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Repeated maternal separation does not alter sucrose-reinforced and open-field behaviors

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Abstract

Repeated separation of rat pups from their mothers has been reported to increase behavioral fearfulness and hypothalamic–pituitary–adrenal (HPA) response to stress. Recently, it was suggested that it might also alter behavioral responses to natural and drug rewards. Here, we studied whether maternal separation (MS) would alter behavioral responses to a sucrose reward. We also tested whether MS would alter behavioral responses in an open-field test using a novel method of analysis [Software for the Exploration of Exploration (SEE)]. Long–Evans rat pups were exposed to either 180 min of MS, 15 min of separation [early handling (EH)] or left undisturbed [nonhandled (NH)] from postnatal day (PND) 3 to 14. The adult male offspring were tested for sucrose solution preference using a two-bottle free-choice test, operant response for sucrose under fixed ratio and progressive ratio (PR) schedules of reinforcement and response to a novel environment (open-field test). MS had no effect on sucrose preference or operant responding for sucrose reward. In the open-field test, NH rats showed a brief decrease in locomotor response, but MS rats did not differ from the NH and EH groups in the other behavioral measures. Thus, under the conditions of the present study, MS did not appear to alter reward-related processes and also had a minimal effect on open-field behavior.

Keywords: Emotionality; Male; Maternal separation; Open-field; Operant response; Rat; Reward; Sucrose preference

1. Introduction

Over the past five decades, it has been repeatedly shown that early postnatal environmental manipulations in rats have profound and long-lasting effects on a variety of biochemical, hormonal and behavioral responses in adulthood (see Denenberg, 1964; Levine, 1957; Meaney et al., 1996). Postnatal handling [early handling (EH)], operationally defined as a brief (up to 15 min) daily separation of the pups from the dams during the preweaning period, decreases behavioral fearfulness and hypothalamic–pituitary–adrenal (HPA) response to stress. In contrast, repeated periods of prolonged (3–6 h/day) maternal separation (MS) are reported to increase "anxiety-like" behavioral responses and HPA axis reactivity (Anisman et al., 1998; Meaney et al., 1996).

Recently, it has been suggested that MS might also alter behavioral responses to natural and drug rewards (Matthews et al., 1996b, 1999). MS, compared to EH, has no effect on the consumption of, or preference for, sucrose solutions over water but leads to blunted negative and positive contrast effects with sucrose as the contrast stimuli (Crnic et al., 1981; Matthews et al., 1996b). Negative and positive consummatory contrasts are defined as behavioral responses elicited by the replacement of a familiar reward with a reward of greater or lesser intrinsic value, respectively. These responses are considered to reflect the perceived discrepancy between the expected reward and the consumed reward (Flaherty, 1982). In addition, Matthews et al. (1996a,b) demonstrated that MS attenuates the anticipatory locomotor response to conditioned appetitive cues. More recently, it was reported that MS rats drank less water–sucrose solution and more ethanol–sucrose solution than EH and normally reared rats (Huot et al., 2001). However, these results are hard to interpret in the context of sucrose preference, because no plain water option was offered to the rats. In addition, it was reported that, as compared with EH, MS rats demonstrate sex- and dose-dependent alterations in cocaine self-administration behavior (Matthews et al., 1999).

The reported changes in the reward system may be the result of changes in the sensitivity of the mesolimbic dopa-

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minergic (DA) reward system (Jones et al., 1990). This hypothesis was supported by demonstrations of decreases in brain dopaminergic and noradrenergic functioning following MS (Matthews et al., 1996a). However, it is important to note that several studies reported contradicting results, wherein MS subjects demonstrate increased sensitivity to dopaminergic agonists (Hall et al., 1999; Lehmann et al., 1998). Thus, although MS affects the mesolimbic dopaminergic system, the precise nature of the changes is not clear. It has been suggested that different separation procedures may account for contradicting findings between studies (Lehmann and Feldon, 2000). Nevertheless, the studies reviewed above used similar methods of MS (repeated random separation between birth and weaning).

It therefore seems that while several studies have explored the relationship between MS and reward sensitivity in adulthood, no clear picture has emerged. In addition, the findings of Matthews et al. (1996a,b) indicate differential effects of MS on the consummatory and appetitive (preparatory) aspects of behavior (Glickman and Schiff, 1967). The distinction between appetitive and consummatory behaviors is important, because many studies have demonstrated that different neuronal substrates mediate these behaviors (e.g., Robbins and Everitt, 1992, Salamone, 1994).

Here, we examined the effects of repeated MS (3 h/day, Days 2–14 postpartum) on the preference for sucrose solutions (consummatory behavior) and on the acquisition and maintenance of sucrose-reinforced lever pressing (appetitive behavior). We have chosen to use the repeated MS on consecutive days procedure, because it has been repeatedly reported to produce physiological and behavioral effects in the adult offspring (e.g., Caldji et al., 2000; Plotsky and Meaney, 1993). Sucrose-reinforced lever pressing was initiated under a fixed ratio-1 (FR-1) schedule of reinforcement. After stable sucrose-reinforced behavior was obtained, we also studied sucrose-reinforced lever pressing under a progressive ratio (PR) schedule. In the PR schedule, the fixed ratio requirements for obtaining a reinforcer (i.e., the response requirement) are progressively increased within a session in order to determine the maximum effort that the subject will exhibit. The final ratio achieved on a PR schedule is thought to provide an index of the reinforcing efficacy of the reinforcer (Hodos, 1961). Finally, there are contradicting reports on the effect of MS on emotionality as expressed in the response to a novel environment, which may be explained by the use of different maternal-separation and behavioral-analysis procedures (Lehmann and Feldon, 2000). Therefore, we also tested the effect of repeated MS on behavior in the open-field test. The resulting data were then subjected to a high-resolution analysis using the Software for the Exploration of Exploration (SEE) (Drai and Golani, 2001). The behavior of the MS rats was compared to two groups: completely undisturbed pups [nonhandling (NH)] serving as a control for the separation effect and an EH group serving as positive control for the NH effect (Feldon and Weiner, 1992) and furthermore as a control for the ''handling'' factor of the separation treatment. The results of the study show no evidence for a robust, prolonged effect of repeated MS on emotionality or response to reward in adult male rats.

2. Methods

2.1. Subjects

Fifteen timed-pregnant Long–Evans rats (Charles River Laboratories, Raleigh, NC) arrived to the animal facility on Day 13 of gestation. Upon arrival, they were individually housed in solid-bottomed breeding cages with wood-shaving bedding and free access to food and water. All dams and pups were housed by litter in the same temperature- and humidity-controlled holding facility (21 °C) under a reversed dark–light cycle (lights on 10:00 pm–10:00 am). The experimental protocols followed the ''Principles of laboratory animal care'' (NIH publication no. 86-23, 1996) and were approved by the Animal Care Committee of NIDA/IRP.

2.2. Separation procedure

On postnatal day (PND) 1, with the day of parturition designated as Day 0, all the pups were weighed and litters were culled to 10 (5 males and 5 females when possible). Litters were randomly assigned to one of the three early-treatment conditions ($n = 5$ litters per treatment). Starting on PND 3–14, litters allocated to the EH group were separated from the dams for a period of 15 min in room temperature. Dams were removed from the home cage and placed into individual cages for the duration of the separation. The pups were then removed from the home cages and placed in plastic mouse cages by litter. Bedding from the home cage was spread in the mouse cages. At the conclusion of the separation period, the pups were returned to the home cage and the dam was then returned. Litters that were allocated to the MS group went through a similar procedure, except that the pups remained away from the dams for 180 min (10:00 am–1:00 pm or 2:00–5:00 pm), and the mouse cages were placed inside a temperature- and humidity-controlled incubator (GQF Manufacturing, Savannah, GA). Temperature inside the incubator was maintained at 33 (PND 3–8) or 31 (PND 9–14) °C. NH litters were left undisturbed from PND 2 to 14, when routine cage maintenance was reinitiated. All animals were weaned on PND 21 by removing the mothers from the cages. One week after weaning, all female pups were removed from the cages and the male pups were group-housed (three per cage) by litter for an additional 6 weeks, after which they were housed individually for the remainder of the experiment. Body weight during the preweaning period was recorded as mean pup weight per litter in order to minimize unnecessary handling of the pups. Body weight was not recorded for the NH pups during the early treatment period (PND 3–14).

2.3. Sucrose preference test

At 10 weeks of age, a sucrose preference test took place in the home cages. Two drinking bottles, similar to the home cage drinking water bottle, were introduced into the cage through the metal mesh top cover. The bottles, one containing a sucrose solution and the other water, were weighed just before the test and immediately following its completion 4 h later (10:00 am–2:00 pm). The relative positioning of the bottles providing sucrose and water was reversed between tests to prevent the development of side preference. Initial relative positioning of the bottles was counterbalanced between groups. The rats were tested over 2 days with two different sucrose concentrations (1% and 3%). Because no group differences emerged, the rats were given another test with a lower concentration (0.5%) in order to determine whether the lack of group differences is due to a ceiling effect of the higher concentrations. Sucrose preference was calculated as the amount of sucrose solution consumed as a proportion of the total fluid intake over the 4-h test period.

2.4. Sucrose-reinforced behavior

2.4.1. Concentration–response test

Testing was conducted in sound-attenuated and ventilated operant chambers (Med Associates, Georgia, VT) when the rats were 3 months old. Each chamber was fitted with a liquid drop receptacle that was connected to a 60-ml syringe attached to the infusion pump (Razel Sci., Stamford, CT). The chambers had two levers located 9 cm above the floor, but only one lever (an active, retractable lever) activated the infusion pump. Presses on the other lever (an inactive, stationary lever) were recorded but had no programmed consequences. The operant chambers were controlled by a Med Associates system. The rats were initially deprived of water overnight and trained to press the active lever for sucrose solution reinforcement under a FR-1 schedule of reinforcement (each lever press is reinforced) for 7 days. Water was made freely available once the rats had acquired the lever-press response (typically within 1 or 2 days). Each session began with the introduction of the active lever into the chamber and the illumination of a white cue light above this lever for 5 s. A red house light was turned on for the entire session (60 min). Sucrose solution (10%) was delivered at a volume of 0.2 ml and a timeout period of 5 s was given after each delivery. During the timeout period, lever presses were not reinforced and the cue light located above the active lever was turned on. On test days (Days 8–11), the sucrose solution concentration was changed daily to 0.3%, 1%, 3% or 10% in a counterbalanced order.

2.4.2. PR test

Following the concentration–response test, sucrose solution was changed back to 10% and the operant requirements were switched to a PR schedule of reinforcement adopted from Roberts and Bennett (1993). The response requirements initially began at 1 and escalated through the PR steps (2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95. . .603). Final ratios, the last ratio successfully completed by the end of the 60-min session, were recorded daily for each rat. After 4 days under the PR schedule, the effect of changes in the concentration of the sucrose reward was studied over 3 test days during which the sucrose solution concentration was changed daily to 0.3%, 1% and 3% in a counterbalanced order.

2.4.3. Open-field behavior

The open-field test was conducted in a gray circular arena (140 cm in diameter) under bright light conditions when the rats were 11 weeks old on rats that were previously tested for sucrose preference. Large cardboard pieces of different geometrical shapes were hung in the rats' field of view and served as spatial cues. Rats were placed into the same location of the arena, one at a time, with their heads facing the arena wall and their activity was recorded for 15 min. At the end of the test, rats were removed from the arena, the number of fecal boli was counted and the floor was washed with detergent and wiped dry. The rats' activity was recorded by a video camera and fed to a PC-based tracking system (Noldus EthoVision, Noldus Information Technology, The Netherlands) that extracted and stored $x–y$ coordinates. The tracking rate was 10 frames per second and the space resolution was about 0.8 cm. Coordinate files were exported from the tracking system and were analyzed by SEE, which was especially developed for the analysis of rodent open-field behavior (Drai and Golani, 2001; Drai et al., 2001). SEE analysis is based on detailed ethological studies (Eilam and Golani, 1989; Golani et al., 1993; Tchernichovski et al., 1998), which showed that the open-field behavior of rats has a well-defined structure. SEE can be used to visualize and quantify this structure and was recently suggested as a tool for characterizing subtle behavioral differences in psychopharmacology and behavior genetics (Benjamini et al., 2001; Drai and Golani, 2001; Drai et al., 2001). In addition, we used SEE to calculate the traditional measures of open-field behavior, i.e., the distance traveled and the time spent at the center of the arena.

SEE analysis is based on the natural distinction between two modes (''gears'') of rodent locomotor behavior: progression and stopping. During ''stopping,'' the rat is not necessarily motionless but may perform many ''local'' movements such as rearing, scanning, sideways and backward steps and even several forward steps. Using the distribution of movement speeds, however, it was shown that such movements constitute a significantly different component (Drai et al., 2000). Using the procedure described in the above study, we computed this distribution separately for each rat and got a bimodal distribution for all rats, with the exception of a small number that did not move from their place at all and thus had no opportunity to use the progression mode. Computed measures that were analyzed were number of ''progression'' segments, median duration of ''progression'' segments and median duration of ''stop'' segments.

2.5. Data analysis

2.5.1. Sucrose preference

Proportions of sucrose consumption were analyzed using ANOVA with the between-subject factor of *Separation* (NH, EH and MS) and the within-subject factor of *Sucrose concentration* (0.5%, 1% and 3%). *Concentration–response*: Data were analyzed separately for total nonreinforced responses on the previously *active* lever and responses on the *inactive* lever. The number of responses was analyzed using ANOVA with the between-subject factor of *Separation* (NH, EH and MS) and the within-subject factor of *Sucrose concentration* (0.3%, 1%, 3% and 10%). *PR*: The ordinal values of the final ratios achieved during the PR tests (the final PR step number) were subjected to ANOVA with the between-subject factor of *Separation* (NH, EH and MS) and the within-subject factor of *Sucrose concentration* (0.3%, 1% and 3%). The ordinal step numbers were used because the actual ratio values were derived from an exponential equation (Roberts and Bennett, 1993) and thus violated the assumption of homogeneity of variance. *Open-field test*: Data were analyzed using ANOVA with the between-subject factor of *Separation* (NH, EH and MS). Post hoc analyses were done with a Fisher PLSD Test (two-tailed) and significant differences are reported for $P<.05$. All analyses were first conducted with a nested factor of *Litter* to account for the use of littermates within the same experimental condition. Because no significant litter effect was found, these data are not shown.

3. Results

3.1. Body weight

There were no significant differences in body weight of the pups at the start of the early treatment (PND 2) or at PND 60 (Table 1). However, ANOVA with repeated measures over Days 2, 21 and 60 showed significant effects of Separation condition [$F(2,12)=4.28$, $P<.05$], Time [$F(2,24)=4880.2$, $P<.001$] and Separation condition × Time interaction [$F(4,24)=2.86$, $P<.05$]. Post hoc tests revealed significant body weight differences in Day 21, with the NH group being heavier than the MS group, which was heavier than the EH group (all P's $<.05$).

Table 1
The effect of the separation condition on body weights (mean ± S.E.M.) at PND 2, 21 and 60 in MS, EH and NH mature male rats

	Body weight (g) by age		
	2 days	21 days	60 days
EH	7.0 ± 0.3	64.9 ± 1.8*	359.6 ± 7.8
NH	6.8 ± 0.1	87.7 ± 0.9*	371.3 ± 10.2
MS	6.9 ± 0.2	75.1 ± 4.8*	348.8 ± 5.3

* Significantly different from the other separation condition groups under the same age ($P<.05$).

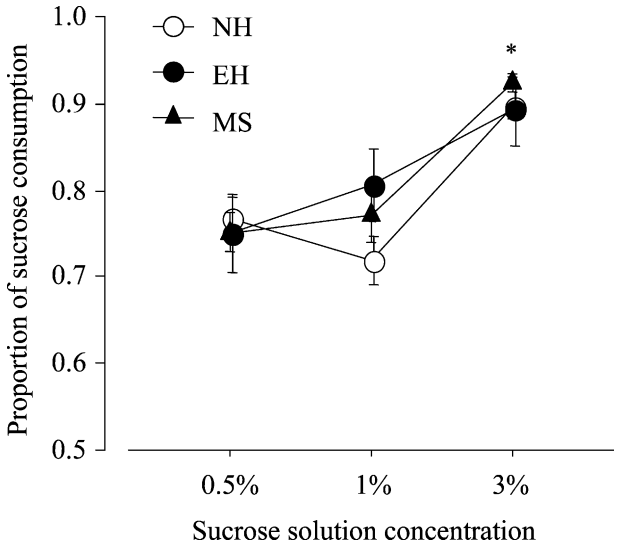

Fig. 1. Two-bottle choice test. The effect of different sucrose concentrations on the proportion of sucrose intake from total liquid consumed (mean ± S.E.M.) in MS, EH and NH mature male rats. * Significantly different from the 0.5% and 1% sucrose concentrations ($P<.05$).

3.2. Sucrose preference test

The numbers of pups tested for sucrose preference were NH = 20, EH = 18 and MS = 19. A significant main effect of sucrose concentration was seen [$F(2,108)=25.09$, $P<.001$], with no significant interaction with the separation condition (Fig. 1). All the rats showed preference for the sucrose solution compared with water and the consumption of the 3% solution was significantly higher than the consumption of 1% and 0.5% solutions (P's $<.001$). Post hoc group differences are indicated on Fig. 1.

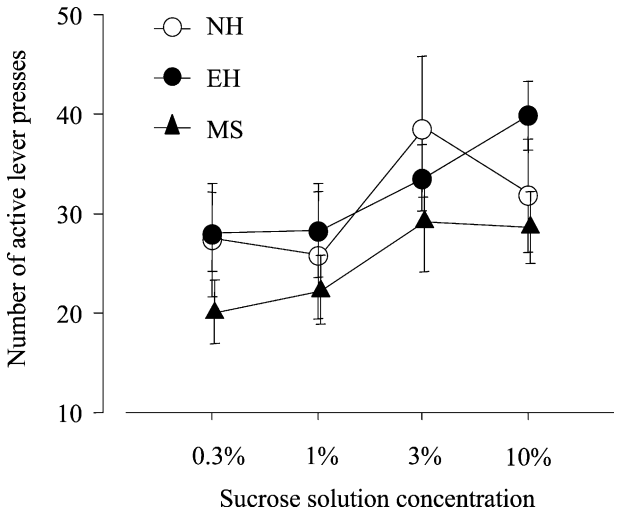

Fig. 2. Sucrose-reinforced behavior: FR-1 schedule of reinforcement. The effect of different sucrose concentrations on the number (mean ± S.E.M.) of sucrose-reinforced active lever responses (60 min) in MS, EH and NH mature male rats.

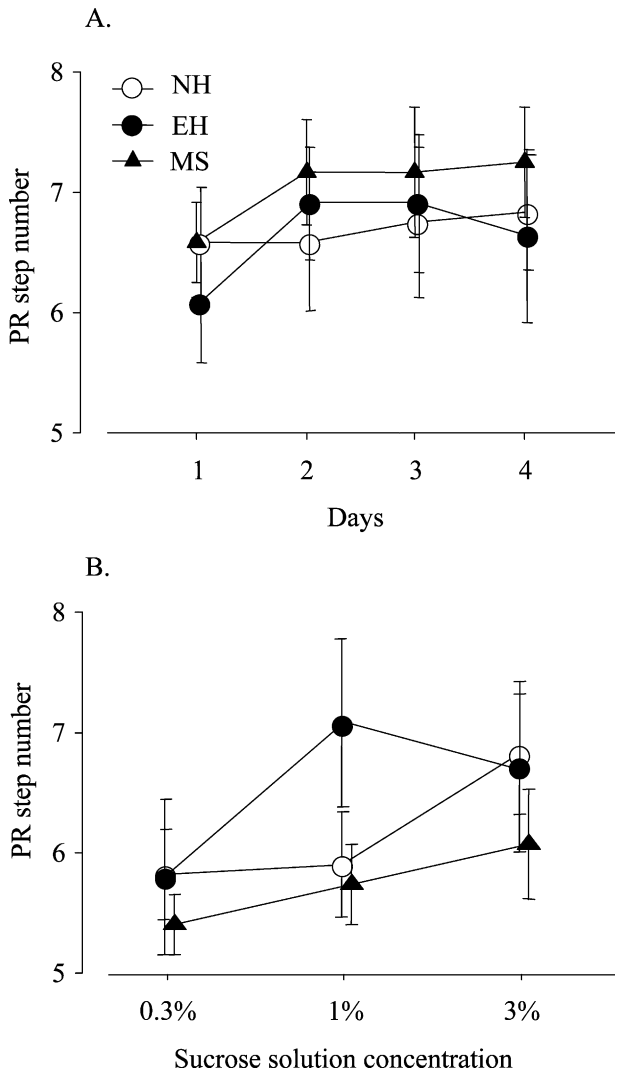

Fig. 3. Sucrose-reinforced behavior: PR schedule of reinforcement. Final PR step numbers (mean ± S.E.M.) in MS, EH and NH mature male rats. (A) Repeated tests with 10% of sucrose. (B) Concentration–response curve conducted following the repeated tests with 10% sucrose.

3.3. Sucrose-reinforced behavior

3.3.1. Concentration–response

The numbers of animals tested were NH = 11, EH = 12 and MS = 12. Lever pressing for sucrose in all groups was positively correlated with the sucrose concentration (Fig. 2). ANOVA of the mean number of reinforcements achieved over the four test sessions revealed only a significant effect of sucrose concentration [$F(3,96) = 6.25$, $P < .001$].

3.3.2. Progressive ratio

The numbers of animals tested were NH = 11, EH = 12 and MS = 12. No differences were observed between the different groups on the final PR step number achieved over the 4 days of training with the 10% sucrose solution (all P's > .1) (Fig. 3A). A concentration–response relationship was established when sucrose concentration was changed to 3%, 1% and 0.3%, such that lower final PR step numbers were achieved for lower sucrose concentrations (Fig. 3B). A repeated-measures ANOVA revealed only a significant effect of sucrose concentration [$F(2,64) = 5.94$, $P < .01$].

3.4. Open-field behavior

The numbers of rats tested were NH = 15, EH = 14 and MS = 16. Threshold speeds between the stopping and progression components of the distribution were similar to those found in Drai et al. (2000), i.e., between 5.1 and 11.1 cm/s for all rats with two components. As in Drai et al. (2000), it was found that behavior episodes in the stopping mode were also localized in space (mean spatial spread for all rats was lower than 4.2 cm), while episodes in the progression mode were used to connect places in the arena (mean spatial spreads for all rats were active enough to have a significant progression mode was higher than 14.3 cm). This indicates that the natural distinction between the stopping and progression modes, which is based on movement speed, is also a distinction between "in place" behavior and "going between places" behavior. The results from the open-field behavior analysis are shown in Table 2. The NH rats appeared to have covered less distance during the first 2 min of the test compared to the EH and MS rats. This observation was partially supported by the ANOVA that showed an effect of separation that approached significance [$F(2,42) = 2.67$, P=.08] and a post hoc analysis that showed that the NH rats covered significantly less distance than EH rats ($P < .04$) but not the MS rats (P>.05). Rats from the NH group also had a significantly higher number of fecal boli compared to the EH rats ($P < .01$).

Table 2
The effect of the separation condition on the behavior in an open-field test in MS, EH and NH mature male rats

	Total distance covered (m)	Distance covered over first 2 min (m)	Time in center[a] (s)	Number of progression segments	Median duration of progression segments (s)	Median duration of stops (s)	Number of fecal boli
EH	41.6 ± 3.9	11.0 ± 0.9	0.46 ± 0.2	128.9 ± 14.2	1.9 ± 0.1	15.8 ± 2.1	1.36 ± 0.4
NH	36.4 ± 4.6	8.8 ± 0.9*	0.26 ± 0.2	129.4 ± 18.0	1.7 ± 0.1	18.0 ± 2.0	3.67 ± 0.7[#]
MS	39.2 ± 4.2	11.7 ± 1.0	0.56 ± 0.3	124.9 ± 16.4	1.8 ± 0.1	15.5 ± 2.0	2.56 ± 0.5

Data are presented as mean ± S.E.M. group scores.

[a] "Center" was defined as an area 20 cm in diameter at the center of the arena.

* Significantly different from EH group ($P < .05$).

[#] Significantly different from EH group ($P < .02$).

4. Discussion

In the present experiment, exposure of rat pups to repeated separation from their mothers had little effect on the growth or behavior of the adult rats. The separation procedure did not reduce body weight gain during the preweaning period or at adulthood, although the pups were kept away from the lactating mother for 3 h/day. In fact, the MS pups were heavier than the EH pups at weaning age (21 days). The reason for these differences in body weight is not clear. It has been suggested that increased (compensatory) maternal care may be the cause for the physical and behavioral changes observed following short-term separation (EH) (Caldji et al., 1998; Liu et al., 1997). However, repeated MS was reported to result in reduced maternal care (Caldji et al., 2000). Moreover, there are many contradicting reports on the effect of MS on the body weight of the offspring. These may be explained by the use of different separation procedures and more specifically the physical severity of the MS manipulation or by the control group used for comparison, i.e., EH, NH or "normal" rats (Lehmann and Feldon, 2000). Although in several studies NH pups were shown to have slower rates of weight gain, this effect was not observed in other studies (see Daly, 1973; Lehmann and Feldon, 2000).

When tested for sucrose preference, all rats showed strong preference for the sucrose solutions. The amount of sucrose intake, as a function of the total liquid intake, rose monotonically with increasing concentrations, commonly considered as an increase in the reward value (Willner et al., 1992). No group differences were observed in this test, indicating that there are no differences in the basic consumption behavior, a result that is in agreement with previous reports (Matthews et al., 1996b). However, it was recently reported that consumption of sucrose solution is reduced in MS rats (Huot et al., 2001), a finding that can be interpreted as a sign of anhedonia (Willner et al., 1992). These authors showed that when presented with a free choice between two bottles, one containing ethanol (8%) in 2.5% sucrose solution and the other containing 2.5% sucrose solution, the MS rats consumed less sucrose solution than the EH and control rats. It is difficult, however, to compare these data to our data. While the separation procedure was similar to the one in this study, no free choice between water and sucrose solution was given to the rats in Huot et al.'s (2001) study. It also was suggested that the sucrose preference technique might lack the sensitivity to detect differences without application of further experimental manipulations, e.g., stress (Zurita et al., 2000). However, because our procedure involved a sharp downward shift of sucrose concentration (sixfold), which is known to activate the HPA axis stress response (Goldman et al., 1973), it seems that our postnatal manipulations were ineffective in inducing changes in sucrose preference even under (mildly) stressful conditions. Finally, Matthews et al. (1996b) found that repeated MS rats demonstrated blunted negative and positive consummatory contrast effects with sucrose as the contrast stimuli, indicating altered reward behavior after MS. Negative and positive consummatory contrasts are defined as behavioral responses elicited by the replacement of a familiar reward with a novel one, either of a lesser or greater intrinsic value. These responses are considered to reflect the comparison of expectancy with reality (Flaherty, 1982). However, because the contrast effect was not evaluated in our study, we cannot preclude the possibility that our MS rats also might show altered contrast effects.

As in the case of the two-bottle sucrose choice test, the three experimental groups did not differ significantly in their instrumental response (lever presses) for a sucrose solution under both fixed ratio and PR schedules of reinforcement. Response rates under the FR-1 schedule (each lever press is reinforced) and the final ratios achieved under the PR schedule rose monotonically with increased sucrose concentrations. The free consumption of palatable rewards has been shown to differ under many conditions from operant performance reinforced by the same rewards (Barr and Phillips, 1999; Mamedov and Bures, 1990). It was further suggested that these behaviors might be under differential control mechanisms (Robbins and Everitt, 1992; Vigorito et al., 1994). Nevertheless, our findings indicate a lack of effect of repeated MS on both the consumption of, and the instrumental response for, sucrose solutions. We can therefore conclude that under the conditions described in this study, MS does not alter reward seeking and consumption.

The effect of the separation condition on response to novelty was examined in an open-field test using a novel, high-resolution assessment method (Drai and Golani, 2001; Drai et al., 2001). Maternally separated rats were not significantly different from the EH rats in any of the recorded parameters and were significantly more active than the NH rats during the first 2 min of the test. The MS rats also did not show significant differences from the other experimental groups in another measure of fearfulness, i.e., number of fecal boli produced during the test (Anderson, 1940). These results are not in agreement with previous reports, which demonstrated a brief reduction in initial exploratory locomotion in MS rats compared to EH rats (Matthews et al., 1996b) and reduced exploration of the center area in MS rats compared to EH but not NH rats (Caldji et al., 2000).

However, it should be pointed out that to the extent that exploratory behavior is a measure of fearfulness, the existing literature is highly inconsistent. Thus, MS was reported to increase fearfulness and reduce exploration (Ogawa et al., 1994; Wigger and Neumann, 1999) or to decrease fearfulness and increase exploration (von Hoersten et al., 1993). These contradictory reports and our failure to observe significant increases in the emotionality of MS rats may, in part, be due to differences in the assessment methods (e.g., photocell beam break, Matthews et al., 1996b, or plus maze, Wigger and Neumann, 1999). In addition, different light/dark schedules in this study vs. others (Caldji et al., 2000; Huot et al., 2001) may explain the different results. Differences between studies may also have arisen from using different "control" groups (e.g., standard husbandry,

unmanipulated (NH) litters, Wigger and Neumann, 1999, or EH pups, von Hoersten et al., 1993) or from to the lack of a consistent separation procedure as argued and demonstrated by Lehmann and Feldon (2000). However, our results also contradict findings from studies using the same separation protocol, strain of rats, sex and assessment methods (Caldji et al., 2000; Huot et al., 2001; Meaney et al., 1996). On the other hand, our results of reduced activity and higher bolus number in the NH group are in agreement with previous findings of higher emotionality in NH rats (Caldji et al., 2000; Levine, 1969; Weizman et al., 1999), indicating that the lack of MS effect observed here is probably not due to procedural differences.

The sensitivity of the developing mammalian brain to early postnatal environmental manipulations has been repeatedly demonstrated over the last decades. MS specifically has been shown to have long-lasting effects on behavioral and physiological responses of rats (e.g., Caldji et al., 2000; Meaney et al., 1996). Subsequently, it has been suggested that the MS manipulation may serve as a potential environmental model for psychiatric disorders such as depression (Huot et al., 2001; Matthews et al., 1996b), schizophrenia (Ellenbroek et al., 1998, but see Lehmann et al., 2000), anxiety disorders (Caldji et al., 2000) and drug abuse (Matthews et al., 1999). Although some of the literature might support this view, we suggest that further consideration should be given to this issue. Our negative findings are in agreement with the conclusions of the extensive review of Lehmann and Feldon (2000), which highlights the contradicting reports, the variety of separation protocols and the statistical caveats of the MS procedure. Furthermore, judging from our experience as well as that of other researchers, we believe that the overall content of the published data might be biased. Naturally, one is reluctant to present negative results following previous positive published results. Thus, it usually follows that studies are repeated several times before the desired effect is demonstrated, a conduct that obviously casts doubt upon the robustness of the effect. We hope that as more data are collected and presented, a more conclusive view of the MS manipulation will be established, leading to a better understanding of its effects.

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