

F. Lévy

Equipe Comportement
Station PRC
UMR 6073 INRA/CNRS/Université
de Tours
37380 Nouzilly, France

A. I. Melo

Centro de Investigación en Reproducción
Animal, CINVESTAV-UAT
Apdo. Postal 62
Tlaxcala, Tlax. 90000, Mexico

B. G. Galef, Jr.

Department of Psychology
McMaster University
Hamilton, Ontario
L8S 4K1, Canada

M. Madden

A. S. Fleming

Department of Psychology
University of Toronto
Mississauga, Ontario
L5L 1C6, Canada

E-mail: afleming@credit.erin.utoronto.ca

Complete Maternal Deprivation Affects Social, but not Spatial, Learning in Adult Rats

ABSTRACT: The effects of maternal deprivation on learning of social and spatial tasks were investigated in female adult rats. Pups were reared artificially and received "lickinglike" tactile stimulation (AR animals) or were reared with their mothers (MR animals). In adulthood, subjects were tested on paradigms of spatial learning and on paradigms involving learning of social cues. Results showed that maternal deprivation did not affect performance on spatial learning, but it did impair performance on the three social learning tasks. The AR animals made no distinction between a new and a previously presented juvenile conspecific. AR animals also responded less rapidly than MR animals at test for maternal behavior 2 weeks after a postpartum experience with pups. Finally, AR animals did not develop a preference for a food previously eaten by a familiar conspecific whereas MR animals did. This study indicates that animals reared without mother and siblings show no deficits in spatial tasks while showing consistent deficits in learning involving social interactions. © 2003 Wiley Periodicals, Inc. *Dev Psychobiol* 43: 177–191, 2003.

Keywords: maternal deprivation; social learning; spatial learning; artificial rearing; maternal behavior

INTRODUCTION

In altricial mammals, the mother and littermates provide a rich stimulus environment that shapes early physiological and cognitive development and later social behavior. Natural variations or active manipulations of the infant–mother relationship have been demonstrated to yield long-term variations in the neurobiology or behavior of the offspring in many species, including monkeys (Berman, 1990; Fairbanks, 1996; Kraemer, 1992, 1997; Maestripieri, Wallen, & Carrol, 1997) and humans (O'Connor & Rutter, 2000; Rutter, Kreppner, & O'Connor, 2001; Scarr & McCartney, 1983; Scarr & Weinberger, 1983). However, not surprisingly, the effects of early experience on

adult behavior have been most intensively explored in rats, where development is rapid, mechanism can be easily studied, and generalizability of effects to other species has been shown (Fleming & Li, 2002; Fleming, O'Day, & Kraemer, 1999).

In rats, early handling consisting of brief daily separation of pup from the mother has consistently been reported to produce robust behavioral effects in young adult rats, including decreased fear-related behavior (Nunez et al., 1995, 1996), and increased selective attention (Weiner, Schnabel, Lubow, & Feldon, 1985). As well, early handling prevents the age-related decline in spatial learning and memory performance in the water maze (Meaney, Aitken, Bhatnagar, & Sapolsky, 1991; Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988).

A different type of postnatal, preweaning manipulation, maternal separation (MS), either in a single 24-hr period or repeated 3- to 6-hr periods, is reported to yield multiple long-term effects in adulthood (Pryce & Feldon, 2003). In comparison to unmanipulated animals, animals that experienced single or repeated separations from mother show increased fear-related behavior (Patchev

Received 11 December 2002; Accepted 22 May 2003

Correspondence to: A. S. Fleming

Contract grant sponsor: NSERC and INRA

Published online in Wiley InterScience

(www.interscience.wiley.com). DOI 10.1002/dev.10131

© 2003 Wiley Periodicals, Inc.

et al., 1997), increased anxiety (Penke et al., 2001), disruption of attentional processes (Ellenbroek & Cools, 1995), and deficits in active avoidance (Lehmann, Pryce, Bettschen, & Feldon, 1999) and spatial learning (Oitzl, Workel, Fluttert, Frosch, & De Kloet, 2000).

In the rat, however, the extent of effects of separation is not always consistent; it depends on a number of factors, including duration of the preweaning separation, timing of separation, number of separations, whether separation is from mother alone or the littermates, gender of the animal, and type of control or comparison group used (for review, see Lehmann & Feldon, 2000). For instance, 3 hr of daily separation from Day 1 to Day 14 induced no effects (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000) whereas in different studies a 24-hr maternal deprivation reduced (Suchecki, Duarte, & Tufik, 2000) or increased (Penke et al., 2001) levels of anxiety in an open-field test.

With respect to the effects on learning, maternal separation also produces variable effects. In a 24-hr maternal-deprivation protocol, adult spatial learning measured in a water maze was disrupted when separation occurred at Day 3 when the pups were removed from the nest and the dam was left with half of the litter (Oitzl et al., 2000), but was improved when separation occurred at Day 9 and when the dam, rather than the pups, were removed from the nest (Lehmann et al., 1999). Other studies, using the water maze task, reported either no change when pups were separated daily from their mothers for 6 hr from Days 12 to 18 (Lehmann et al., 2002) or an improvement when the same kind of separation occurred between Days 15 to 21 (Frisone, Frye, & Zimmerberg, 2002).

Although not always consistent, the maternal separation or deprivation paradigms clearly can have long-term behavioral effects. Less clear is to what to attribute these effects—whether to the absence of mother and nest-related cues during the separation period, to the behavior of the mother on reunification of mother and pups, or to “stress effects.” As well, whether the control group is a totally undisturbed group or a colony husbandry group also influences one interpretation of the separation effects (Pryce & Feldon, 2003). These periodic separation paradigms involve not only the separation from the mother and, sometimes, the littermates but also produce physiological stresses associated with changes in body temperature and periods of nutritional deprivation. In addition, in the maternal separation paradigms, on reunification of mother and pups, there is evidence that if mothers were left alone during the separation period, when pups are returned to the nest, dams engage in very active pup licking, providing intensive stimulation similar to that received by pups that are simply “handled” (Hofer, Brunelli, & Shair, 1993; Plotsky & Meaney, 1993).

An alternative approach to the study of maternal separation effects is to study animals that experience

complete separation from the mother and littermates (Hall, 1998). In this situation, pups can be raised artificially on a pump, and both body temperature and nutrition can be closely regulated. Moreover, there is no “reunification” and hence no additional maternal stimulation; however, it is possible to “reinstat” in a controlled fashion aspects of maternal behavior by providing the isolated pup with additional “lickinglike” stimulation, nest odors, and access to peers. In the few studies that have used this type of “separation” paradigm, there is some evidence of long-term behavioral effects. For instance, artificial rearing increases anxiety measured in an open-field test (Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001) and alters social and maternal behavior in adult animals (Gonzalez et al., 2001; Kaneko, Riley, & Ehlers, 1996/1997). Finally, there is some evidence that in males artificial rearing impairs spatial learning (Wainwright et al., 1999).

In light of the variable effects of short-term, periodic maternal separations on learning and the paucity of studies on artificial-rearing effects, in this study we were interested in exploring the effects on a variety of types of learning of this artificial-rearing procedure, which permits a more extensive period of maternal separation than occurs during MS, but without some of the other confounds associated with separation procedures. In particular, the artificial-rearing procedure is associated with social isolation and is consequently likely to affect reactivity to social stimuli. Therefore, we hypothesized that being reared without the mother and littermates could affect learning tasks that utilized social cues or social interactions. By comparing the performance of artificially reared rats and mother-reared rats in nonsocial spatial learning and in social learning, we could assess the possibility that the deficits of learning are specific to situations involving social stimulation, but without making any assumption about the possible mechanisms involved. To our knowledge, this is one of very few studies to investigate the effects of MS on learned social recognition.

For nonsocial tasks, we tested spatial learning because it is known to be affected by a variety of early-experience manipulations (Frisone et al., 2002; Lehmann et al., 2002) and is dependent on the hippocampus, a brain structure that is particularly susceptible to effects of handling and MS (Lehmann et al., 2002; Morris, Garrud, Rawlins, & O’Keefe, 1982; Olton, Becker, & Handelmann, 1979; Vazquez, Van Oers, Levine, & Akil, 1996). Water maze and radial maze tasks were used because they differentially tax motivation (aversive vs. appetitive), motor systems (swimming vs. running), and memory systems (reference memory vs. working memory).

Three social learning tasks were used involving spontaneous behavioral responses of animals that are exposed

to juveniles, pups, or adults. Moreover, two of these tasks—the social transmission for food preference paradigm (Alvarez, Lipton, Rebecca, & Eichenbaum, 2001; Bunsey & Eichenbaum, 1995; Winocur, 1990; Winocur, McDonald, & Moscovitch, 2001; Winocur & Moscovitch, 1999) and social recognition (Kogan, Frankland, & Silva, 2000)—depend at least in part on the hippocampal region whereas the maternal memory paradigms involve nonhippocampal limbic structures (Ferguson, Aldag, Insel, & Young, 2001; Li & Fleming, 2002).

The social recognition paradigm involves exposing the experimental animal on 2 consecutive days to either the same juvenile animal or to two different juveniles. It uses a habituation-dishabituation procedure that has been the most common technique used to investigate the capacity of animals to discriminate and recognize familiarity (Dluzen, Muraoka, Engelmann, & Landgraf, 1998; Engelmann, Ebner, Wotjak, & Landgraf, 1998; Gheusi, Bluthé, Goodall, & Dantzer, 1994; Ploeger, Willemsen, & Cools, 1991; Popik & van Ree, 1998). This form of memory, although short-lasting, can last up to 3 days (Fleming, Kuchera, Lee, & Winocur, 1994). The memory processes involved are based on chemosensory cues (Sawyer, Hengehold, & Perez, 1984), involve the olfactory system (Dluzen et al., 1998), and are disrupted by lesions of the hippocampus (Kogan et al., 2000).

The social transmission for food-preferences paradigm consists of exposing a naïve observer rat to a recently fed conspecific (demonstrator). Then the observer exhibits an enhanced preference for whatever food its demonstrator ate (Galef & Wigmore, 1983). This relies on the combination of the odor of the recently eaten food with carbon disulfide, a natural odorant in rat's breath, but does not involve individual identification of a conspecific. Interestingly, although this task also involves simple exposure learning to conspecific and olfactory cues, it too is disrupted by lesions of the hippocampus (Alvarez et al., 2001; Bunsey & Eichenbaum, 1995; Clark, Broadbent, Zola, & Squire, 2002; Winocur, 1990; Winocur et al., 2001).

The last social learning task used, the maternal memory paradigm, refers to the ability of the parturient mother to maintain her responsiveness to offspring for at least 10 days after experiencing only 60 min of interaction with pups at the time of birth (Orpen & Fleming, 1987). This memory relies on the acquisition of multisensory cues from pups, including olfactory and somatosensory stimulation (Morgan, Fleming, & Stern, 1992), and hippocampal lesions have no effect on this task (Lee, Li, Watchus, & Fleming, 1999). Therefore, artificially reared female rats and mother-reared female rats were tested, in adulthood, after their first parturition and interactive contact with pups.

MATERIALS AND METHODS

Animals and Housing

The animals were obtained from a population of primiparous Sprague-Dawley rats bred at the University of Toronto at Mississauga, from a stock originally obtained from Charles Rivers Farms in St. Constant, Quebec. The animals were housed individually in clear Plexiglas cages (22 × 44 × 30 cm). Animals were provided with wood shavings and had ad-lib access to Purina Rat Chow food and water. The animals were maintained on a 12:12 hr light:dark cycle, with lights on at 0800 hr. The room temperature and humidity were maintained at 24°C and 40 to 50%, respectively.

Procedure

To create a population of artificially reared pups for all but the last social-task studies, female dams gave birth, and on the day of parturition (PND1) their litters were culled to 4 males and 6 females. On PND4, 5 females were removed from the nest, 4 of the females underwent a surgical procedure called a gastrostomy, and a fifth was marked with diluted food coloring and returned to the nest (intact control, Mother-Reared, MR-CTRL group). The dye was applied to the pups' dorsal surface every second day until Day 14, at which point ear-punch identification holes were applied. Three of the 5 females that underwent surgery were raised artificially from PND4 to 21 (see below for description of these groups). The fourth had the gastrostomy tube cut off just outside the skin and was returned to the nest after being marked with a different food color (Mother-Reared SHAM, MR-SHAM group).

Pup Surgery

All animals were weighed prior to surgery. The surgical animals were anesthetized in a bell jar with approximately 1 to 2 ml of methoxyflurane (Metofane, CDMV, Inc). The surgery involved inserting a leader wire (stainless steel, 0.25 mm in diameter), sheathed in Silastic tubing (Dow Corning, VWR Scientific) and PE-10 (Clay Adams) tube into the pups' mouth and down the esophagus. When the end of 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 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 outer wall of the pup and held in place with a small amount of super glue. Neosporin antibacterial cream was applied topically at the site of penetration. The implantation usually took no more than 90 s, and the pups awakened within 3 to 5 min. This procedure has been successfully used (Diaz, Moore, Petracca, Schacher, & Stamper, 1981), and none of the animals in our study had infections or died.

Rearing and Weaning

Following the gastrostomy, pups were housed individually in plastic cups (11 mm in diameter × 20 mm deep) which fit into a

second weighted cup that floats in temperature-controlled water bath (aquarium filled with water maintained at 36°C). The cups were filled with corncob bedding (Renseed), and the lids of the cups remained open to allow the gastrostomy tubing to emerge and to connect to nearby syringes containing milk formula. Syringes containing the formula diet (Messer diet, adapted from the University of Iowa; were mounted on timer-controlled infusion pumps (Series PHD 2000, Harvard Apparatus Syringe Pumps). The pumps were programmed to infuse the diet for 10 min every hour, 24 hr daily. The amount of diet the pump was calibrated to deliver was based on a specified fraction of the mean pup weight for the pumps (For the first day, the amount was 33% of the mean body weight. This amount slowly increased to a maximum of 40% of mean body weight.) Each of the two pumps maintained 10 pups, for a total of 20 pups per cohort. The diet was made every week, refrigerated, and consisted of a mineral mix and a formula mix. The mineral mix consisted of 0.214 g of zinc sulfate (ZnSO₄), 0.12 g of copper sulfate (CuSO₄), 0.22 g of iron sulfate (FeSO₄), 2.0 g of potassium chloride (KCl), and 2.0 g of magnesium chloride (MgCl), which were mixed together and dissolved in 50 ml of double-distilled water. The formula mix consisted of 1500 ml (four cans) of Carnation evaporated milk, 450 ml of double-distilled water, 70 g of Purina 710 protein, 130 ml of Mazola corn oil, 2.0 g of tryptophan, 10 g of a vitamin mix, 11 g of tricalcium phosphate, and 0.2 g of deoxycholic acid.

Each morning, the pups were removed from the cups, weighed, and had their tubing flushed with 0.1 cc of distilled water. The infusion syringes were replaced with new syringes containing fresh diet, and the pumps were recalibrated according to the new mean pup weight per pump. The two sets of control pups also were removed from the litter and weighed at this time.

One group of artificially reared animals (artificially reared with minimal stimulation: AR-MIN group) was stimulated twice a day (the required minimum for stimulating urination and defecation) with a warm, wet paintbrush swiping their anogenital regions in a up-and-down vertical motion for 30 to 45 s to stimulate urination and defecation. A second group (artificially reared with maximal stimulation: AR-MAX group) was stroked five times a day in the same region and on the dorsal surface of the body with the same pattern of motion, but the stimulation lasted 2 min per pup. In some of the studies, a third group (artificially reared maximal stimulation and social stimulation: AR-SOC group) received the same pattern of stimulation, but these pups were raised with a social companion (a female of no relation) of the same age. Social partners were returned to their own dams after 12 hr and replaced by freshly social partner pups. A particular pup used as a social partner was deprived for a 12-hr period on two to three occasions during the first 18 days of life, and each time they were then returned to their lactating mothers and allowed to feed. Animals used as social partners survived and maintained adequate body weights. This stimulation manipulation was carried out from the day the pups were placed on the pumps (PND3–4) to the day of weaning from the pumps (PND18). MR-SHAM and MR-CTRL pups remained with the dam and were left undisturbed.

On PND18, AR pups were removed from the pumps and the experimental conditions maintained. AR pups were placed in small (24 × 18 × 12 cm) cages and given free access to crushed

cat chow mixed with formula and water. The cages were placed on heating pads to maintain at a temperature of 36°C. On PND21, all AR and MR animals were weaned and placed in 40 × 20 × 18 cm cages. Each AR animal was paired with a conspecific of the same sex and age, and each MR-SHAM animal was housed with one MR-CTRL animal until adulthood (60–120 days of age) when they were tested.

Observers were blinded to the animals' rearing condition on all behavioral tasks.

Nonsocial Spatial Learning Tasks

Water Maze Task: Small Pool. A first cohort of female rats (AR-MIN group: $n = 9$, AR-MAX group: $n = 8$, MR-SHAM group: $n = 5$, MR-CTRL group: $n = 5$) was tested in a small pool (120 cm diameter, 60 cm depth). This test used a circular tank constructed of opaque plastic and filled with water (20–22°C) rendered opaque by the addition of soluble nontoxic white paint. The pool was located in a test room in which there were extramaze spatial cues, including posters on the walls and laboratory furniture around the pool. The pool was divided into four quadrants, and four equally spaced points at the border of the pool were used at the starting points for swim trials. The rats were required to locate the hidden platform (11 cm diameter) situated at a fixed position in the center of one quadrant and 1 cm below the surface of the water. There was one testing session per day, with five trials per session. On each trial, the rat was placed, facing the wall, in one of the four quadrants in the tank and allowed to swim for a maximum of 120 s. Once the rat found the platform, it remained there for 10 s before being returned to its cage. If the rat failed to find the platform in that time, it was placed for 20 s onto the platform before being returned to its cage. Each trial conducted each day was started from a different quadrant, with the order determined pseudorandomly (not twice from the same quadrant) and varying from day to day. The inter-trial interval was 10 min from the end of one trial to the beginning of the next, and at this time, animals were dried off with a towel. On the last day, after the last trial, the platform was removed from the pool, and each animal was allowed to search for the platform for 120 s (probe test). The pool was cleaned between test trials. Animals were tested for 4 days with five trials per day. In this place version of the task, a significantly shorter latency to find the platform is considered evidence of reference memory (Morris et al., 1982).

Water Maze Task: Large Pool. Since no deficit in performance in the small pool was observed in artificially reared animals, the difficulty of the task was increased by increasing the size of the water maze. Therefore, a second cohort of female rats (AR-MIN group: $n = 6$, AR-MAX group: $n = 6$, AR-SOC group: $n = 6$, MR-SHAM group: $n = 6$, MR-CTRL group: $n = 6$) was tested in a larger pool (180 cm diameter with black side walls 80 cm high) and with fewer trials per day (four trials a day for 5 days). In addition, we explored the ability of these animals to retain this learning over a period of months. Therefore, this second cohort of animals was retested 10 months later for 2 consecutive days (four trials per day). Finally, to assess more closely the possible cognitive deficits of the maternally deprived rats, such as persistence or perseveration of behavior, reversal

