, Lundeberg, Bruzelius, & Alster, 1993). These physiological effects are accompanied by decreased locomotor activity in open field (Uvnäs-Moberg et al., 1996). So, it is possible that the effects of isolation in AR-SOCIAL females were reversed by the tactile stimuli obtained from the conspecific (in addition to experimenter-provided stimulation).

As mentioned above, we have previously found that AR produces increased locomotor activity in the open field (Gonzalez et al., 2001) and deficits in the sensorimotor gating, an automatic aspect of attention (Lovic & Fleming, 2004). These effects were ameliorated in AR pups provided with extra tactile stimulation. Furthermore, Lovic and Fleming (2004) reported a positive correlation between measures of attention and maternal behavior. In the present experiments, rats that were maternally deprived early in life spent less time taking care the pups (Experiment 1), and sniffing the body and the face of Demonstrator rats (Experiment 2). Therefore, it is entirely possible that deficits observed in the present study are due to an increased locomotor activity and poor sensorimotor gating, both of which could lead to a reduction of allocation in attentional resources. Although it is unknown which mechanisms of attention are affected by our manipulations, the above data support the hypothesis that the effects found in this study are due to the reduction in attentional resources.

Another system that could be involved in the behavioral abnormalities of maternal deprivation is oxytocin. It has been shown that oxytocin facilitates bonding, attachment, and increases the amount of social contact between animals (Witt, Winslow, & Insel, 1992). Oxytocin is released by the activation of somatosensory afferents in response to social contact and grooming in adult rats. Thus, there are several types of nonnoxious stimuli such as touch (stroking), warm temperature, vibration, and electroacupuncture that increase oxytocin levels in plasma and in cerebrospinal fluid (Agren, Lundeberg, Uvnäs-Moberg, & Sato, 1995; Uvnäs-Moberg et al., 1993). The fact that social contact during isolation reverses some of the social negative effects in adulthood, suggest that stimuli from the partner inside the cup could induce the release of endogenous oxytocin, and in this way reverse the negative effects of isolation. Consistent with this interpretation are the data of Francis, Young, Meaney, and Insel (2002) showing that pups that received high levels of licking stimulation early on have, as adults, higher densities of oxytocin receptors in brain. Furthermore, adult female rats who receive injection of a oxytocin antagonist (i.p.) on their first day of birth, show decreased pituitary oxytocin levels (Young, Carter, Cushing, & Caldwell, 2005). The same treatment in monogamous prairie vole causes a low partner preference in adults (Bales & Carter, 2003) and a reductions in parental care in juveniles (Bales, Pfeifer, & Carter, 2004). Acute effects of oxytocin have been reported in female prairie voles on vocalizations. So, a single injection of an oxytocin antagonist on PND 1, decreased vocalization while repeated treatment (from 1 to 7 PNDs) produced an increased in vocalization in response to social isolation in pups of 8 days old (Kramer, Cushing, & Carter, 2003).

In summary, these data support the hypothesis that stimulation by the mother and littermates, which would normally occur in the nest context, has effects on the development of behavioral systems involved in the regulation of maternal and social-learning behaviors. AR precludes these effects, whereas “replacement” tactile and social stimulation partially reinstates them.

NOTES

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REFERENCES

- Agren, G., Lundeberg, T., Uvnäs-Moberg, K., & Sato, A. (1995). The oxytocin antagonist 1-deamino-2-D-Tyr (Oet)-4-Thr-8-Orn oxytocin reverses the increase in the withdrawal response latency to thermal, but not mechanical nociceptive stimuli following oxytocin administration or massage-like stroking in rats. *Neuroscience Letter*, 187, 49–52.
- Alberts, J. R. (1978). Huddling by rat pups: Group behavioral mechanisms of temperature regulation and energy conservation. *Journal of Comparative Physiology Psychology*, 92, 231–240.
- Alberts, J. R. (1979). Huddling by rat pups: Multisensory control of contact behavior. *Journal of Comparative Physiology Psychology*, 92, 220–226.
- Alberts, J. R., & May, B. (1984). Nonnutritive, thermotactile induction of filial huddling in rat pups. *Developmental Psychobiology*, 17, 161–181.
- Araki, T., Ito, K., Kurosawa, M., & Sato, A. (1984). Responses of adrenal sympathetic nerve activity and catecholamine secretion to cutaneous stimulation in anesthetized rats. *Neuroscience*, 12, 289–299.
- Bales, K. L., & Carter, C. S. (2003). Developmental exposure to oxytocin facilitates partner preferences in males prairie voles (*Microtus ochrogaster*). *Behavioral Neuroscience*, 117, 854–859.
- Bales, K. L., Pfeifer, L. A., & Carter, C. S. (2004). Sex differences and developmental effects of manipulations of oxytocin on alloparenting and anxiety in prairie voles. *Developmental Psychobiology*, 44, 123–131.
- Barnett, S. A., & Burn, J. (1967). Early stimulation and maternal behavior. *Nature*, 14, 150–152.
- Beach, F. A., & Jaynes, J. (1954). Effects of early experience upon the behavior of animals. *Psychological Bulletin*, 51, 239–263.
- Blass, E. M. (1986). The development of olfactory control over behavior. In: W. T. Greenough & J. M. Juraska (Eds.). *Developmental Neuropsychobiology, Behavioral Biology* (pp. 423–447), An international Series. London: Academic Press, Inc., Harcourt Brace Jovanovich, Publishers.

- Brake, S. C., Shair, H., & Hofer, M. A. (1981). Exploiting the nursing niche, sucking and feeding in the context of the mother-infant interaction. In: E. M. Blass (Ed.), *Developmental Psychobiology and Behavioral Ecology and Book of Behavioral Neurobiology*. (Vol. 9, pp. 347–388). New York & London: Plenum Press.
- Carden, S. E., & Hofer, M. A. (1992). Effect of a social companion in the ultrasonic vocalization and contact responses of 3-day-old rat pups. *Behavioral Neuroscience*, 106, 421–426.
- Calhoun, J. G. (1962). *The ecology and sociology of the Norway rat*. Bethesda, MD: Department of Health, Education and Welfare.
- Cirulli, F., Gottlieb, S. L., Rosenfeld, P. & Levine, S. (1992). Maternal factors regulate stress responsiveness in the neonatal rat. *Psychobiology*, 20(2), 143–152.
- Diaz, J., Moore, E., Petraccia, F., Schacher, J., & Stamper, C. (1981). Artificial rearing of preweaning rats: The effectiveness of direct intragastric feeding. *Physiology & Behavior*, 27, 1103–1105.
- Fairbanks, L. A. (1996). Individual differences in maternal style: Cause and consequences for mothers and for offspring. In J. S. Rosenblatt & C. T. Snowdon (Eds.), *Advances in the study of behavior* (pp. 579–611). San Diego: Academic Press.
- Fillion, T. J., & Blass, E. M. (1986). Infantile experience with suckling odors determines adult sexual behavior in male rats. *Science*, 231(4739), 729–731.
- Fleming, A. S., O'Day, D. H., & Kraemer, G. W. (1999). Neurobiology of mother-infant interactions: Experience and central nervous system plasticity across development and generations. *Neuroscience*, 23, 673–685.
- Fleming, A. S., Kraemer, G. W., Gonzalez, A., Lovic, V., Ress, S., & Melo, A. I. (2002). Mothering begets mothering: The transmission of behavior and its neurobiology across generations. *Pharmacology Biochemistry & Behavior*, 73, 61–75.
- Francis, D. D., Young, L. J., Meaney, M. J., & Insel, T. R. (2002). Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: Gender differences. *Journal of Endocrinology*, 14(5):349:353.
- Galef, B. G. J., & Wigmore, S. W. (1983). Transfer of information concerning distant foods: A laboratory investigation of the “information-centre” hypothesis. *Animal Behavior*, 20, 11–14.
- Gheusi, G., Goodall, G., & Dantzer, R. (1997). Individually distinctive odours represent individual conspecifics in rat. *Animal Behavior*, 53, 935–944.
- Gonzalez, A., & Fleming, A. S. (2002). Artificial rearing causes changes in c-fos expression in juvenile female rats. *Journal of Neurosciences*, 116, 999–1013.
- Gonzalez, A., Lovic, V., Ward, G. R., Wainwright, P. E., & Fleming, A. S. (2001). Intergenerational effects of complete maternal deprivation and replacement stimulation on maternal behavior and emotionality in female rats. *Developmental Psychobiology*, 38, 11–32.
- Hall, F. S. (1998). Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences. *Critical Reviews in Neurobiology*, 12, 129–162.
- Hall, W. G. (1975). Weaning and growth of artificially reared rats. *Science*, 397, 726–735.
- Hofer, M. A. (1994). Early relationships as regulators of infant physiology and behavior. *Acta Paediatric Supplement*, 397, 9–18.
- Hofer, M. A., Brunelli, S., & Shair, H. (1993). The effects of 24-hr maternal separation and of litter size reduction on the isolation-distress response of 12-day-old rat pups. *Developmental Psychobiology*, 26, 483–497.
- Hofer, M. A., & Shair, H. (1978). Ultrasonic vocalization during social interaction and isolation in 2 week old rats. *Developmental Psychobiology*, 11, 495–504.
- Johanson, I. B., & Hall, W. G. (1981). The ontogeny of feeding: V. Influence of texture, home odor, and sibling presence on ingestive behavior. *Journal of Comparative Physiology Psychology*, 95, 837–847.
- Kramer, K. M., Cushing, B. S., & Carter, C. S. (2003). Developmental effects of oxytocin on stress response: Single versus repeated exposure. *Physiology & Behavior*, 79, 775–782.
- Kuhn, C. M., & Schanberg, S. M. (1998). Responses to maternal separation: Mechanism and mediators. *International Journal of Development & Neuroscience*, 16, 261–270.
- Kurosawa, M., Suzuki, A., Utsugi, K., & Araki, T. (1982). Response of adrenal effect nerve activity to non-noxious mechanical stimulation of the skin in rats. *Neuroscience Letter*, 34, 295–300.
- Lehmann, J., & Feldon, J. (2000). Long-term biobehavioral effects of maternal separation in the rat: Consistent or confusing? *Reviews in Neurosciences*, 11, 383–408.
- Levine, S. (2001). Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. *Physiology & Behavior*, 73, 255–260.
- Levine, S., Haltmeyer, G. C., Karas, G. G., & Denenberg, V. H. (1967). Physiological and behavioral effects of infantile stimulation. *Physiology & Behavior*, 2, 55–59.
- Lévy, F., Melo, A. I., Galef, B. G. Jr., Madden, M., & Fleming, A. S. (2003). Complete maternal deprivation affects social, but not Spatial, learning in adult rats. *Developmental Psychobiology*, 43, 177–191.
- Lovic, V., & Fleming, A. S. (2004). Artificial-rearing female rats show reduced prepulse inhibition and deficits in the attentional set shifting task-reversal of effects with maternal-like licking stimulation. *Behavioral Brain Research*, 148, 209–219.
- Messer, M., Thoman, E. B., Galofre, A., Dallman, T., & Dallman, P. R. (1969). Artificial feeding of infant rats by continuous infusion. *Journal of Nutrition*, 98, 404–410.
- Pankseep, J. (1981). The ontogeny of play in rats. *Developmental Psychobiology*, 14, 327–332.
- Pedersen, C. A., & Blass, E. (1981). Olfactory control over suckling in albino rats. In: R. N. Aslin, J. R. Alberts, & M. R. Petersen (Eds.), *The development of perception: Psychobiological perspectives* (pp. 359–381). New York: Academic Press.

- Polan, H. J., & Hofer, M. A. (1998). Olfactory preference for mother over home nest shavings by newborn rats. *Developmental Psychobiology*, 33, 5–20.
- Randall, P.K., & Campbell, B. A. (1976). Ontogeny of behavioral arousal in rats: Effect of maternal and sibling presence. *Journal of Comparative Physiology Psychology*, 90, 453–459.
- Rosenblatt, J. S., & Lehrman, D. S. (1963). Maternal behavior of the laboratory rat. In: H. L. Rheingold (Ed.), *Maternal Behavior in Mammals*, (pp 8–57), New York: Wiley.
- Schank, J. C., & Alberts, J. R. (2000). The developmental emergence of coupled activity as cooperative aggregation in the rat pups. *Proceedings of the Royal Society London B*, 267, 2307–2315.
- Shah, A., Oxley, G., Lovic, V., & Fleming, A. S. (2002). Effects of preweaning exposure to novel maternal odors on maternal responsiveness and selectivity in adulthood. *Developmental Psychobiology*, 41, 187–196.
- Shishelova, A. (2000). The socially enriched environment in early age alters the exploratory activity and ability for learning in rats. *Zh Vyssh Nerv Deiat Im I P Pavlova* 50, 667–675.
- Smart, B. J. L., Stephens, D. N., & Katz, H. B. (1983). Growth and development of rat artificially reared on a high or a low plane of nutrition. *British Journal of Nutrition*, 49, 497–506.
- Smith, G. J., & Spear, N. E. (1978). Effects of the home environment on withholding behaviors and conditioning in infant and neonatal rats. *Science*, 202, 327–329.
- Sokoloff, G., & Blumberg, M. S. (2001). Competition and cooperation among huddling infant rats. *Developmental Psychobiology*, 39, 65–75.
- Sullivan, R. M., & Hall, W. G. (1988). Reinforcers in infancy: Classical conditioning using stroking or intraoral infusions of milk as UCS. *Developmental Psychobiology*, 21, 215–223.
- Uvnäs-Moberg, K. (1997). Physiological and endocrine effects of social contact. *Annals of New York Academic of Sciences*, 807, 146–163.
- Uvnäs-Moberg, K., Alster, P., Lund, I., Lundeberg, T., Kurosawa, M., & Ahlenius, S. (1996). Stroking of the abdomen causes decreased locomotor activity in conscious male rats. *Physiology & Behavior*, 60, 1409–1411.
- Uvnäs-Moberg, K., Bruzelius, G., Alster, P., & Lundeberg, T. (1993). The antinociceptive effect of non-noxious sensory stimulation is partly mediated through oxytocinergic mechanisms. *Acta Physiologica Scandinavia*, 149, 199–204.
- Wilson, D. A., & Sullivan, R. M. (1994). Neurobiology of associative learning in the neonate: Early olfactory learning. *Behavioral Neural Biology*, 61, 1–18.
- Witt, D. M., Winslow, J. T., & Insel, T. R. (1992). Enhanced social interactions in rats following chronic, centrally infused oxytocin. *Pharmacology, Biochemistry & Behavior*, 43, 855–886.
- Wiesner, B. P. & Sheard, N. M. (1933). *Maternal behavior in the rat*. Edinburgh, London: Oliver & Boyd.
- Young, E., Carter, C. S., Cushing, B. S., & Caldwell, J. D. (2005). Neonatal manipulation of oxytocin alters oxytocin levels in the pituitary of adult rats. *Hormone, Metabolites & Reseachers*, 37, 397–401.