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Pharmacology, Biochemistry and Behavior 70 (2001) 397–409

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Early social isolation, but not maternal separation, affects behavioral sensitization to amphetamine in male and female adult rats

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Received 11 January 2001; received in revised form 7 June 2001; accepted 23 July 2001

Abstract

Early life stressful manipulations, such as maternal separation (MS) or social isolation (SI), can influence the neurobiological development of rats and alter the response of adult animals to drugs of abuse. The present study examined the acute and sensitized behavioral responses (locomotor activity (LMA) and stereotypy) induced by amphetamine after MS or SI in male and female rats. In addition, the hypothesis that the combination of SI and MS could lead to additional effects on the behavioral response to amphetamine was tested. After the repetitive, intermittent administration of 1.5 mg/kg D-amphetamine over five consecutive days, the behavioral expression of sensitization to a challenge injection was assessed following a 2-day withdrawal period. In both sexes, MS and SI did not alter the acute locomotor activating effects of amphetamine as measured in the open-field environment after the first administration of the drug. Whereas SI altered the expression of sensitization to amphetamine in both sexes, MS did not affect it. Finally, in none of the behavioral variables measured did MS and SI interact to further modify the behavioral profile of the animals. The present results suggest that a postweaning manipulation of the environment (SI) is more effective than a preweaning manipulation (MS) in modifying the expression of sensitization to amphetamine.

Keywords: Maternal separation; Social isolation; Sex differences; Sprague–Dawley strain; D-amphetamine; Sensitization; Locomotor activity; Stereotypy

1. Introduction

Alterations of social interactions with congeners in early life can induce profound behavioral, neurochemical, as well as endocrinological, changes that persist throughout adulthood (for review, see Hall, 1998). For example, early social isolation (SI) from weaning (21 days of age) to adulthood is a procedure in which the animals are deprived of social contact with conspecifics during development. The so-called SI syndrome is characterized by increased spontaneous locomotor activity (LMA) (Domeney and Feldon, 1998; Gentsch et al., 1988; Rebouças and Schmidek, 1997; Weiss et al., 1999, 2000), disruption of prepulse inhibition (Domeney and Feldon, 1998; Geyer et al., 1993; Varty and Geyer, 1998; Varty and Higgins, 1995; Weiss et al., 1999, 2000, 2001; Wilkinson et al., 1994) but not of latent inhibition (Feldon et al., 1990; Weiss et al., 2001; Wilkinson et al., 1994), alterations of motivational behaviors (Morgan and Einon, 1975) and perseverative tendencies (Jones et al., 1991; Morgan et al., 1975). A further example of environmental manipulation is preweaning maternal separation (MS), which consists of separating pups from their mothers for a long period of time (1–24 h). Although MS procedures vary considerably across laboratories, this manipulation is widely considered as a neonatal stressor (Francis and Meaney, 1999; Zimmerberg and Shartrand, 1992) and has been reported to give rise to profound behavioral and neuroendocrinological modifications in adult animals (Lehmann and Feldon, 2000). Maternally separated animals show altered spontaneous LMA (Kaneko et al., 1994; Lehmann et al., 1999, 2000b;

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0091-3057/01/$ – see front matter
PII: S0091-3057(01)00626-8

Zimmerberg and Shartrand, 1992), increased sensitivity to apomorphine (Ellenbroek and Cools, 1995; Rots et al., 1996) and altered hypothalamo-pituitary–adrenal (HPA) axis functioning with enhanced corticosterone responsiveness to stressors (Meaney et al., 1996; Stanton et al., 1988). Finally, MS enhances latent inhibition (Lehmann et al., 1998, 2000b; Weiss et al., 2001) but does not affect prepulse inhibition (Feldon et al., 2000; Lehmann et al., 2000a; Weiss et al., 2001).

Drugs of abuse are known to have variable behavioral consequences depending on the biological background of the animals. For example, within the same population of subjects, genetic and biological factors such as strain (Haney et al., 1994; Leith and Kuczenski, 1982; Sircar and Kim, 1999; Stöhr et al., 1998), sex (Camp and Robinson, 1988; Haney et al., 1994; Robinson et al., 1982b; Sircar and Kim, 1999) or age (Fujiwara et al., 1987; Kolta et al., 1990) may contribute to individual differences in sensitivity to psychostimulants. In addition, environmental factors during development have been suggested to influence sensitivity to psychoactive compounds. For example, social isolates demonstrate increased amphetamine-induced stereotypy (Sahakian et al., 1975) or not (Smith et al., 1997), but their self-administration profile does not differ from that of grouped controls (Schenk et al., 1988; Zimmerberg and Brett, 1992). Moreover, acquisition of intravenous cocaine self-administration has been reported to be impaired (Phillips et al., 1994), increased (Schenk et al., 1987) or unchanged by SI (Boyle et al., 1991; Bozarth et al., 1989), depending on the dose of cocaine administered (Howes et al., 2000). The effects of SI in the conditioned place preference paradigm are also equivocal: SI impairs conditioned place preference induced by cocaine (Schenk et al., 1986), whereas it has either no influence (Schenk et al., 1986) or prevents amphetamine-induced place preference (Wongwitdecha and Marsden, 1995).

Although isolates are generally reported to be spontaneously hyperactive in response to a novel environment, they do not always differ from grouped rats with respect to the locomotor activating effects of psychostimulants (Einon and Sahakian, 1979; Sahakian et al., 1975; Schenk et al., 1988). However, in the study of Bardo et al. (1995), isolates showed increased susceptibility to develop amphetamine sensitization after repeated drug injections. Furthermore, Ahmed et al. (1995) demonstrated that adult rats housed in SI develop amphetamine sensitization at lower doses (0.5 and 0.75 mg/kg) than socially reared rats, which needed a dose of 1 mg/kg.

Preweaning MS represents a severe early life stressor that influences the addictive vulnerability of animals when they become adults. Maternally separated rats demonstrated blunted locomotor response to amphetamine (Matthews et al., 1996a; Zimmerberg and Shartrand, 1992). However, two studies performed in our laboratory using Wistar rats, but different MS procedures, demonstrated either no effect (Lehmann et al., 1998) or an increased sensitivity to the locomotor activating effects of amphetamine in MS rats (Pryce et al., 2001). Taking all of the above reports into consideration, it is difficult to draw a clear picture of the effects of MS on amphetamine behavioral sensitivity. The discrepancies may in part stem from differences in the strain of rats, the MS procedure or the route of administration. In addition, the temperature used during the MS procedure is known to alter the drug sensitivity profile, with MS performed under warm conditions (34 °C) reducing and MS performed under cold conditions (20 °C) enhancing the amphetamine-induced locomotor hyperactivity (Zimmerberg and Shartrand, 1992). As the effect of MS on the behavioral expression of amphetamine sensitization (LMA and stereotypy) had never been investigated, we thought that it could provide new insights regarding the influence of maternal deprivation on the predisposition of rats to psychostimulants.

Because spontaneous hyperactivity is shown by social isolates in novel environments, it is not always clear from the literature whether changes purportedly induced by drug treatments have clearly been distinguished from the already existing predrug locomotor activation. Therefore, one of the goals of the present study was to determine if the increased sensitivity of isolates to amphetamine, as reported in the literature, could be explained by the predrug LMA levels. In order to test this possible relationship, we used rats from the Sprague–Dawley (SD) strain, which do not show increased spontaneous activity consequent to SI (Geyer et al., 1993; Weiss et al., 2000). In addition, we compared, in the same study, the sensitization profile of males and females from three perspectives. First, the sex influence on sensitivity to drugs of abuse can be a very important issue for human research and only few reports have actually investigated this point (Robinson, 1988; Zimmerberg and Brett, 1992). Second, it has been widely demonstrated that females are more sensitive than males to D-amphetamine treatment (Lehmann et al., 1998; Robinson, 1984; Zimmerberg and Brett, 1992). Third, several studies performed in our laboratories suggest that males are more sensitive to environmental manipulations than females and thus the use of males in the study of animal models using environmentally induced changes in addictive behavior may be important (Feldon et al., 2000). Finally, based on the knowledge that psychostimulants and stressors can induce cross-sensitization by activating similar brain pathways (Ahmed et al., 1995; Antelman et al., 1980; Herman et al., 1984; MacLennan and Maier, 1983), we investigated the effects of a combination of two early life chronic stressors, i.e., preweaning MS and postweaning SI, on amphetamine sensitivity. For the latter, male and female SD rats were first tested for their spontaneous locomotor responses to a novel environment. Then the locomotor-activating effects and stereotyped behavior produced by the acute administration of amphetamine, as well as the expression of sensitization following its repeated, intermittent administration were assessed in the same animals.

2. Methods

2.1. Subjects and housing conditions

The present studies were conducted on SD rats [Zur:SD (Crl:CD®(SD)BR)], bred at the Research Unit, Schwerzenbach, Switzerland. Animals were maintained under standard conditions, in temperature (21 ± 1.0 °C) and humidity (55 ± 5%)-controlled rooms, on a 12–12-h reversed light/dark cycle (lights off at 07:00 h). During the studies, animals had access to food (Nafag, 9431, Nafag Ecossan, Gossau, Switzerland) and water ad libitum. All experiments were carried out in accordance with Swiss federal regulations for animal experimentation.

2.2. Environmental manipulations

For the MS procedure, mating of male and female SD rats was performed in the laboratory such that all the mothers gave birth within 1 week. Immediately after birth, all litters were culled in order to have four males and four females in each litter. The mothers and their pups were reared in solid bottom Macrolon cages containing sawdust (dimensions 59.0 × 38.5 × 20.0) with water and food given ad libitum. Following the culling, the mothers were randomly divided into two experimental groups. One group underwent the MS procedure (MS group), and one control group was left undisturbed from postnatal day (PND) 1 to 21 (NMS group). The MS procedure consisted of separating the rat pups from their mothers for 4 h per day, from PND 1 to 21. The separation was regularly performed each day between 10:00 and 14:00 h. During these 4 h of separation, each pup was kept separately from its littermates on heated sawdust at 28–30 °C.

At weaning (PND 21), NMS and MS pups were separated from their mothers and further divided and reared either in SI (ISO; one rat per cage) or in social groups (GRP; three or four rats per cage), so that for each sex, the four experimental groups (NMS/GRP, NMS/ISO, MS/GRP and MS/ISO) were each composed of seven to eight rats originating from seven to eight different mothers. The latter was observed to avoid any genetic or behavioral bias due to maternal care (“litter effects”; Lehmann et al., 2000a). All animals were reared in solid-bottom Macrolon cages containing sawdust (dimensions 48.0 × 27.0 × 20.0 cm for isolates and 59.0 × 38.5 × 20.0 cm for group-housed). Animals were only disturbed for cleaning purposes, which consisted of changing the cage once a week for isolates and twice a week for grouped animals. Female and male rats were kept in two separate animal rooms, but isolates and grouped rats from the same sex were housed in the same holding room so that isolated rats maintained visual, auditory and olfactory contact with the other animals throughout the studies. At no time were animals handled during the period before experimental manipulations commenced.

2.3. Apparatus

The LMA of the animals was assessed in an open-field environment and was expressed as the total distance traveled in the entire arena in centimeters. The apparatus consisted of eight square arenas (76.5 × 76.5 × 49 cm) made of dark grey plastic, which were located in two experimental rooms illuminated by low light (12 lx). Two video cameras, each fixed above four arenas and relayed to a monitor and a video tracking motion analysis system (Ethovision, Noldus Information Technology BV, Wageningen, The Netherlands) allowed the LMA recording, which was measured in bins of 10 min. Males and females were tested in separate experimental rooms, in counterbalanced groups of four. After 1 h of habituation to the test room, each rat was individually placed into the center of the open-field arena. Because of the large number of animals, we tested all rats in a 4-day shift.

2.4. Drug

D-amphetamine sulphate (Sigma, Switzerland) was prepared as the salt in a saline solution (NaCl 0.9%) and was administered intraperitoneally (ip) in a volume of 1 ml/kg body weight at a concentration of 1.5 mg/kg.

2.5. Procedure

2.5.1. Acute response to amphetamine

Before any experimental testing commenced, all animals were kept in the appropriate housing condition for 12 weeks after weaning. To investigate the behavioral activation induced by an acute amphetamine injection, on Day 1, all rats (for each sex, seven to eight rats in each of the four experimental groups) were tested in a first open-field test composed of three sessions. First, the spontaneous LMA of the rats in response to novelty was recorded during 30 min without any drug treatment (Habituation Session 1). Second, all animals received an intraperitoneal injection of saline (NaCl 0.9%, 1 ml/kg body weight) and returned immediately to the open-field arena for a further 30-min session (Saline Session 1). Third, the LMA response of the rats to an acute amphetamine treatment was recorded for a further 2 h after an intraperitoneal injection of 1.5 mg/kg D-amphetamine (Amphetamine Session 1). Thus, all animals were used as their own controls. After completion of the experiment, an experimenter blind to the experimental treatments scored, on videotapes, several stereotyped behaviors for the first 30 min of the amphetamine session. The reason for choosing this period of time will become clear after presentation of the results on the locomotor activating effects of amphetamine (see Results). The stereotyped behaviors were scored as follows: *Rearing* was scored when the two front paws left the floor, excluding grooming behavior. *Head movements* made in the two directions, either horizontally (left–right) or vertically (up–down) were scored as such.

Sniffing was scored when it was directed either towards the floor or a wall of the open-field arena. For each rat, once every 10 s, it was decided if one (at most) of any type of stereotyped behavior had occurred. Based on a total possible score of 180 occurrences, the score for each stereotyped behavior was calculated as a percentage.

2.5.2. Development of amphetamine sensitization

To induce sensitization to the psychostimulant, on Days 2–5, all rats (males and females) received an intraperitoneal injection of D-amphetamine in the home cage, around 12:00 h. No behavioral assessment was performed during these 4 consecutive days.

2.5.3. Expression of amphetamine sensitization

After 2 days of withdrawal from amphetamine (Days 6 and 7), during which the animals remained undisturbed in the animal rooms, the effects of the repeated amphetamine treatment were assessed on Day 8 in a second open-field session (i.e., 72 h after the last injection). This second open-field test was similar to the first one: the animals were first tested for 30 min without any drug treatment (Habituation Session 2). Then, they were injected with saline and their LMA was further recorded for 30 min (Saline Session 2). Directly after the saline session, the expression of sensitization to the locomotor activating and stereotypy-producing effects of amphetamine was investigated by challenging all rats with an intraperitoneal injection of 1.5 mg/kg D-amphetamine. Activity was monitored during the following 2-h test (Amphetamine Session 2) and stereotyped behaviors for the first 30 min of this session.

2.6. Statistical analysis

The data were analyzed using the StatView software (Abacus Concepts Inc., Berkeley, CA, 1992). Animals were weighed at weaning and at the beginning of the experiment (adult age) and data analyzed using a $2 \times 2 \times 2$ analysis of variance (ANOVA) consisting of main factors of Sex (male versus female rats), MS (MS versus NMS) and SI (isolated versus group-reared rats).

We investigated the influence of sex on the behavioral profile induced by MS and/or SI. LMA effects during the habituation and saline sessions was assessed using overall $2 \times 2 \times 2 \times 2 \times 3$ ANOVAs consisting of three between-subjects main factors of Sex (males versus females), MS (MS versus NMS) and SI (isolated versus group-reared rats) and of two repeated measurement factors of Treatment (acute versus challenge amphetamine injection) and Time Bin (three bins of 10 min). In addition, LMA effects during the amphetamine sessions were assessed using $2 \times 2 \times 2 \times 2 \times 12$ ANOVAs consisting of three between-subjects main factors of Sex, MS (MS versus NMS) and SI (isolated versus grouped) and of two repeated measurement factors of Amphetamine Treatment (acute versus challenge injection) and Time Bin (12 bins of 10 min). Finally, the effects of the different factors on stereotyped behaviors (rearing, head movement, sniffing) were assessed using a $2 \times 2 \times 2 \times 2$ ANOVA consisting of three between-subjects main factors of Sex, MS and SI and a repeated measurement factor of Amphetamine Treatment. The use of Amphetamine Treatment (acute versus challenge amphetamine injection) as a within-subjects factor for all rats served to compare the acute versus the sensitized responses (LMA and stereotyped behavior) induced by amphetamine. The aforementioned analyses yielded large sex differences in the behavior of the animals (see Results). Since these sex differences could have masked subtle effects of either SI or MS, we conducted separate ANOVAs for males and females. Whenever an interaction between two factors was significant, a post hoc *t* test, using the Fisher's protected least significant difference (PLSD) test was applied.

3. Results

3.1. Weight of the rats at weaning (PND 21)

There was no effect of MS on the body weight of the pups at weaning, $F(1,54) = 1.6$, $P > .21$. Furthermore, the sex of the pups did not affect the body weight of the animals, $F(1,54) = 2.3$, $P > .13$.

3.2. Weight of the rats at the beginning of the experiment

The body weight of the animals was significantly different between the sexes, $F(1,54) = 469.5$, $P < .001$, with males (483.0 ± 8.0 g) being heavier than females (288.8 ± 3.8 g). Neither MS nor SI had a differential effect on the body weight of the animals.

3.3. Habituation sessions

3.3.1. Sex effect

Females showed much higher levels of LMA (as expressed in distance traveled in centimeters) relative to their male counterparts, $F(1,54) = 55.5$, $P < .001$ (see Fig. 1). All animals demonstrated habituation to the open-field environment, as reflected by a gradual decrease of their LMA throughout the 30-min test session (significant main effect of time bin, $F(2,108) = 226.0$, $P < 0.001$; see Fig. 1), which was similar for the two sexes. As explained above, because of the large sex difference, we conducted separate analyses for males and females to investigate the effects produced by MS and/or SI.

3.3.2. Males

A significant LMA reduction was demonstrated for the second habituation session (performed after repeated amphetamine treatment) relative to the first session (naive animals), $F(1,27) = 20.8$, $P < .001$ (see Fig. 1), this differ-

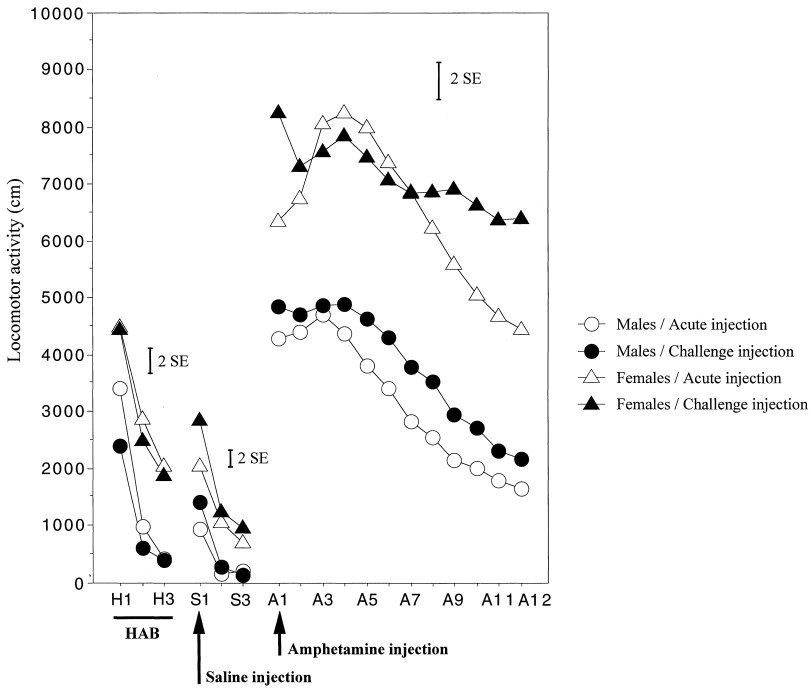

Fig. 1. Sex effect on the LMA profile of SD rats. The total distance traveled (cm) is presented in 10-min bins for the habituation period of 30 min (H1 to H3), the 30 min following saline injection (S1 to S3) and the 2 h following the injection of 1.5 mg/kg D-amphetamine (A1 to A12), in males ($n=31$) and females ($n=31$). Data are presented for the open-field Test 1 (acute injection of amphetamine; white symbols) and for the open-field Test 2 performed after repeated amphetamine treatment followed by a 2-day drug withdrawal period (challenge injection of amphetamine; black symbols). Data are collapsed over the factors of MS and SI. The upper bars represent two standard errors (S.E.) derived from the ANOVA for each phase.

ence being significant for the first 20 min, Treatment × Time Bin interaction, $F(2,54)=13.1$, $P<.001$. MS rats were significantly more active than their NMS counterparts, irrespective of the habituation session, $F(1,27)=14.0$, $P<.001$ (see Fig. 2). SI did not affect the LMA of the rats during habituation (see Fig. 3), and there was no MS × SI interaction.

3.3.3. Females

The habituation profile of females was similar for the two habituation sessions (before and after repeated amphetamine treatment; see Fig. 1). Furthermore, a trend towards increased LMA levels in MS animals was apparent, $F(1,27)=4.0$, $P=.06$ (see Fig. 2). Finally, SI did not affect the LMA of the rats during habituation (see Fig. 3), and there was no MS × SI interaction.

3.4. Saline sessions

3.4.1. Sex effect

A large effect of sex on LMA was maintained during the saline sessions, with females demonstrating greater levels of LMA relative to their male counterparts, $F(1,54)=57.5$, $P<.001$ (see Fig. 1). All animals demonstrated habituation to the open-field environment as reflected by a gradual decrease of their LMA throughout the 30-min test session, $F(2,108)=149.1$, $P<.001$, which was similar for the two sexes. Due to a large sex effect observed in the saline sessions, all consequent data were analyzed separately for males and females.

3.4.2. Males

Although for all males, LMA was increased during the second saline session relative to the first, $F(1,27)=8.3$, $P<.01$ (see Fig. 1), this effect was restricted to the first 10 min. Fisher's PLSD test, $P<.05$, indicated an increased behavioral reaction to the saline injection after the repeated treatment with amphetamine, Treatment × Time Bin interaction, $F(2,54)=7.0$, $P<.01$. MS rats demonstrated increased LMA relative to NMS rats, $F(1,27)=7.9$, $P<.01$ (see Fig. 2), irrespective of the saline session. Finally, SI did not modify the LMA response of the rats during the saline treatment (see Fig. 3), and no MS × SI interaction was found.

3.4.3. Females

Similar to the data observed for males, females showed increased LMA in the second saline session relative to the first, $F(1,27)=4.7$, $P<.05$, with this effect being restricted to the first 10 min, Treatment × Time Bin interaction $F(2,54)=3.3$, $P<.05$; Fisher's PLSD test, $P<.01$ (see Fig. 1). Although a main effect of MS revealed an increase in LMA in MS relative to NMS (see Fig. 2), this effect was significant only for the first 10 min of the session,

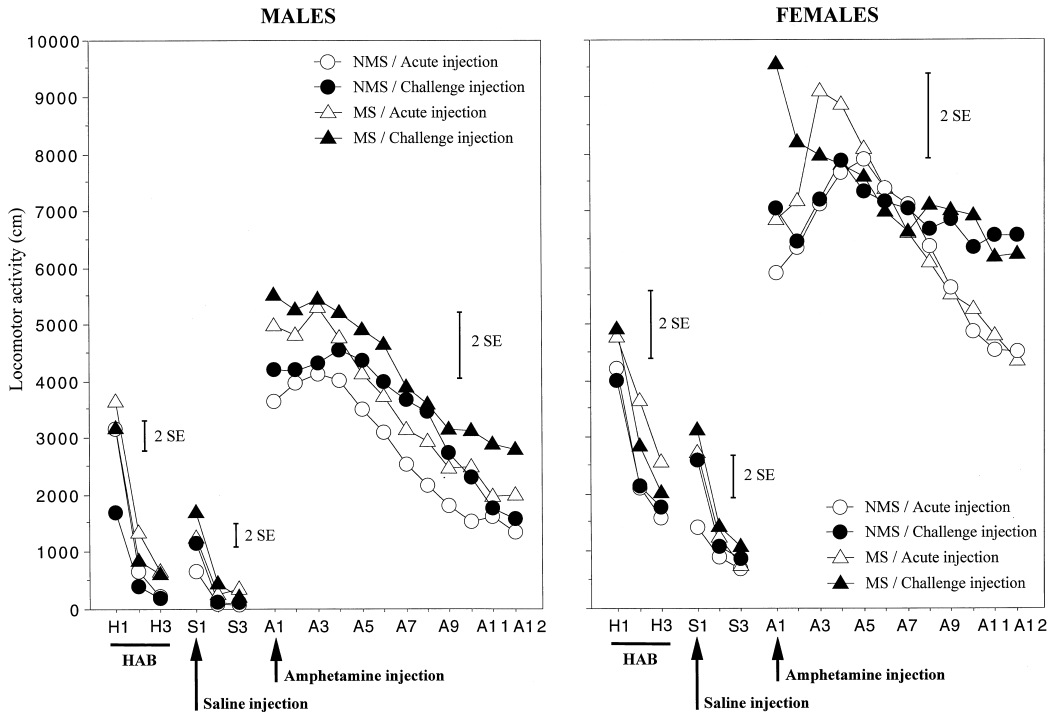

Fig. 2. Effect of MS on the LMA profile in male (left panel) and female (right panel) SD rats. The total distance traveled (cm) is presented in 10-min bins for the habituation period of 30 min (H1 to H3), the 30 min following saline injection (S1 to S3) and for the 2 h following the injection of 1.5 mg/kg D-amphetamine (A1 to A12), in males maternally separated (MS; $n=15$) or nonmaternally separated (NMS, $n=16$) and in females maternally separated (MS; $n=15$) or nonmaternally separated (NMS; $n=16$). Data are presented for the open-field Test 1 (acute injection of amphetamine; white symbols) and for the open-field Test 2 performed after repeated amphetamine treatment followed by a 2-day drug withdrawal period (challenge injection of amphetamine; black symbols). Data are collapsed over the factor of SI. The upper bars represent two standard errors (S.E.) derived from the ANOVA for each phase.

Fisher's PLSD test, $P<.01$. Finally, there was no effect of SI on LMA after saline injection in females (see Fig. 3), as well as no MS × SI interaction.

3.5. Amphetamine sessions

3.5.1. Sex effect

A large sex difference in the LMA was retained during the 2-h test after amphetamine injection, $F(1,54)=53.9$, $P<.001$ (see Fig. 1). A general increase in LMA after the challenge injection of amphetamine (Session 2) relative to the acute response (Session 1), $F(1,54)=10.5$, $P<.01$, reflected the existence of behavioral sensitization to the repeated amphetamine treatment (i.e., increased LMA to a challenge injection of amphetamine after a period of drug withdrawal). However, the expression of sensitization to amphetamine was different between male and female rats, Sex × Treatment × Time Bin interaction, $F(11,594)=5.6$, $P<.001$. Males expressed sensitization towards the end of the test session from Bin 7 to Bin 10, whereas females showed sensitization for Bin 1 and then from Bin 9 to Bin 12 (see Fig. 1).

3.5.2. Males

Although a significant main effect of treatment, $F(1,27)=7.1$, $P<.02$, revealed the existence of sensitization to the drug for all males, a significant SI × Treatment × Time Bin interaction, $F(11,297)=2.8$, $P<.01$, reflected the fact that only grouped rats exhibited sensitization to the locomotor activating effects of amphetamine (Session 2). The latter was demonstrated by the prolonged activity of grouped rats as compared with their acute response to the drug (significant from Bin 6 to Bin 12; see Fig. 3). In contrast, isolates showed absolutely no LMA differences before and after repeated amphetamine treatment. Furthermore, the acute administration of amphetamine enhanced the LMA of grouped rats to a greater extent than for isolates, but only at the beginning of the test session, Fisher's PLSD test, $P=.07$ for Bin 1 and $P=.05$ for Bin 2 (see Fig. 3). In addition, after the challenge injection of amphetamine, isolates showed reduced LMA relative to grouped rats, mainly at the end of the test session, Fisher's PLSD test, $P<.05$ for Bin 8 and $P<.03$ for Bin 10 (see Fig. 3).

Finally, MS animals demonstrated a trend towards generally higher levels of LMA relative to their NMS counterparts, $F(1,27)=3.8$, $P=.06$ (see Fig. 2). However, the MS procedure had no influence on amphetamine sensitization (lack of MS × Treatment × Time Bin interaction). Finally, no MS × SI interaction was found.

3.5.3. Females

After the challenge injection of amphetamine, a trend towards increased LMA levels as compared with the acute

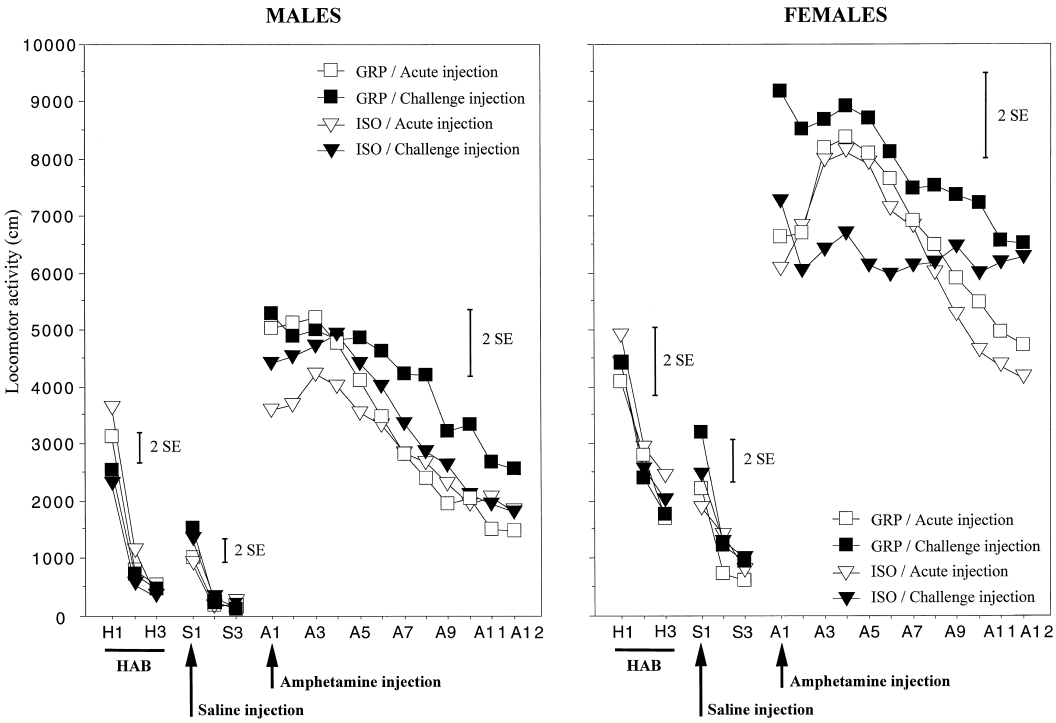

Fig. 3. Effect of SI on the LMA profile in male (left panel) and female (right panel) SD rats. The total distance traveled (cm) is presented in 10-min bins for the habituation period of 30 min (H1 to H3), the 30 min following saline injection (S1 to S3) and for the 2 h following the injection of 1.5 mg/kg D-amphetamine (A1 to A12), in males isolated (ISO; $n=16$) or grouped (GRP; $n=15$) and in females isolated (ISO; $n=15$) or grouped (GRP, $n=16$). Data are presented for the open-field Test 1 (acute injection of amphetamine; white symbols) and for the open-field Test 2 performed after repeated amphetamine treatment followed by a 2-day drug withdrawal period (challenge injection of amphetamine; black symbols). Data are collapsed over the factor of MS. The upper bars represent two standard errors (S.E.) derived from the ANOVA for each phase.

response to the drug was apparent, Treatment main effect, $F(1,27)=4.1$, $P=.05$. However, the significant SI × Treat-Treatment × Time Bin interaction, $F(11,297)=2.0$, $P<.03$, revealed a different expression of amphetamine sensitization between grouped and isolated rats (see Fig. 3). In grouped animals, the expression of sensitization was twofold. First, grouped females showed a shift to the left of the peak response, Fisher's PLSD test, $P<.01$, for Bin 1 as compared with Bin 1 of the acute response, and did not show the characteristic drug-induced inverted U shape due to the maintenance of very high LMA levels for the first 50 min of the test session (Bins 1–5). Second, grouped females exhibited prolonged LMA towards the end of the test session relative to their gradual LMA decrease after the acute injection, Fisher's PLSD test, $P<.03$ for Bins 10 and 12, $P=.06$ for Bins 9 and 11 (see Fig. 3). In contrast, for isolated females only Bins 11 and 12 were higher relative to the corresponding bins during the acute response, $P=.06$ and $P<.03$, respectively (Fisher's PLSD test). However, as depicted in Fig. 3, isolated females showed constant LMA levels during the entire 2 h of the test session, whereas grouped females demonstrated a gradual LMA decrease throughout the test session. Furthermore, although grouped and isolated females expressed similar LMA responses to the acute amphetamine injection, isolates exhibited reduced LMA levels relative to grouped controls after the challenge injection, Fisher's PLSD test, $P<.04$ for Bins 2 and 5, $P=.06$ for Bins 3 and 6 (see Fig. 3).

In addition, the MS × Time Bin interaction, $F(11,297)=3.0$, $P<.001$, revealed that MS rats were slightly more active than NMS rats, but only for the first 10 min of the session, Fisher's PLSD test, $P<.03$ (see Fig. 2). However, analysis of covariance revealed that this apparent increased activity in MS females following amphetamine was entirely explicable in terms of baseline activity in these animals. The MS procedure did not influence the expression of sensitization after chronic amphetamine treatment (lack of MS × Treatment × Time Bin interaction). Finally, there was no MS × SI interaction.

3.6. Stereotyped behaviors

In the present study, by using a low dose of amphetamine and a large open-field environment, we clearly favored locomotion and probably reduced the likelihood for animals to be engaged in stereotyped behavior. Indeed, we observed that intense stereotyped behavior frequently appeared when the rats were in the corners of the open field (more restricted areas). Nevertheless, our results based on the locomotor activating effects of amphetamine demonstrated differences in the LMA profile between isolated and group-reared rats following the injection. Therefore, we wanted to examine

Table 1
Percentage ± S.E.M. of stereotyped behavior during the first 30 min following the injection of 1.5 mg/kg D-amphetamine

	Rearing (%)		Head movements[a] (%)		Sniffing (%)	
	Acute	Challenge	Acute	Challenge	Acute	Challenge
Males[b]	25.3 ± 3.3	31.8 ± 3.3	39.7 ± 2.1	42.7 ± 2.5	2.0 ± 0.7	4.0 ± 1.3
Females[b]	39.7 ± 3.9	53.6 ± 3.7[c]	29.7 ± 2.5	22.6 ± 2.4[c]	2.3 ± 1.4	10.1 ± 2.4[c]
Males						
NMS/GRP	31.0 ± 7.4	30.4 ± 7.4	39.0 ± 4.6	36.1 ± 5.7	2.3 ± 1.2	3.3 ± 1.9
NMS/ISO	15.6 ± 6.2	25.6 ± 7.3	40.8 ± 4.7	46.9 ± 6.0	3.0 ± 2.1	8.0 ± 4.7
MS/GRP	31.5 ± 5.5	38.8 ± 7.9	43.7 ± 3.8	41.5 ± 5.4	0.0 ± 0.0	1.5 ± 0.6[c]
MS/ISO	23.8 ± 6.4	33.4 ± 4.0	36.0 ± 3.8	45.9 ± 1.7[c]	2.3 ± 1.5	2.8 ± 0.9
Females						
NMS/GRP	53.2 ± 9.8	67.3 ± 5.7	24.5 ± 5.3	15.9 ± 3.6	1.3 ± 0.8	5.9 ± 2.7
NMS/ISO	26.6 ± 4.7	47.1 ± 7.2[c]	35.5 ± 4.2	23.3 ± 3.6[c]	5.9 ± 5.4	13.0 ± 7.6
MS/GRP	43.4 ± 6.6	65.9 ± 4.1[c]	25.2 ± 4.2	16.0 ± 1.8	1.3 ± 0.9	6.6 ± 2.2[c]
MS/ISO	35.2 ± 6.9	31.4 ± 4.3	34.0 ± 5.7	36.7 ± 5.9	0.8 ± 0.5	15.5 ± 5.1[c]

NMS, nonmaternally separated rats; MS, maternally separated rats; GRP, group-reared rats; and ISO, isolation-reared rats.
[a] Total of horizontal and vertical head movements.
[b] Data are collapsed over the four experimental groups.
[c] Significantly different from the acute response ($P < .05$).

whether this could be explained by the fact that isolates were more engaged in stereotypy behaviors at the beginning of the test session, which would also be an expression of sensitization to amphetamine.

3.6.1. Sex effect

During the first 30-min test session, the challenge injection of amphetamine after withdrawal significantly enhanced rearing, $F(1,54) = 12.8$, $P < .001$, and sniffing, $F(1,54) = P < .001$, behavior relative to the levels obtained after the acute amphetamine injection (see Table 1). Furthermore, sex differences were apparent. Over the two amphetamine sessions and in contrast to males, females were engaged almost half of the time in rearing, $F(1,54) = 22.4$, $P < .001$. However, the picture was reversed when head movements (total of horizontal and vertical head movements) were considered. Indeed, males showed higher levels of head movements relative to females (see Table 1). Finally, no sex differences were apparent for the occurrence of sniffing behavior during the first 30-min test session following amphetamine injection.

3.6.2. Males

Male rats did not demonstrate any significant increase in stereotyped behaviors (rearing, head movements or sniffing) during the first 30-min test session following the challenge injection of amphetamine as compared with the same period after acute drug injection (see Table 1). Moreover, neither MS nor SI influenced the stereotyped behaviors of the males during the first 30 min of the amphetamine session (see Table 1).

3.6.3. Females

A significant effect of the repeated amphetamine treatment was seen on the stereotyped behavior of females, as reflected by an increase in stereotyped activities following the challenge injection of amphetamine relative to the first session, $F(1,27) = 11.5$, $P < .01$ for rearing, $F(1,27) = 8.5$, $P < .01$ for total head movements and $F(1,27) = 16.0$, $P < .001$ for sniffing (see Table 1). In addition, SI reduced rearing behavior, $F(1,27) = 18.8$, $P < .001$, but increased head movements, $F(1,27) = 10.3$, $P < .01$, during the first 30-min test session. In contrast, MS did not modify stereotyped behavior in the female rats. Finally, both MS and SI did not influence sniffing behavior (see Table 1).

4. Discussion

The present study was designed to investigate the consequences of a combination of two early life stressful manipulations (i.e., MS and SI) in male and female SD rats on (1) the spontaneous LMA response to a novel open-field environment, (2) the acute locomotor response to D-amphetamine and (3) the expression of sensitization to a challenge injection of D-amphetamine following a 2-day withdrawal period from its repeated administration over five consecutive days. We report here distinctive effects of MS (= preweaning environmental manipulation) and SI (= postweaning environmental manipulation) on spontaneous LMA and on the expression of sensitization to the behavioral effects of amphetamine. In both males and females, MS enhanced spontaneous LMA, whereas SI did not affect it. In contrast, MS did not modify the expression of amphetamine sensitization, whereas SI prevented it in males and considerably affected it in female rats. More generally, the sensitization profile was modified by the rats' sex, in the direction of females demonstrating sensitization to a larger extent than males. Although the effects of MS and SI on sensitivity to the locomotor activating effects of

an acute injection of amphetamine were very weak, the response profiles were different. Indeed, MS rats exhibited a marginal increase in LMA restricted to the very beginning of the session (which could be attributed to the already preexisting higher spontaneous LMA levels), whereas male social isolates demonstrated a slight reduction of their LMA at the beginning of the session. Nevertheless, we are unable to establish that the two environmental manipulations used in the present study affected the sensitivity of SD rats to the acute administration of amphetamine. Finally, in the entire set of data, no interaction between the effects of MS and SI was found, suggesting that the combined manipulation of MS and SI does not represent a relevant model for the study of developmental/environmental influence on sensitivity to amphetamine. The general statement from the literature is that MS reduces and SI enhances the sensitivity to amphetamine (Bardo et al., 1995; Matthews et al., 1996a). However, Hall et al. (1999) demonstrated that amphetamine increases extracellular levels of DA in the nucleus accumbens similarly in adult maternally separated and socially isolated rats. Consequently, if amphetamine-mediated activation of the meso-accumbens dopaminergic system is somehow similar in MS and SI rats, the difference in behavioral sensitization seen between the two groups might be explained by a modulation of other neurotransmitter systems.

4.1. Expression of amphetamine sensitization and 'conditioning' to the intraperitoneal injection

In the present study, sensitization to amphetamine did not express itself as a shift upwards in LMA relative to the acute response but rather as the maintenance of very high LMA levels over a longer period of time. The environmental conditions during repeated amphetamine or cocaine treatments (injections in home cage or testing system) are known to influence the magnitude of sensitization to the drug, with injections in the testing system facilitating the induction and expression of sensitization (Browman et al., 1998b; Robinson et al., 1998). Therefore, we cannot exclude that the particular expression of sensitization in our study is not related to the fact that the repeated injections of amphetamine were administered in a distinctly different environment (home cage) from the one used to assess the expression of the sensitization (open field).

During the saline session, in the second testing conducted after the repeated amphetamine treatment, all rats demonstrated significantly enhanced LMA for the first 10 min relative to the same period during the first open-field testing. This effect seems to be injection-dependent rather than context-dependent. Indeed, rats did not show an increased reaction to the open-field arena itself, but rather demonstrated reduced LMA levels in the second habituation session as compared with the first, reflecting a between-tests habituation to the open-field environment. This 'conditioning to the injection' has already been reported after amphetamine (Badiani et al., 1997) or cocaine (Browman et al., 1998a) pretreatment and supports the contention that environmental cues can influence the behavioral response to a drug. Indeed, this effect of injection on activity is not discernible from the locomotor activating effects specific to the drug. Moreover, Kalivas and Duffy (1990) showed that saline injection, administered after repetitive, intermittent cocaine pretreatment, can significantly enhance dopamine in the nucleus accumbens of rats.

4.2. Effect of SI on behavioral activity in response to a new environment and after acute D-amphetamine treatment

In both males and females, non-drug-treated social isolates did not differ from group-housed rats in their spontaneous LMA in the open-field environment. In other words, isolates did not react differently than social rats to two potentially stressful events: (a) confrontation with a completely novel environment during the first habituation session and (b) stress of an intraperitoneal injection during the two saline sessions. The lack of locomotor hyperactivity in isolates from the SD strain has already been reported by Geyer et al. (1993), as well as in a recent study performed in our laboratory (Weiss et al., 2000). Consequently, this specific effect seems to be an intrinsic characteristic of the SD strain, and it is not dependent on the rats' sex. In response to an acute injection of amphetamine, the effect of SI on the LMA response of the animals was weak in males (tending towards a reduction) and clearly absent in females. Furthermore, isolates generally demonstrated less rearing behavior than group-housed controls. We can thus conclude that SI did not affect the sensitivity of the animals to an acute administration of amphetamine in the present study, thereby confirming our previous findings using Wistar rats (Weiss et al., 1999). This finding is also in line with the study of Einon and Sahakian (1979), who also failed to demonstrate a difference in sensitivity to the acute LMA effects of 1.5 mg/kg D-amphetamine between isolated and grouped rats from the SD strain. Similarly, studies using different rat strains such as hooded (Sahakian et al., 1975) or Long–Evans (Schenk et al., 1988) have reported the same lack of a SI effect on the locomotor activating effects of a D-amphetamine dose ≥ 1 mg/kg. However, our results contradict the findings of Sahakian et al. (1975) and Einon and Sahakian (1979), who found increased D-amphetamine induced stereotypy in isolates following administration of 0.5–1.5 and 0.5 mg/kg, respectively. Nevertheless, methodological differences, including the period of investigation (only the first 30 min after amphetamine injection in our study) and the method used to assess stereotyped behavior, may account for these apparent discrepancies. Moreover, as suggested by Sahakian et al. (1975), LMA and stereotyped behaviors

may be mediated by different mechanisms and, therefore, would not necessarily be modified concomitantly.

4.3. Effect of SI on the expression of D-amphetamine sensitization

SI considerably altered the expression of sensitization to amphetamine, both in male and female rats. In males, sensitization to amphetamine was totally absent in social isolates, whereas in females, SI modified its expression. Although the LMA levels of isolated females were lower relative to grouped controls, these LMA levels were maintained during the entire testing session, demonstrating thus a prolonged sensitivity of isolated females to the drug. It would have been interesting to extend the LMA measurement beyond the 2-h to investigate the duration of this hypersensitivity of isolated females to the challenge injection. Grouped rats of both sexes demonstrated amphetamine sensitization, mainly by maintaining high LMA levels towards the end of the session. In comparison with grouped controls, isolates of both sexes showed reduced LMA levels to the challenge injection, although their LMA profiles were very similar following the acute injection. The reduced response to amphetamine in isolates following the challenge injection of the drug could have been explained by an increase in stereotyped behavior. In males, during the first 30 min of the test session, no difference in stereotypy was found between isolated and grouped rats, supporting the lack of a SI effect on sensitization to the locomotor activating effects of amphetamine. In contrast, in isolated females, the increase in stereotyped head movements may have partly limited the locomotor hyperactivity induced by the challenge injection of amphetamine relative to grouped controls.

The present findings partly contradict those of Bardo et al. (1995), which showed that only isolates expressed amphetamine sensitization. Although Bardo et al. (1995) also used male rats from the SD strain, methodological differences, such as a withdrawal period of 6 days or a control group composed of rats reared in an enriched environment, may explain some of the discrepancies. In addition, by using an open-field environment in the present study, we favored locomotion and probably did not have the optimal conditions to trigger stereotyped behavior. Finally, the dose of D-amphetamine, as well as the route of administration (subcutaneous versus intraperitoneal) may render the comparison between studies difficult. Indeed, a pharmacological threshold for the induction of sensitization may exist, since in the study of Ahmed et al. (1995), rats reared in isolation as adults developed sensitization to lower D-amphetamine doses (0.5–0.75 mg/kg) than did grouped rats, whereas at 1.0 mg/kg, there was no difference between the two groups. Thus, we cannot exclude that in our study, the dose of 1.5 mg/kg D-amphetamine was too high to show a SI-induced increased sensitization in males.

4.4. Effect of MS on behavioral activity in response to a new environment and after acute D-amphetamine treatment

During the habituation and saline sessions, MS males and females demonstrated increased levels of spontaneous activity in the open-field environment relative to their NMS counterparts. However, previous studies from our laboratory, using several MS procedures but always Wistar rats, failed to demonstrate a significant effect of MS on spontaneous LMA (Lehmann et al., 1999, 2000b). Moreover, several other laboratories, using different rat strains, report either no effect of MS (Matthews et al., 1996a, with Lister hooded rats; Zimmerberg and Shartrand, 1992, with Long–Evans rats) or decreased LMA in a novel environment (Koch and Arnold, 1972, with albino rats; Matthews et al., 1996b, with Lister hooded rats). The latter may suggest that the MS-induced spontaneous locomotor hyperactivity is more specific to the SD rat strain. Of relevance in this context is the study of Kaneko et al. (1994), in which a spontaneous locomotor hyperactivity was also found following MS. In parallel, as already reported above, the well-described locomotor hyperactivity of isolates is not apparent in the SD strain. Therefore, the hyperactivity of MS rats and the lack of a SI-induced effect on spontaneous LMA seem to be characteristic of the SD strain. If this observation holds true, it reveals that for a still unknown reason, environmental manipulations in the SD strain lead to LMA profiles that are opposite to those of other rat strains.

After amphetamine treatment, MS males and females demonstrated slight increases in LMA. Nevertheless, this MS effect was very weak and was entirely explicable by the preexisting locomotor hyperactivity in MS rats as compared with NMS rats. The lack of effect of MS on sensitivity of rats to a dose $\geq$ 1.0 mg/kg D-amphetamine has already been demonstrated in several laboratories, using different rat strains and MS procedures (Lehmann et al., 1998; Matthews et al., 1996a).

4.5. Effect of MS on the expression of D-amphetamine sensitization

The present findings clearly show that MS had no effect on the expression of sensitization to the locomotor or stereotypy effects produced by repeated administration of amphetamine. This finding is in line with the lack of a MS effect on the acute behavioral response to amphetamine. Moreover, this lack of MS influence on the expression of sensitization was consistent across both sexes and on a background of MS-induced spontaneous hyperactivity. This result is in direct contrast with the effects induced by SI. In fact, SI alters the expression of amphetamine sensitization without affecting spontaneous levels of activity. To the best of our knowledge, we are the first to present the effects of MS on the expression of amphetamine sensitization.

4.6. Sex differences

In the present study, sex differences were apparent across all experimental phases. In response to a novel environment, females demonstrated consistently higher spontaneous LMA relative to their male counterparts, confirming studies reported in the literature (Joseph and Gallagher, 1980; Lehmann et al., 1998; Slob et al., 1981). Furthermore, females showed increased locomotor responses to both acute and repeated amphetamine treatments relative to males. Although the present findings support the contention that females are more sensitive to psychostimulants than males (Camp and Robinson, 1988; Robinson, 1988; Robinson et al., 1982a; Sircar and Kim, 1999) even when the pharmacokinetic variables are taken into account, the reason for such a difference is not completely understood. A possible explanation for this sex difference could be related to the already existing important difference between males and females in spontaneous LMA. Indeed, it has been reported that hyperactive animals are more prone to show sensitization to psychostimulants (Bevins et al., 1997; Hooks et al., 1991). Another possible explanation that may account for several behaviors mediated by the dopaminergic system such as LMA is related to gonadal hormones. Indeed, in females but not in males, estrogens have been reported to increase dopaminergic activity in the striatum and nucleus accumbens. Thus, estrogens may facilitate sensitization to psychostimulants in females relative to males (Becker, 1990; Becker and Cha, 1989; Diaz-Veliz et al., 1994; Sircar and Kim, 1999). Although we did not control the estrous cycle of females in the present study, this is reflected by the statistical data variations around the mean and may explain the larger errors bars for the data on females relative to males during amphetamine treatment.

Whereas no influence of sex was seen on the effects produced by MS or SI on LMA during habituation and after saline injection, the effects of SI on the expression of amphetamine sensitization were sex dependent, reflecting a direct influence of sex on the behavioral effects produced by this environmental manipulation. The reason why the SI-induced effect on the expression of amphetamine sensitization was modified by sex is not known. However, it is likely to involve combined effects of this environmental manipulation with the effects of gonadal hormones on the dopaminergic system.

In summary, the present study demonstrates that the preweaning manipulation of MS enhances, both in males and females, spontaneous LMA but does not affect hyperactivity induced by amphetamine or the expression of sensitization to its repeated, intermittent administration. In contrast, the postweaning manipulation of SI prevents in males and considerably affects in females the expression of amphetamine sensitization, without affecting either spontaneous activity or the acute effects of amphetamine on LMA. Finally, the combination of MS and SI does not further modify the behavioral profile that is observed when the two environmental manipulations are conducted separately, suggesting, therefore, that the combined manipulation of MS and SI does not represent a relevant model for the study of developmental/environmental influences on the sensitivity of adult rats to amphetamine.

Acknowledgments

This study was supported by the Swiss Federal Institute of Technology (Zurich, Switzerland). Isabelle Weiss was further supported by a grant from F. Hoffmann-LaRoche, (Basel, Switzerland). We would like to thank Dr. Isabelle Allmann-Iselin, Natascha Häni, Thomas Karich and Sandra Egger for their care of the animals, Peter Schmid for his technical assistance and Bonnie Strehler for her secretarial help. Isabelle Weiss is also grateful to Dr. Olivier Raineteau and Gaël Hedou for their insightful comments on the manuscript and to Maud Seguy for her invaluable help during the study.

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