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## 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; Maestri-pieri, 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

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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 “reinststate” 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 (ZnSO<sub>4</sub>), 0.12 g of copper sulfate (CuSO<sub>4</sub>), 0.22 g of iron sulfate (FeSO<sub>4</sub>), 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

learning was tested for 2 consecutive days when the platform was moved to a position diagonally across from the initial location.

### Data Collection and Analysis

For each trial, the (a) latency to find the platform, i.e., the time it took the animal to find the platform and climb onto it; (b) time spent in the correct quadrant, i.e., the quadrant where the platform was located; and (c) time spent close to the walls, i.e. the head of the rat was less than 15 cm away from the wall, were recorded. All rats swam with no apparent difficulty using the characteristic set of adult swimming postures.

When the small pool was used, only the latency to find the platform was recorded. Data were analyzed by using a series of 2 (rearing condition)  $\times$  4 or 5 (days) ANOVAs with a repeated measurement factor for days. Separated analyses were done for acquisition, retention, and reversal training.

**Radial Arm Maze Task.** Spatial working memory was tested in the eight-arm radial maze. In this maze, working memory was tested by measuring the ability of the animal to remember previously visited arms within the same trial. 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:  $n = 6$ ) tested in the large water maze also were tested in the radial arm maze. To avoid order effects on the two tasks, testing on tasks was counterbalanced.

The eight-arm radial maze was made of wood painted black and consisted of eight arms (58 cm long) radiating horizontally from an octagonal platform (37 cm diameter). A Plexiglas sliding door that could be raised or lowered was located at the entrance to each arm. The arms were bordered by clear side walls 2 cm high. A dish was placed 2 cm in diameter and 4 cm high at the distal end of each arm, and held a single Froot Loop cereal. The entire apparatus was elevated (70 cm) above the floor. The maze was placed in the center of a testing room. Spatial cues included a variety of posters on the walls and laboratory furniture around the maze.

Rats received daily three habituation sessions in which pieces of Froot Loop cereal were scattered throughout the apparatus including the central platform, each arm, and the food cups. Rats were placed individually in the central platform for 20 s and then allowed to explore the maze for 10 min. At the end of the third session, all rats ate the Froot Loop cereal. Testing began on the fourth day. For the test trials, each rat was placed individually on the central platform for 20 s. A trial was initiated by raising the doors at the entrance to each arm and allowing the rat to choose freely from among the eight arms. The animal was removed after all eight arms had been entered or after 10 min had elapsed. One such trial was administered daily for 14 consecutive days. The arena was cleaned between test trials using 70% alcohol.

**Data collection and analysis.** For each trial, an arm was recorded as having been entered when all four of the rat's paws were in the arm. An error was scored whenever a rat reentered an arm that had been previously scored as entered (working memory error). The time taken to enter the eight arms (find and

consume all eight food rewards) and the number of errors were recorded.

### Social Learning Tasks

**Social Recognition Task.** Groups consisted of AR-MIN ( $n = 7$ ), AR-MAX ( $n = 9$ ), and MR-CTRL and MR-SHAM combined ( $n = 9$ ). Animals were tested only on this task.

Animals were given daily vaginal smears to establish stage of cycle and to insure that exposure to the juvenile conspecific occurred on the day of metestrus and that test occurred on the day of diestrus, when estrogen levels are reduced. This procedure eliminates potential effects of steroids on any aspect of memory. On the day prior to exposure, all animals were transferred to a large cage (45.9  $\times$  45.9  $\times$  29 cm) to facilitate behavioral observations. They remained in this cage during exposure and test. On the day of exposure, a juvenile 20- to 25-day-old male rat was placed into the female's cage for 30 min. Nosing (gently pushing against the nose and face area or flank of another animal with the snout) and sniffing (same as nosing, but without direct contact) of the stimulus juvenile by the female were recorded on a computer-based event recorder for the first 5 min of exposure. At the end of the 30-min exposure period, juveniles were removed from the cage and returned to their home cages. The second test took place 24 hr after exposure. At test, half of the animals within each rearing condition were exposed to the same juvenile they had encountered on the previous day (SAME) and half were exposed to a different juvenile male (DIFF). Observations of social investigation were undertaken on the interaction for a 5-min period.

**Data collection and analysis.** Time spent in social investigation (total time spent sniffing and nosing the juvenile conspecific) was analyzed at exposure and at test using a repeated measure three-way ANOVA in a 2  $\times$  2  $\times$  2 (Rearing Condition: AR vs. MR  $\times$  Juvenile Familiarity: SAME, DIFF  $\times$  Time: Exposure, Test) design.

### Maternal Memory Task

Six groups of animals were tested. These included groups AR-MIN ( $n = 10$ ), AR-MAX ( $n = 9$ ), AR-SOCIAL ( $n = 6$ ), MR-SHAM ( $n = 10$ ), and MR-CTRL ( $n = 10$ ) that received a maternal experience for 24 hr at parturition. As well, 7 inexperienced MR-CTRL animals also were tested.

**Maternal Experience (Pup Exposure) Test.** Subjects were allowed to interact with their offspring for the first 24 hr postpartum. Ten-minute tests for maternal behavior were undertaken at the end of the 24-hr exposure period and during 5-min "spot checks" at 25-min intervals over a 2-hr period. At the time of observations at the end of the exposure period, mothers' pups were removed from the cage and 5 min later 6 pups were placed into one corner of the cage opposite to the subject's nest site. The occurrence of the following behaviors was recorded using a computer-based event-recorder: (a) pup retrieval: A rat picked a pup up in her mouth and carried it across at least one quadrant, usually back into her nest; (b) pup

sniffing: A rat poked her snout close to or touch on pups; (c) pup licking: A female opened her mouth and placed her tongue on the pups, including the total body licking and anogenital licking; (d) hovering/crouching: A rat positioned herself over the pups and arched her back, (e) nest building: A rat picked up nesting material in her mouth and transported it back to the nest site or pushed the material with her forepaws toward the nest site.

**Maternal Retention Test.** Subjects were tested for maternal behavior on Days 15 to 16 postpartum after 2 weeks of separation from pups and the initial pup exposure at parturition. On the first test day, 6 newly fed 1- to 6-day-old foster pups taken from a lactating "donor" mother were placed opposite the female in her cage. Using a computer-based event recorder, all maternal behaviors were recorded for an initial 10-minute period. After the observation period, two spot checks at least 2 hr after the test and at the end of the day were made in which the position of the female in the cage (which of the four equally sized quadrants) and what the female was doing were recorded. The foster pups were left with the female overnight and removed the following morning. They were returned to donor mothers and exchanged for a recently fed litter of 6 foster pups that were later placed into the female's cage at the initiation of the next 10-min observation period. As in the maternal experience observations, pup retrieval, pup licking, nest building, and hover/crouch were recorded. Regardless of the performance of the experimental animals, foster pups were always returned to their mothers in the morning. Since donor pups were obtained from two donor litters, the maximum number of times donor pups were used in the test was twice and at a 96-hr interval. In general, however, pups were used only once. No loss of pups was observed using this paradigm.

Maternal retention tests continued until a female was designated maternal or for a maximum of 11 days (the first day of induction testing, Day 0). A female was said to reach criterion for maternal behavior if she retrieved all pups into the nest during the 10-min observation period on 2 consecutive days and was observed to be with pups in the nest either in a crouch or lactating posture during the test or during spot checks. The latency, in days, to become maternal was calculated as the first day of 2 consecutive days of full maternal behavior. If an animal failed to respond within the 10-day test period, it was assigned a score of 11.

**Data collection and analysis.** As maternal latencies and durations of pup retrieval, pup licking, nest building, and hover/crouching were not normally distributed, all data were analyzed using the nonparametric Kruskal-Wallis for multigroup comparisons and post hoc comparisons.

**Social Learning of a Food Preference.** 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:  $n=6$ ) tested in the water maze and in the radial arm maze also were tested in the food preference task. These animals are called the observers.

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 needed.

The demonstrator animals (Demo) were females unrelated to subjects, but had resided with the observer animals for 2 days in a test cage ( $40 \times 20 \times 18$  cm). The Demo were food deprived for 23 hr, removed to a room away from their respective observer, and fed either COC or CIN for 60 min. Then each Demo was placed back into the test cage with the associated observer. The Demo remained with the observer for 30 min. After removal of the Demo, two food cups were placed into the front left and right corners of the test cage and counterbalanced across animals. Each dish contained 50 g of a scented chow diet, one of which was identical with the diet eaten by the Demo. Observer animals were allowed to eat the two diets undisturbed for 2 hr, at which time the two diets were weighed. To remove debris, fecal pellets, or other matter from the food cups, food was strained through a sieve prior to weighing.

**Data collection and data analysis.** The proportion of the total diet intake that was the same as the Demo diet was computed for each animal (proportional intake of Demo diet = Demo diet intake/(total food intake). One-way ANOVAs comparing the MR and AR groups in their proportional Demo intake were computed.

All procedures used in these experiments were approved by the Local and University Animal Care Committees of the University of Toronto.

## RESULTS

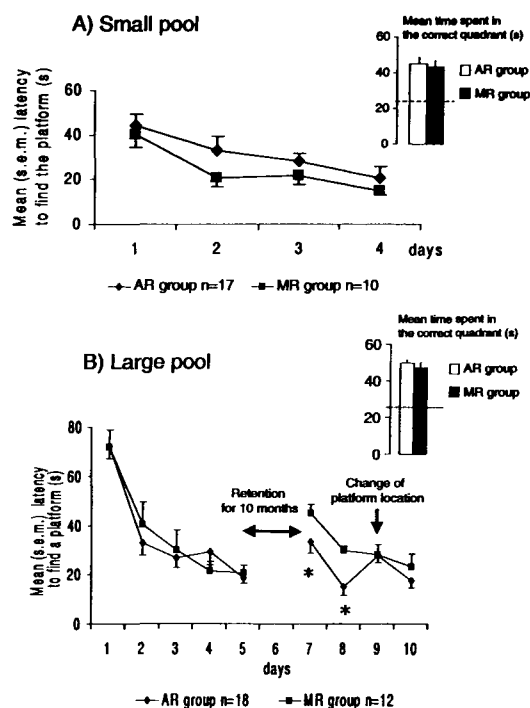
### Spatial Learning Tasks

The AR-MIN and AR-MAX (and where relevant, AR-SOC) groups did not differ significantly in their performance on any phase of the water maze or the radial arm maze tasks. Data obtained from these groups were combined into a single AR group; as well, the two MR groups did not differ significantly from one another and were combined into a single MR group. All statistical comparisons were therefore between AR and MR animals.

### Water Maze Task: Small Pool

**Acquisition.** The  $2 \times 4$  repeated measures ANOVAs showed a significant main effect of days,  $F(1, 25) = 18$ ,  $p < 0.001$  (Figure 1A), but no main effect of rearing and No Days  $\times$  Rearing interaction. Both groups showed a reduction of latency to find the platform over days, AR group: Day 1 versus Day 4,  $p = 0.05$ ; MR group: Day 1 versus Day 2,  $p = 0.02$ ; Day 1 versus Day 3,  $p = 0.03$ ; Day 1 versus Day 4,  $p = 0.02$ .

During the probe trial, the time spent in the platform quadrant exceeded chance level in both groups,  $t$  test,  $p < 0.004$ , and this duration of time did not differ between the AR and MR groups ( $46.2 \pm 3.0$  s vs.  $45.3 \pm 4.0$  s, respectively) (Figure 1A).



**FIGURE 1** Effects of complete maternal deprivation on spatial learning in a water maze. (A) Mean (SEM) latency to find the hidden platform in a small pool (120 cm diameter) during 4 days (five trials/day). For each animal, latency to find the platform of the four trials on each day was grouped into one block. Inset: Mean percentage of time spent in the correct quadrant when the platform was removed during a 2-min test (probe trial). No significant difference between groups was observed. (B) Mean (SEM) latency to find the hidden platform in a large pool (180 cm diameter) during 5 days (four trials/day) after a retention time of 10 months and after a change of platform location. For each animal, latency to find the platform of the five trials on each day was grouped into one block. \*Difference between groups  $p < 0.05$ . Inset: Mean percentage of time spent in the correct quadrant when the platform was removed during a 2-min test (probe trial). No significant difference between groups was observed.

### Water Maze Task: Large Pool

**Acquisition.** In latency to find the platform, the  $2 \times 5$  repeated measures ANOVA again showed a significant main effect of days,  $F(1, 28) = 121, p < 0.001$  (Figure 1B) and no main effect of rearing condition or a Days  $\times$  Group interaction. The days effect again demonstrated overall learning across the 5 days in all groups, AR group: Day 1 versus Day 2, 3, 4, or 5,  $p < 0.001$ ; MR group: Day 1 versus Day 3, 4, or 5,  $p < 0.01$ .

Similar results were obtained in the percentage of time spent either in the quadrant where the platform was located or near the walls (data not shown), with no rearing condition differences. However, a significant effect of

days was obtained for both measures, indicating for all groups an increase of time spent in the correct quadrant,  $F(1, 28) = 43.6, p < 0.001$ , and a decrease in the time spent close to the walls,  $F(1, 28) = 23.9, p < 0.001$ .

**Probe Trial.** The time spent in the platform quadrant exceeded chance levels in both groups,  $t$  test,  $p < 0.001$ , and this duration of time did not differ between the AR and MR groups ( $50.5 \pm 0.1$  vs.  $47 \pm 0.09$ , respectively) (Figure 1B).

**Long-Term Retention.** Groups were retested 10 months after the initial learning for their latency to find the platform on 2 consecutive days. The 2 (rearing)  $\times$  2 (days) ANOVA revealed a significant main effect for days,  $F(1, 27) = 10, p < 0.01$ , and rearing condition,  $F(1, 27) = 8.5, p < 0.01$ , but no Rearing  $\times$  Time interaction. The days effect again demonstrated overall learning, but only for the AR group, Day 1 versus Day 2,  $p = 0.01$ . When the two groups were compared on each day separately, the AR animals took less time to find the platform than did the MR animals, a difference that was most evident during the second day of retesting,  $t$  test,  $p = 0.05$ , Day 1,  $p < 0.01$  Day 2 (Figure 1B). However, when latencies obtained on the last day of the original testing were compared with the first day of retesting, there was only a significant effect of time, with all groups taking longer on retest,  $F(1, 28) = 23.1, p < 0.001$ . There were no main effects of rearing condition or interaction. Consistent with these latency effects, during the 2 days of retesting, there was a significant increase in time spent in the correct quadrant,  $F(1, 28) = 9.4, p < 0.005$ , and a decrease in the time spent close to the walls,  $F(1, 28) = 13.8, p < 0.001$ ; there were no effects of rearing condition or a Day  $\times$  Group interaction (data not shown).

**Reversal Test.** When the platform was moved to a position diagonally across from the initial location, comparisons were made between performance on the last day of initial training and the first day after platform reversal. A 2 (rearing condition)  $\times$  2 (days) repeated measures ANOVA revealed, for the latency to find the platform, a significant main effect of rearing condition,  $F(1, 27) = 4.7, p < 0.04$  (Figure 1B), but no effect of days or interaction. When the two groups were compared on each day separately, the AR group exhibited a shorter latency only the day before but not just after the change of platform location, reflecting the marginal interaction of the ANOVA.

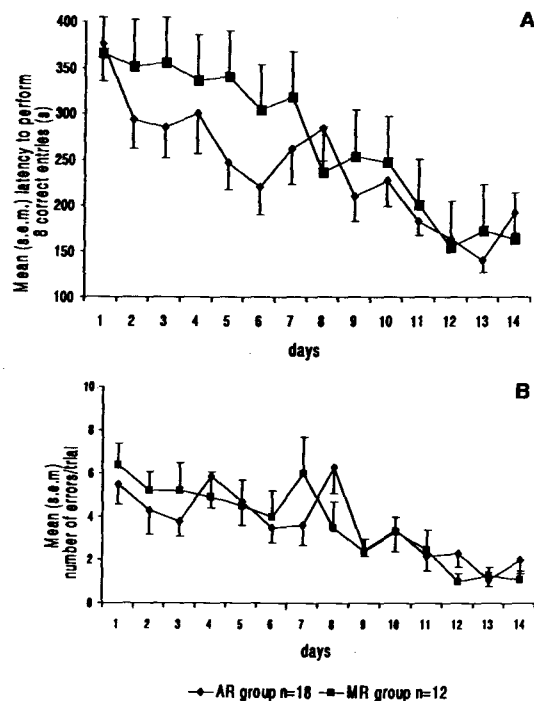
As for the percentage of time spent in the correct quadrant, the 2 (rearing condition)  $\times$  2 (days) repeated measures ANOVA revealed only a main effect of days,  $F(1, 27) = 8.4, p < 0.01$ , indicating that animals spent less time in the correct quadrant after the change of platform location (data not shown). With regard to the time spent

close to the walls, there were no main effects of days or of rearing condition, but there was a significant Days  $\times$  Rearing Condition interaction,  $F(1, 27) = 10, p < 0.01$ , reflecting an increase in time spent close to the walls in the AR group and a decrease in the MR group (data not shown).

For the 2 days of reversal testing, the 2 (rearing condition)  $\times$  2 (days) repeated measures ANOVA revealed no significant main effects or interaction for the latency to find the platform or the time spent close to the walls. Training animals spent an increasing amount of time over the 2-day period.

### Radial Arm Maze Task

For the latency to enter the eight arms, the 2 (rearing condition, AR vs. MR)  $\times$  14 (days) repeated measures ANOVA did not yield a significant main effect of rearing condition or a Day  $\times$  Group interaction; however, there was a significant effect of days,  $F(1, 28) = 92.7, p < 0.001$  (Figure 2A), demonstrating the overall learning across the 14 days, AR group: Day 1 versus Day 11, 12, 13, or 14,  $p < 0.02$ ; Day 2 or Day 4 versus Day 12,  $p < 0.05$ ; Day 1,



**FIGURE 2** Effects of complete maternal deprivation on spatial learning in an eight radial arm maze. (A) Mean (SEM) latency to perform eight correct entries during 14 days (1 trial/day). (B) Mean (SEM) number of errors per trial during 14 days. There was no significant difference between groups.

3, 4, 5, or 8 versus Day 13,  $p < 0.05$ ; MR group: Day 1 versus Day 8, 12, or 14,  $p < 0.01$ ; Day 2 or Day 3 versus Day 5,  $p < 0.05$ .

When the average latency to enter the eight arms across the first 7 days was compared with the average latency across the last 7 days, again there were no differences between rearing condition, although there was a marginal interaction of rearing condition and days,  $F(1, 28) = 3.4, p = 0.07$ , with the AR group taking less time to enter the eight arms than the MR group before having entered the eight arms: There were no significant main effects of rearing conditions or a Day  $\times$  Group interaction, but a significant effect of days,  $F(1, 28) = 36, p < 0.001$  (Figure 2B); AR group: Day 1 versus Day 13,  $p < 0.02$ ; MR group: Day 1 versus Day 12,  $p < 0.05$ ; Day 2 versus Day 14,  $p < 0.05$ .

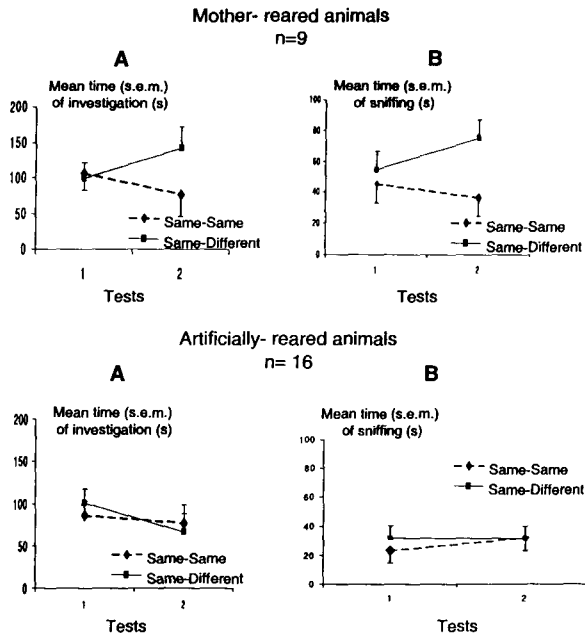
### Social Learning Tasks

**Social Recognition Task.** Time spent in social investigation (total time spent sniffing and nosing the juvenile conspecific) was analyzed at exposure and at test using a repeated measure three-way ANOVA, in a 2  $\times$  2  $\times$  2 (Rearing Condition: AR vs. MR)  $\times$  Juvenile Familiarity: SAME, DIFF  $\times$  Time: Exposure, Test) design. Analyses on social investigation showed no main effects, but a significant three-way interaction,  $F(1, 21) = 5.7, p < 0.026$ . As can be seen in Figure 3A, MR animals showed a different pattern of responding than did AR animals. MR animals showed a decrease in investigation from the first to the second exposure when the stimulus animal was the same on the two tests, but an increase from the first to the second test when the juvenile stimulus animal was different. AR animals, showed no significant change in investigation time from the first to the second tests under both stimulus conditions, when the juveniles were the same or different on the two tests.

Analyses of individual behaviors comprising the investigation measure showed that sniffing the body of the juvenile was a major component of investigation (Figure 3B). When time spent body sniffing was analyzed in a three-way ANOVA, as described earlier, there was not only a significant three-way interaction,  $F(1, 21) = 10.0, p < 0.005$ , but also a main effect of rearing,  $F(1, 21) = 5.84, p < 0.02$ , where MR animals spent more time than AR animals sniffing the stimulus juvenile's body, regardless of the familiarity status of the juvenile ( $53.5 \pm 14$  s vs.  $31.5 \pm 2$  s).

**Social Learning of a Food Preference.** The proportion of total intake comprised of the diet eaten by the Demo (proportion = Demo food intake/Demo and novel food



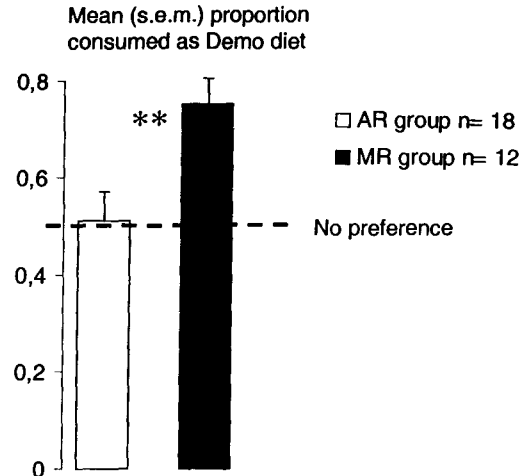


**FIGURE 3** Effects of complete maternal deprivation on the recognition of a juvenile. (A) Mean (*SEM*) time spent investigating familiar (same) and unfamiliar (different) juveniles between first and second exposure in mother-reared and in artificially reared animals. (B) Mean (*SEM*) time spent sniffing (same) and unfamiliar (different) juveniles. MR animals showed an increase in investigation or in sniffing when the juvenile was different whereas AR animals did not.

intake) did not differ significantly among the different AR groups (AR-MIN group:  $0.41 \pm 0.12$ ; AR-MAX group:  $0.53 \pm 0.05$ ; AR-SOC group:  $0.59 \pm 0.12$ ) or among the two MR groups (MR-SHAM group:  $0.76 \pm 0.06$ ; MR-CTRL group:  $0.75 \pm 0.07$ ). The three AR groups were combined, and the two MR groups were combined. AR subjects performed at chance level ( $0.51 \pm 0.25$ ,  $p > 0.05$ ) whereas the MR subjects selected the Demo food at above-chance level ( $0.75 \pm 0.16$ ,  $p < 0.01$ ) and significantly more than did the AR subjects,  $F(1, 28) = 8.27$ ,  $p < 0.02$  (Figure 4).

#### Maternal Memory Task

**Maternal experience phase.** Analyses comparing AR and MR groups or across the three AR and two MR groups showed no significant differences for any of the behaviors during either the initial 10-min observations or during any of the other 5-min observations over a 2-hr period. All animals in all groups retrieved pups, adopted a crouch posture over pups, and spent some proportion of the observation period engaged in general and genital body licking and in nest-building behavior. Thus, based on a single 10-min observation and spot checks on Day 1

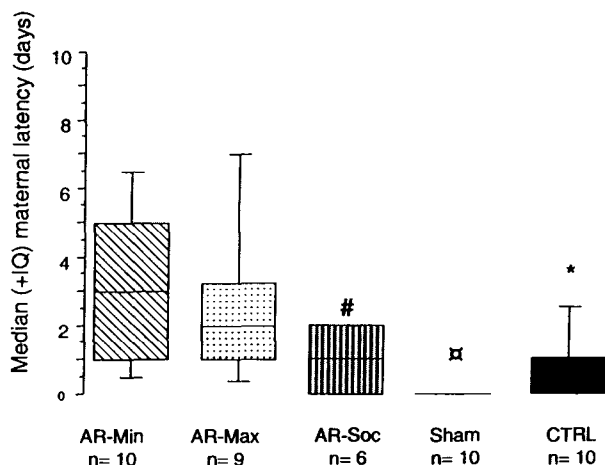


**FIGURE 4** Effects of complete maternal deprivation on a social learning for a food preference. Mean (*SEM*) proportion of total diet consumed as the preexposed diet in mother-reared and in artificially reared animals. \*\*Difference between groups  $p < 0.01$ .

postpartum, there were no significant group differences in the quality of the experiences or stimulation received (Note that maternal behavior differences between AR vs. MR animals are normally found between Days 4 and 10 postpartum, and not at this earlier time; Gonzalez et al., 2001).

**Maternal retention phase.** To illustrate the facilitative effect of maternal experience in unmanipulated animals, in the first analysis all MR animals that did ( $n = 20$ ) and did not ( $n = 7$ ) receive a maternal experience postpartum were compared in their maternal latencies at test. Consistent with previous studies, experienced animals had a significantly lower latency to respond to pups than did inexperienced animals, median latency of experienced = 0, median latency of inexperienced = 4.0;  $U = 26.5$ ,  $n_1 = 7$ ,  $n_2 = 20$ ,  $p < 0.007$ .

In the next analysis, since SHAM and CTRL groups differed from one another in their maternal onset latencies,  $U = 20$ ,  $n_1 = 10$ ,  $n_2 = 10$ ,  $p < 0.01$ , the AR-MR two-group analysis was not undertaken. Instead, Kruskal-Wallis comparisons across all five groups (AR-MIN, AR-MAX, AR-Social, SHAM, and CTRL groups) were undertaken and showed a significant effect of rearing condition,  $\chi^2 = 21.6$ ,  $df = 4$ ,  $p < 0.001$  (Figure 5). AR-MIN and AR-MAX animals had the longest onset latencies and did not differ from one another. Both groups differed significantly from the two MR groups, AR-MIN versus SHAM:  $U = 5$ ,  $n_1 = 10$ ,  $n_2 = 10$ ,  $p < 0.001$ ; AR-MIN versus CTRL:  $U = 18$ ,  $n_1 = 10$ ,  $n_2 = 10$ ,  $p < 0.02$ ; AR-MAX versus SHAM:  $U = 5$ ,  $n_1 = 9$ ,  $n_2 = 10$ ,



**FIGURE 5** Effects of complete maternal deprivation on maternal memory. Median and quartiles of latency (in days) to exhibit maternal behavior in animals receiving 2 hr of maternal experience following parturition and tested 10 days after parturition. #Significant difference with AR-MIN group,  $p < 0.05$ ; αsignificant difference with the other groups,  $p < 0.05$ ; \*significant difference with AR-MIN and AR-MAX groups,  $p < 0.05$ .

$p < 0.001$ ; and AR-MAX versus CTRL:  $U = 20$ ,  $n_1 = 9$ ,  $n_2 = 10$ ,  $p < 0.05$ . However, social stimulation during rearing resulted in intermediate latencies between the AR and MR groups. AR-Social animals had significantly shorter latencies than did AR-MIN animals,  $U = 12$ ,  $n_1 = 6$ ,  $n_2 = 10$ ,  $p < 0.05$ , and had longer latencies than did SHAM animals,  $U = 15$ ,  $n_1 = 6$ ,  $n_2 = 10$ ,  $p < 0.02$ .

Behaviors exhibited on the first day of retention were consistent with the latency data, in that animals showing short latencies showed higher levels of crouching and licking on the first test day. There were significant differences between the five groups in hovering/crouching over young,  $\chi^2 = 16.9$ ,  $df = 4$ ,  $p < 0.01$ , and marginally body licking,  $\chi^2 = 8.4$ ,  $df = 4$ ,  $p = 0.07$ . Time spent hovering/crouching over young in AR-MAX animals was significantly lower than in CTRL, SHAM, or AR-Social groups,  $U = 4$ ,  $n_1 = 7$ ,  $n_2 = 8$ ,  $p < 0.01$ ;  $U = 9$ ,  $n_1 = 7$ ,  $n_2 = 10$ ,  $p < 0.05$ ;  $U = 2$ ,  $n_1 = 4$ ,  $n_2 = 7$ ,  $p < 0.05$ , respectively (For 2 animals of the CTRL group and 1 of the AR-Social group, data were missing).

## DISCUSSION

In this set of studies, animals were raised artificially without a mother and littermates (AR) until weaning and with a single conspecific from weaning until adulthood. Consistent with the majority of the studies that investigate the effects of more limited maternal separation, these studies showed that even this more severe form of

maternal/sibling deprivation has no negative effect on the acquisition of spatial learning in adulthood. However, AR animals showed clear and consistent deficits in their ability to learn both familiar ("it is a familiar juvenile," social recognition) and general ("it's a pup," maternal memory) characteristics of a conspecific and specific olfactory cues provided by a conspecific (food preference task), hence, tasks that have a social component.

In their performance on spatial tasks, AR and MR animals acquired criterion performance with the same response latencies (Figures 1 and 2) and with the same number of errors (Figure 2). Additional tests, designed to challenge the animals' abilities, also showed no deficits in the AR animals: No AR-MR differences were found in tests of simple reversal learning or of long-term retention over a 10-month period in the water maze (Figure 1B). In fact, in a number of these instances, the AR animals outperformed mother-reared animals on the initial tests, achieving marginally faster acquisition in the radial arm maze (Figure 2A) and on long-term retention in the water maze (Figure 1B). Interestingly, this somewhat-enhanced performance of AR animals also has been reported for animals with more limited maternal separations. Female rats that were repeatedly isolated from their mothers 6 hr daily for 7 days showed a more rapid acquisition in a water maze than controls when tested as adults (Frisone et al., 2002). Similar improvements in water maze reversal learning have been reported for animals that experienced a single 24-hr maternal separation (Lehmann et al., 1999).

The absence of a deficit in spatial learning with artificial rearing is quite surprising given that our animals were maternally deprived for 24 hr/day throughout the preweaning period, and in their "cup" environment received minimal spatial experience. Our results would not have been predicted by the elegant results of Cramer (1988), who reported that if rat pups are prevented from exploring their mothers' ventrums and of locating "nipples," they show deficits in adulthood in spatial behavior. It may well be that being reared in cups in the absence of virtually all spatial cues, and hence in the absence of any spatial experience, permits the space processing system to develop normally; however, being reared in a spatial environment where some spatial experiences are acquired (but animals do not experience the usual and biologically "expected" spatial cues) disrupts the normal development of the spatial system. This clearly happens in other sensory domains, as in the visual system, where being reared without any visual input results in the development of binocular cells in the visual cortex whereas being reared with limited monocular input redirects development so that normally binocular cells become monocular (Blakemore, 1974).

Given that there were no spatial deficits in the water maze and the radial arm maze and no deficits in long-term

