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Long-Term Effects of Repeated Maternal Separation on Three Different Latent Inhibition Paradigms

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LEHMANN, J., T. STÖHR, J. SCHULLER, A. DOMENEY, C. HEIDBREDER AND J. FELDON. *Long-term effects of repeated maternal separation on three different latent inhibition paradigms.* PHARMACOL BIOCHEM BEHAV **59**(4) 873–882, 1998.—In the present study we investigated the effect of repeated maternal separation on postnatal days 12, 14, 16, and 18 for 6 h/day on Wistar rats on three latent inhibition (LI) paradigms: two-way active avoidance, conditioned emotional response (CER), and conditioned taste aversion (CTA). In addition, hyperactivity induced by *d*-amphetamine and stereotypies induced by apomorphine were evaluated. In all three LI experiments, the control animals showed only marginal LI, whereas the maternally separated animals showed enhanced LI (only males in CTA). In two-way active avoidance within the nonpreexposed condition maternally separated animals showed improved acquisition of avoidance learning compared with the control animals. Sensitivity in response to amphetamine and apomorphine was not altered by the maternal separation procedure. Thus, maternal separation in this study, contrary to previous reports, but in line with results obtained following early handling before weaning, led to enhancement of the LI phenomenon as assessed in each of the three procedures. As our maternal separation procedure (6 h on days 12, 14, 16, and 18) led to behavioral outcomes that differed from those reported by Ellenbroek and Cools (24 h on day 10), it is suggested that maternal separation regimens that are dissimilar may lead to different and sometimes opposite behavioral effects. © 1998 Elsevier Science Inc.

Rat Maternal separation Latent inhibition Stereotypy Open field Amphetamine Apomorphine

EARLY environmental manipulations in rats have been reported to have profound effects on both neurodevelopment [e.g., (3,27,37)] and on behavior in adulthood [e.g., (21,25)]. Neurodevelopmental and behavioral studies in rodents have used various methods for stressing the neonates before weaning. From the mid-1950s onwards, the major methodological manipulation employed was that of postnatal handling [e.g., (13,19,26,42,43)]. Early postnatal handling generally consists of a brief (3 to 30 min) daily separation of the experimental subjects from the mother and littermates, whereas the control animals are left completely undisturbed. More recently, maternal separation has been used as the neonatal stress procedure [e.g., (12,17,22,33)]. This technique consists of removal of the pups from the dam for a longer period of time than that employed above (1 to 24 h) and at varying time points after birth, but littermates are left together.

Behavioral differences following different neonatal rearing manipulations have often been attributed to differences in emotional reactivity (1,11,21,28). Thus, the application of the latent inhibition (LI) paradigm to evaluate specific learning impairments following environmental manipulations in neonates is appropriate because it tests learning behavior that is devoid of motivational-emotional components (43). LI refers to the fact that prior preexposure to a nonreinforced stimulus leads to subsequent retardation of conditioning to that stimulus compared to nonpreexposure in controls. Further, it has been stated (23) that LI is an ubiquitous phenomenon, and one that is found across a wide range of learning procedures.

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In procedures in which early handling is employed as the environmental manipulation, numerous studies using the LI paradigm have been performed. The most frequently investigated LI procedures are the two-way active avoidance (43) and the conditioned emotional response (CER) paradigm (14,42). It has been shown, using both procedures, that early handled subjects show enhancement of LI, whereas the nonhandled males show a disruption (13,14,30,42,43).

In a recent study, Ellenbroek and Cools (12) reported that after a single 24-h separation procedure on postnatal day 10 the maternally separated subjects showed a disruption of LI in the conditioned taste aversion (CTA) paradigm. These authors further reported that early maternal separation also enhanced the susceptibility of rats for the dopamine agonist apomorphine, i.e., the gnawing response was significantly greater in maternally separated subjects after an injection of 1.5 mg/kg apomorphine–HCl.

Thus, there are two possible explanations for these findings: first, that the different manipulation procedures (maternal separation vs. early handling) produce opposite effects [e.g., (15,27)]. Second, it could be argued that LI in the CTA procedure used by Ellenbroek and Cools is different from LI as measured using other procedures, and may indeed be measuring something different, which is undetected by the other LI procedures. Indeed, this view has been suggested as an explanation for the fact that aspiration lesions of the hippocampus enhanced LI when the CTA procedure was employed (32), while in other procedures hippocampal aspiration lesions have been reported typically to attenuate LI [for a review, see (41)].

To investigate the above findings in more detail we decided to use a modification of the maternal separation procedure used by Ellenbroek and Cools (12), and to investigate the long-term effects of this manipulation procedure on behavior in all three LI paradigms (active avoidance, CER, and CTA). In addition, we evaluated the behavioral sensitivity of animals to a single pharmacological challenge with amphetamine and the dopamine agonist apomorphine.

METHOD

Animals

Maternal separation procedure. It was our original intention to use the same maternal separation procedure as used by Ellenbroek and Cools (12). However, due to high mortality rates of the pups during the 24 h of maternal separation in a pilot study, we adopted the procedure described below.

Immediately after birth all litters were culled to the same litter size of four males and four females per litter where possible. To perform the maternal separation procedure all pups from half of the litters were removed from their mothers on days 12, 14, 16, and 18 after birth for a total of 6 h/day (1000–1600 h), during which time the litters were kept in an incubator at around 25°C. The remaining litters served as a control and were left throughout weaning with the mother and subjected to normal animal husbandry (i.e., the cages were cleaned twice a week and the animals were given food and water). At weaning (day 21) all rats were placed in group cages (type IV 59.0 × 38.5 × 20.0 cm) with four of the same sex animals per cage, each of the four animals originating from different litters.

Experimental design. All experiments were performed on male and female adult Wistar rats [Zur:Wist(HanIbm), Institute of Toxicology, Zürich, Switzerland], having a minimum age of 3 months at the start of the studies and 12 months at the end. Animals were housed individually throughout the studies (unless otherwise stated) in Macrolon cages (48 × 27 × 20 cm) under reversed cycle lighting (lights on 1900–0700 h) in a temperature (21 ± 1°C) and humidity (55 ± 5%) controlled animal facility. Food (Nafag 9431, Eberle Nafag AG, Gossau, CH) and water were available ad lib in the home cages except for when indicated below.

All experiments were performed on two independent groups of animals: group 1 was used for the two-way active avoidance LI and the conditioned taste aversion paradigm; group 2 was tested in a conditioned emotional response LI paradigm, as well as for amphetamine-induced activity and apomorphine-induced stereotypies (Table 1).

Two-Way Active Avoidance

Apparatus. The apparatus consisted of four identical Coulbourn Instruments (Allentown, PA) shuttleboxes (Model E10-16TC), each set in a ventilated sound- and light-attenuating shell (Model E10-20). The internal dimensions of each chamber were 35 × 17 × 21.5 cm as measured from the raised grid floor. The box was divided by an aluminium hurdle (17 cm long, 4 cm high). The barrier was very thin to prevent animals from balancing on it and thus avoiding shock. Scrambled shocks were delivered from a constant current shock generator (Coulbourn Instruments, Model E13-14) and scanner (Model E13-13) set at 0.5 mA. The chambers were illuminated during the experimental session with two diffuse light sources (house lights), mounted 19 cm above the grid floor in the middle of the side walls. The conditioned stimulus (CS) was an 85-dB[A] tone (10 s) produced by a 2.9-kHz tone module (Model E12-02) placed behind the shuttle box on the floor of the shell.

Animals and Procedure

For the two-way active avoidance LI paradigm 24 male and 24 female 6-month-old adult Wistar rats were tested. The animals were housed three rats per cage (Macrolon Type IV 59.0 × 38.5 × 20.0 cm). The procedure included three stages given 24 h apart.

Familiarization session. Each animal was placed in the shuttlebox with the house light on for a period of 60 min.

Preexposure session. Each rat was placed in the experimental chamber. Preexposed (PE) animals received 50 presentations of the appropriate stimulus, with a variable interstimulus interval of 50 s. The nonpreexposed (NPE) animals were con-

TABLE 1

	Group 1	Group 2
Experiment/age	Two-way active avoidance/6 months (n = 48)	Conditioned emotional response/3 months (n = 64)
Experiment/age	Conditioned taste aversion/11 months (n = 48)	Amphetamine-induced activity/9 months (n = 44)
Experiment/age		Apomorphine-induced stereotypies/ 11 months (n = 40)

fined to the chamber for an identical period of time without receiving the tones.

Test session. Each animal was placed in the shuttlebox and received 100 avoidance trials with a variable interval of 50 s ranging from 10 to 90 s. Each avoidance trial began with a 10-s tone followed by a 2-s 0.5-mA shock, the tone remaining on with the shock. If the animal crossed the barrier to the opposite compartment during the tone, the stimulus was terminated and no shock was delivered (avoidance response). A crossing response during shock terminated the tone and the shock (escape response). If the animal failed to cross during the entire tone–shock trial, the trial terminated after 12 s. The number of avoidance responses was recorded in 10 trial blocks.

Data Collection and Analysis

Test session: avoidance responses. The 100 avoidance trials were divided into 10 blocks of 10 trials each. The number of avoidance responses per 10 trials was calculated for each of the 10 blocks. These data were analyzed by a $2 \times 2 \times 2 \times 10$ ANOVA with main factors of gender (male, female), treatment (maternally separated, control), and preexposure (0, 50) and a repeated measurement factor of blocks (1–10) each consisting of ten trials.

Conditioned Emotional Response

Apparatus. The apparatus consisted of four Coulbourn Instruments test cages (Model E10-10), each set in a ventilated sound-attenuating Coulbourn Instruments isolation cubicle (Model E10-20). A drinking bottle with a tube opening of 3-mm diameter was inserted into the chamber through a 3×4 cm hole located in the center of the right wall of the chamber, 1.5 cm above the grid floor. Licks were detected by a Coulbourn Instruments infrared optical lickometer (Model E24-01). The preexposed, to-be-conditioned stimuli (CS) were generated from a 28-V, 40-mA houselight located on the right wall of the chamber 26 cm above the grid floor. The experiment was conducted in a dark chamber, and the CS was a 5-s steady houselight. Shock was delivered through the cage floor from a Coulbourn Instruments shocker (Model E13-12) and scanner (Model E13-13) set at 0.5 mA.

A Coulbourn Instruments infrared activity monitor (Model E24-61) was mounted on the ceiling. It was operated in the "movement unit" mode, in which a 10-ms pulse is produced each time the monitor detects a change in the animal's infrared heat pattern. This results in a series of pulses ("activity counts") at a frequency proportional to the amount of movement made by the animal. Equipment programming and data recording were controlled by a Compaq IBM-compatible personal computer (486/DX2/ 66).

Animals and Procedure

The experiment was performed on 32 male and 32 female Wistar rats with a minimum age of 3 months at the start of the experiment.

One week prior to the start of the experiment animals were handled for approximately 3 min per day and a 23-h water restriction schedule was initiated. This was followed by 5 days of training during which each rat was put into the experimental chamber for 20 min. Throughout the experiment the following data were recorded: total number of licks made by the animal, latency to first lick, and total activity counts.

The LI procedure included the following stages.

Preexposure session. Each rat was placed in the experimental chamber and allowed to drink. Because the results of the two-way active avoidance experiment suggested that maternal separation leads to enhancement of latent inhibition, a relatively low number of preexposures (20) were used to avoid a maximal LI effect in the controls as might have been obtained with 40 preexposures (unpublished observations in this laboratory). The lower number of preexposures used allows the measurement of both enhancement and attenuation of the LI phenomenon. Preexposed (PE) animals therefore received 20 presentations of 5-s steady houselight, with an interstimulus interval of 25 s. The nonpreexposed (NPE) animals were confined to the chamber for an identical period of time without receiving the light stimuli (i.e., in darkness).

Conditioning session. Each rat was placed in the experimental chamber and allowed to drink. Two light-shock pairings were given 5 and 10 min after the start of the session. Light parameters were identical to those used in preexposure. The 1-s shock (0.5 mA) immediately followed light termination. After the second pairing, rats were left in the experimental chamber for an additional 5 min.

Rebaseline session. Each rat was given a drinking session identical to the training sessions.

Test session. Animals were tested on 2 consecutive days. Each animal was placed in the chamber and allowed to drink. The light was presented to each of the four rats after it completed 275 licks, and lasted for 15 min. The following times were recorded: time to first lick, time to complete licks 1–250, time to complete licks 251–275 (pre-CS) and time to complete licks 276–300 (CS period).

Preexposure, conditioning, rebaseline, and the two test sessions were given 24 h apart.

Data Collection and Analysis

Licking during the rebaseline session: latency to first lick. Latency to first lick during the rebaseline session was submitted to a logarithmic transformation to allow a parametric analysis. The data were analyzed by a $2 \times 2 \times 2$ ANOVA with main factors of gender (male, female), treatment (maternally separated, control), and preexposure (0, 20).

Test session: time to complete 25 licks prior to stimulus presentation. The time (in seconds) taken by the animals to make the 25 licks prior to the presentation of the stimulus was analyzed by a $2 \times 2 \times 2 \times 2$ ANOVA with main factors of gender (male, female), treatment (maternally separated, control), and preexposure (0, 20), and a repeated measurement factor of days (1, 2).

Test session: suppression ratio. The degree of lick suppression as a result of stimulus presentation was measured using a suppression ratio: time A/(time A + time B), where time A is the period (in seconds) to complete 251–275 licks (pre-CS period) and time B is the period (in seconds) to complete licks 276–300 (CS period). A suppression ratio of 0.00 indicates complete suppression, and a ratio of 0.50 indicates no change in lick rate after stimulus onset. The suppression ratio was analyzed by a $2 \times 2 \times 2 \times 2$ ANOVA with main factors of gender (male, female), treatment (maternally separated, control), and preexposure (0, 20), and a repeated measurement factor of days (1, 2).

Conditioned Taste Aversion

For the CTA procedure the animals were housed individually in Macrolon cages (Type III $42.5 \times 26.6 \times 15.0$ cm) designed in such a way that a drinking tube could be attached

and the spout inserted through a hole in the anterior part of the cage. The water intake of each animal was recorded by measuring the weight of the tube before and after each drinking session.

Animals and Procedure

The experiment used 24 male and 24 female Wistar rats that were approximately 11 months old and had been previously tested in the two-way active avoidance paradigm. The animals were transferred from same-sex group caging (three animals per cage) to individual CTA cages. Forty-eight hours after transferring the subjects to individual cages the drinking tubes were removed. During the subsequent 14 days the rats were allowed to drink, by reattachment of the drinking tubes, only for 15 min in the morning (between 0900 and 1100 h in groups of eight rats per drinking group) and for 45 min in the evening (1700–1745 h). Water consumption of each animal was recorded for every session.

Baseline session. From day 1 to 5 the animals were habituated to the schedule of water deprivation.

Preexposure session. For preexposure, animals were divided into preexposed (PE) and nonpreexposed (NPE) groups in a counterbalanced manner in relation to their PE/NPE allocation in active avoidance and based on their drinking behavior during baseline. From day 6 to day 8 all PE animals received a 0.1% saccharin solution instead of water during the morning session and water in the evening. The NPE animals received water during morning and evening sessions.

Conditioning session. On day 9 all rats received a 0.1% saccharin solution during the 15-min morning session followed immediately by an intraperitoneal injection of lithium chloride (LiCl; Sigma Chemical Company, Switzerland) solution (0.14 M; 1.5% of body weight).

Rebaseline session. On day 10 each rat was given a drinking session identical to the baseline sessions.

Test session. Animals were tested over 4 consecutive days, during which time all rats received saccharin during the morning session and water in the afternoon.

Data Collection and Analysis

Fluid intake was calculated by subtracting the weight of a water tube after a session from its weight before the session. These data were analyzed by a $2 \times 2 \times 2 \times 4$ ANOVA with main factors of gender (male, female), treatment (maternally separated, control), preexposure (0, 3), and a repeated measurements factor of days (1–4).

Amphetamine-Induced Activity

Open-field apparatus. The open field consisted of four rectangular boxes ($76.5 \times 76.5 \times 49$ cm) made of gray PVC and located in a dimly illuminated room (12 lx as measured at the bottom of the open-field boxes). Open-field behavior (distance travelled in cm) was measured via a computerized animal observation system (Ethovision, Noldus, The Netherlands) which was connected to a camera mounted on the ceiling above the open-field boxes.

Animals and Procedure

The experiment used 22 male and 22 female 9-month-old Wistar rats that had previously been used in the CER LI. Before the start of each session the animals were habituated to the testing room for 30 min. All animals were subjected to open-field testing on 2 consecutive days for 1 h/day without prior injection. On day 3 all animals received an injection of saline (1 ml/kg IP) before being placed in the open field for 1 h. Immediately afterwards all rats received an injection of *d*-amphetamine sulphate (0.5 or 1.0 mg/kg IP) followed by further testing in the open field for 1 h.

Data Collection and Analysis

The computer software calculated the distance the rat travelled while in the arena. The activity data were analyzed for the effect of amphetamine vs. saline injections using a $2 \times 2 \times 2 \times 2$ ANOVA with main factors of gender (male, female), treatment (maternally separated, control), dose (0.5, 1.0), and a repeated measurements factor of drug (saline, amphetamine).

Apomorphine-Induced Stereotypies

Apomorphine-induced stereotypies were measured in individual Plexiglas cages, $25 \times 20 \times 20$ cm (Acti +, Viewpoint, Lyon, France), which were held in banks of 40.

Animals and Procedure

The experiment was performed on 20 male and 20 female Wistar rats aged 11 months that had previously received amphetamine (0.5 or 1.0 mg/kg IP) followed by open-field testing. All animals first received an injection of the vehicle for apomorphine, 1 ml/kg 0.01% sodium metabisulphite ($Na_2S_2O_5$) SC and were then placed individually in observation boxes and scored for stereotypies (Table 2) over 30 min (15-min intervals). Immediately afterwards all rats were injected with 1.5 mg/kg apomorphine SC and stereotyped behavior was scored at 15-min intervals for 165 min. Rating was carried out by two experienced observers blind to the identity of the animals.

Data Collection and Analysis

Because there was no significant difference between observer ratings, for data analysis the mean value of both raters for each time point was taken and analyzed by a $2 \times 2 \times 2$ ANOVA with main factors of gender (male, female), treatment (maternally separated, control), and a repeated measurements factor of drug (saline, apomorphine) for the effect of drug, with a $2 \times 2 \times 11$ ANOVA with main factors of gender (male, female), treatment (maternally separated, control), and a repeated measurements factor of interval (1–11) for apomorphine-induced stereotypies and with a $2 \times 2 \times 2$ ANOVA with main factors of gender (male, female), treatment (maternally separated, control), and a repeated measurements factor of interval (1–2) for saline-induced stereotypies.

TABLE 2

STEREOTYPY SCORING SYSTEM (ACCORDING TO COSTALL ET AL. (7))

Score	Behavior Observed
0–0.5	No stereotyped behavior (not different from normal animals)
1–1.5	Periodic sniffing and/or repetitive head/limb movements
2–2.5	Continuous sniffing and/or repetitive head/limb movements
3–3.5	Periodic licking/chewing/biting (oral stereotypies)
4–4.5	Continuous licking/chewing/biting

Drug Challenge Studies

Apomorphine hydrochloride (Research Biochemicals Inc., Switzerland), calculated as free base, was prepared in solution of sodium metabisulphite ($Na_2S_2O_5$) 0.01% (Sigma Chemical Company, Switzerland) to protect the apomorphine from oxidation, and administered by SC injection in the flank.

Amphetamine sulphate (Sigma Chemical Company, Switzerland), used as the salt was prepared in normal saline and administered by intraperitoneal injection.

RESULTS

Two-Way Active Avoidance LI

Avoidance responses. The 2 × 2 × 2 × 10 ANOVA revealed a highly significant main effect of blocks, $F(9, 360) = 52.24$, $p < 0.0001$, indicating an overall increase of avoidance responses as a function of progressive training. In addition, the analysis revealed a significant main effect of preexposure, $F(1, 40) = 5.82$, $p < 0.025$, as well as a significant interaction of preexposure × blocks, $F(9, 360) = 2.47$, $p < 0.01$, supporting the conclusion that overall, the NPE subjects acquired the avoidance response faster and to a higher level compared with the PE subjects, i.e., the existence of the latent inhibition phenomenon. However, the significant preexposure × treatment × blocks interaction, $F(9, 360) = 2.27$, $p < 0.02$, suggests that the difference in performance of the NPE compared to the PE subjects, i.e., LI, was greater in maternally separated subjects than in control subjects. This is shown in Fig. 1, which depicts the percentage of avoidance responses in 10 blocks of 10 trials each. It should be noted that this enhanced LI was primarily due to improved avoidance in the maternally separated NPE group compared to the control NPE group. The conclusion that LI was more evident in the maternal separation condition compared with the control condition was further statistically supported by 2 post hoc 2 × 10 ANOVAS, which compared PE and NPE groups within each condition (maternal separation, control). Whereas for the maternal separation condition both the main effect of preexposure and the preexposure × blocks interaction were significant, $F(1, 20) = 8.01$, $p < 0.02$; $F(9, 120) = 3.48$, $p < 0.001$, respectively, in the control condition both the main effect of preexposure and the preexposure × blocks interaction were nonsignificant, $F(1, 20) = 0.65$, $p > 0.05$; $F(9, 180) = 1.41$, $p > 0.05$, respectively.

Conditioned Emotional Response

Licking during the rebaseline session: latency to first lick. The 2 × 2 × 2 ANOVA yielded only a significant main effect of treatment, $F(1, 56) = 4.54$, $p < 0.04$, reflecting the shorter latencies of maternally separated groups to start licking in the rebaseline session (mean latencies: controls 9.7 ± 1.2 s, maternally separated animals 6.5 ± 1.1 s).

Licking during the rebaseline session: total licks. A 2 × 2 × 2 ANOVA yielded only a significant main effect of gender, $F(1, 56) = 5.34$, $p < 0.03$, reflecting the overall larger number of licks for males (mean value 1781 ± 96) compared with females (mean value 1494 ± 81).

Test session: time to complete 25 licks prior to the stimulus presentation. The 2 × 2 × 2 × 2 ANOVA revealed no significant main effects or interactions. The overall mean time taken to complete licks 251-275 was 5.83 ± 0.55.

Test session: suppression ratio. The 2 × 2 × 2 × 2 ANOVA revealed a significant effect of days, $F(1, 56) = 10.04$, $p < 0.003$, reflecting the overall decreased suppression on day 2 compared to day 1. However, as there were no significant interactions of this factor with any of the other factors or interactions, a 2 × 2 × 2 ANOVA collapsed over the factor of days was carried out. This analysis revealed a significant main effect of preexposure, $F(1, 56) = 10.40$, $p < 0.003$, demonstrating the overall existence of latent inhibition, i.e., decreased suppression of drinking in the PE compared with the NPE groups. The significant main effect of treatment, $F(1, 56) = 4.59$, $p < 0.04$, reflects the fact that maternally separated animals were less suppressed than control animals. Although the interaction of preexposure × treatment only came close to the acceptable level of significance, $F(1, 56) = 3.44$, $p < 0.07$, a post hoc *t*-test was performed to establish whether there was latent inhibition in each condition, controls: $t(62) = 1.24$, NS;

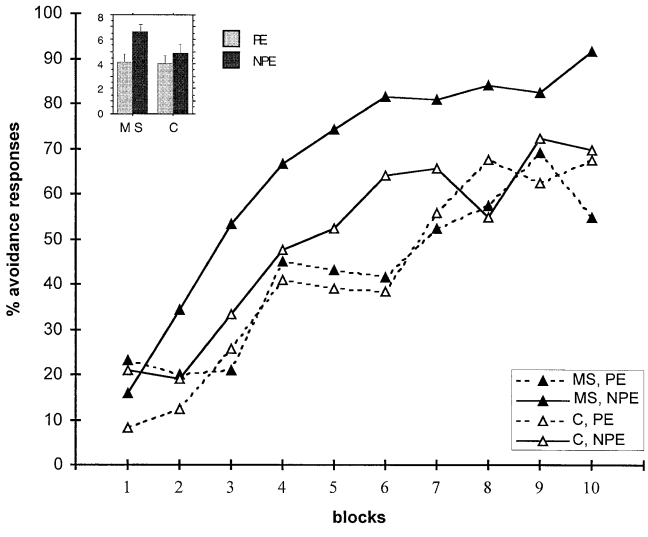

FIG. 1. Mean percentage of avoidance responses over 10 blocks of 10 acquisition trials in the test stage in maternally separated (MS) and control (C), preexposed (PE) and non-preexposed (NPE) animals. The overall number of avoidance crossings in all four conditions is depicted in the top left-hand corner.

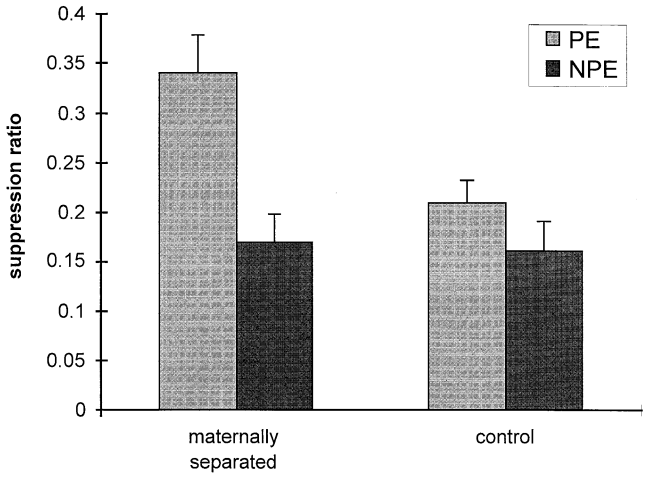

FIG. 2. Means and standard errors of suppression ratios for the preexposed (PE) and non-preexposed (NPE) group of the two experimental condition in the CER paradigm.

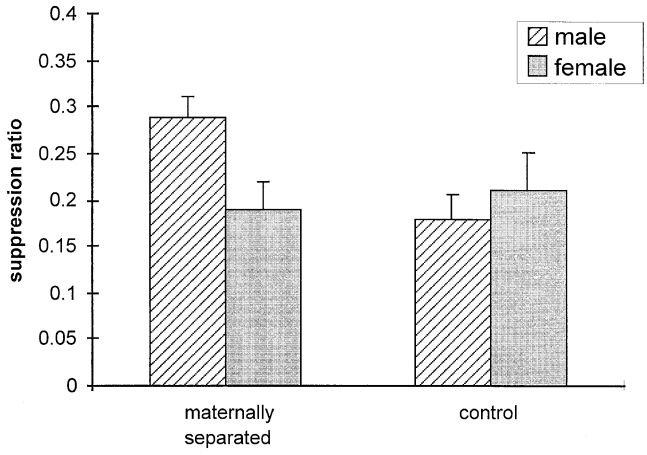

FIG. 3. Means and standard errors of suppression ratios for maternally separated and control male and female rats, representing the interaction of treatment × gender in the CER paradigm.

maternally separated: $t(62) = 3.49$, $p < 0.001$. That maternally separated animals were less suppressed than control animals tended to be more evident in the PE compared to NPE animals. LI was more clearly evident in the maternally separated rats compared to their controls (see also results of *t*-test) (Fig. 2). Additionally, there was a significant gender × treatment interaction, $F(1, 56) = 4.24$, $p < 0.05$, reflecting that maternal separation led to decreased suppression of licking more in males than in females (Fig. 3).

Conditioned Taste Aversion

Test session: amount of saccharin consumed. The 2 × 2 × 2 × 4 ANOVA revealed a significant effect of days, $F(3, 120) = 30.51$, $p < 0.001$, reflecting an overall decreased taste aversion from day 1 to 4. The significant main effect of preexposure, $F(1, 40) = 44.83$, $p < 0.001$, reflects the overall existence of LI, i.e., decreased suppression of saccharin intake in the PE compared with the NPE groups. In addition, there was a significant days × gender × treatment interaction, $F(3, 120) = 2.91$, $p < 0.04$, depicted in Fig. 4, which shows that while the maternally separated females recovered from the aversion effect faster than the control females, no such difference existed between the maternally separated and the control males. A separate analysis within males and females revealed a significant effect of days × preexposure × treatment, $F(3, 60) = 3.09$, $p < 0.035$, for males but not for females, $F(3, 60) = 0.67$, $p > 0.05$, reflecting enhanced LI in the maternally separated males compared to the control group, whereas no influence of treatment could be found in females (see Fig. 5).

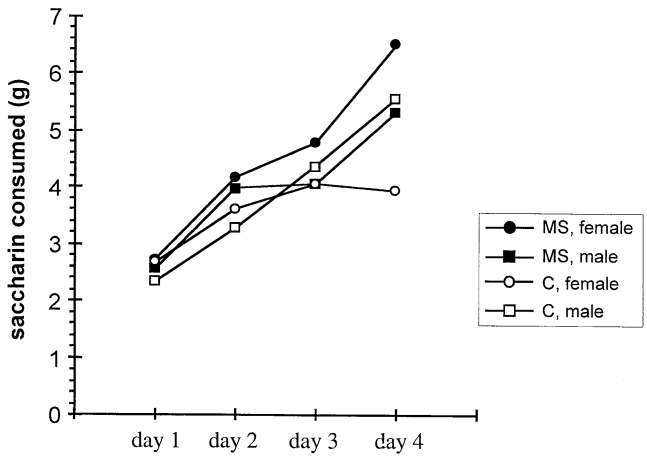

FIG. 4. Time course of the mean saccharin consumption (g) over days for male and female rats of the two experimental groups (MS = maternally separated, C = control) in the CTA paradigm.

Amphetamine-Induced Activity

The 2 × 2 × 2 × 2 ANOVA revealed a significant within subject main effect of drug, $F(1, 36) = 183.60$, $p < 0.001$, reflecting that amphetamine increased overall activity compared to saline. There was also a significant main effect of dose, $F(1, 36) = 4.51$, $p < 0.05$, indicating an increased response to the higher dose of amphetamine. The significant

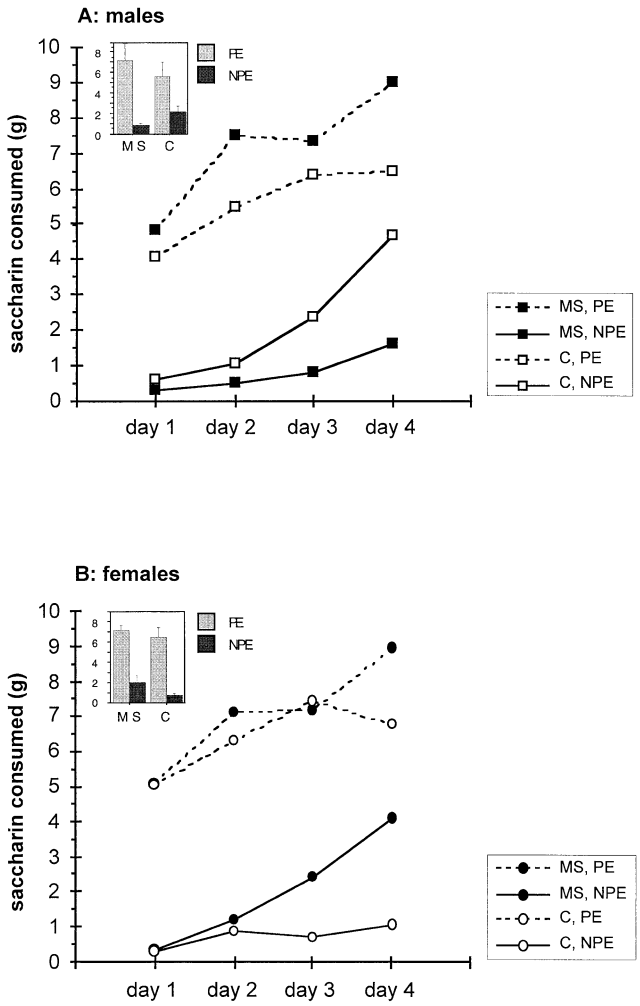

FIG. 5. Time course of the mean saccharin consumption (g) over days separated for A: male and B: female rats of the preexposed (PE) and non-preexposed (NPE) group of the two experimental conditions (MS = maternally separated, C = control). The overall means and standard errors of saccharin consumption in all four conditions is depicted in the top left-hand corner of each graph.

main effect of gender, $F(1, 36) = 39.05$, $p < 0.001$, reflects that females had a higher level of overall activity, and in addition there was an interaction of drug × gender, $F(1, 36) = 32.12$, $p < 0.001$, reflecting that females showed a larger increase of activity after the injection of amphetamine compared to the male groups.

However, the interactions of drug × dose × treatment and of drug × dose × treatment × gender approached significance, $F(1, 36) = 3.08$, $p < 0.09$; $F(1, 36) = 3.02$, $p < 0.095$, respectively, indicating that the increased response to the higher dose of amphetamine was particularly evident in the maternally separated females compared with the other three groups (Fig. 6).

Apomorphine-Induced Stereotypies

The 2 × 2 × 11 ANOVA revealed a significant main effect of gender, $F(1, 36) = 28.51$, $p < 0.0001$, reflecting an overall longer lasting effect of apomorphine in male rats compared with female rats. In addition, there was a significant effect of the repeated measurements factor interval, $F(10, 360) = 146.08$, $p < 0.0001$, reflecting the initial increase (during the first hour) and the later decrease of stereotypies over time. The significant interaction of gender × interval, $F(10, 360) = 37.60$, $p < 0.0001$, indicates that the overall increased level of stereotypies observed in male rats is due to the different time course in the response compared to that of females, i.e., male rats irrespective of treatment condition responded for longer than female rats to apomorphine (Fig. 7).

No significant effect of maternal separation was found.

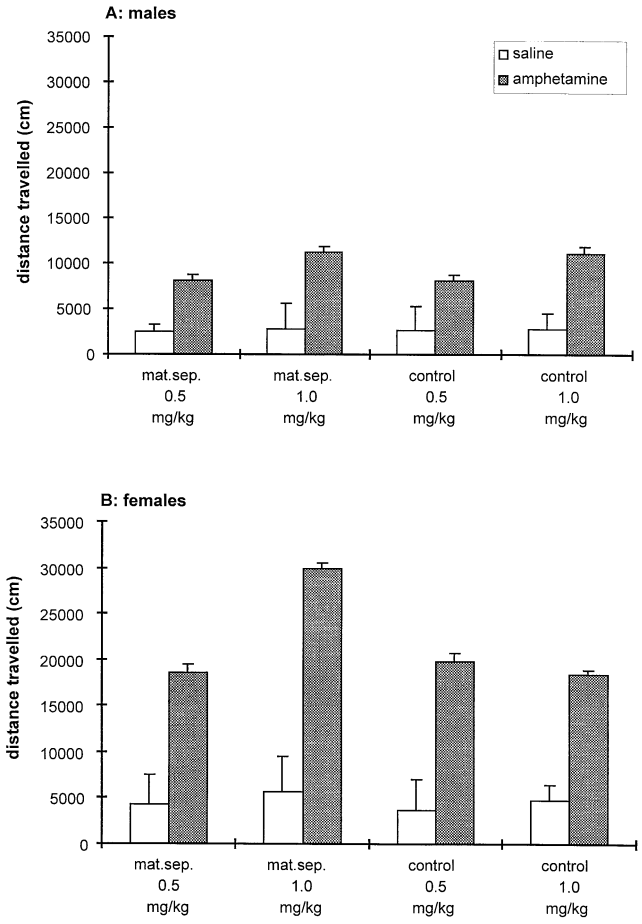

FIG. 6. Means and standard errors of distance travelled in cm following saline (open bars) or amphetamine (shadowed bars) injection in A: male and B: female, maternally separated and control rats.

DISCUSSION

Our data suggest that repeated maternal separation before weaning in rats has long-term consequences for both learning in general, and selective attention in particular. In the present studies maternal separation affected LI in a similar manner in all three experimental paradigms. Thus, one can assume that all three paradigms measure the same characteristic of selective attention, as has already been stated by Lubow (23). This implies that the explanation for the effects of the hippocampal lesions on LI provided by Purves et al. (32) to explain the discrepancy in findings using CTA as opposed to other behavioral procedures, may indeed only apply to consequences of hippocampal lesions. The LI experiments revealed that maternally separated animals showed enhanced LI in the two-way active avoidance paradigm, as well as in the CER paradigm in comparison to the control group. In the CTA paradigm, enhanced LI was also found, but this effect was restricted to the maternally separated males. Further, the maternally separated animals showed improved avoidance acquisition in the NPE condition and faster extinction of the CTA response (females only). Although all animals showed an increase in activity after an injection of amphetamine, only maternally separated females showed significantly greater sensitivity to the highest dose tested. Maternal separation had no influence on the intensity of the stereotypic response to apomorphine, although there was a gender difference in that males showed a prolonged stereotyped response to apomorphine compared with females.

That the control animals in the present study showed only marginal LI in the CER paradigm and the active avoidance paradigm is probably a consequence of the parameters chosen for the preexposure procedure. Based on the results obtained for LI in the active avoidance task, the subsequent CER experiment was designed in such a way that enhancement (as found in the active avoidance paradigm) of LI, as well as its attenuation [as reported by Ellenbroek and Cools (12)] in the maternally separated group could be observed, i.e., a relatively low number of preexposures (20) was used such that the control animals were at the threshold level for demonstrating LI. In a series of unrelated experiments it has been shown that a higher number of preexposures (e.g., 40) lead to such a robust LI effect in the control group that enhancement can be difficult to see against this background (unpublished observation in our laboratory). Furthermore, although it can be suggested that the reduced LI seen in the control animals of the present study reflects a procedure similar to nonhandling (the males of which have been reported to demonstrate reduced LI) [for review, see (41)], we do not believe that this is the case here, as the control animals were subjected to normal animal husbandry.

The observation in the present study that there is an overall enhancement of LI in CTA in the maternally separated males is further corroborated by the results for the active avoidance LI and CER LI. This is in contrast to those reported following a single 24-h maternal separation procedure on postnatal day 10 (12), in which it was shown that LI in the CTA paradigm was reduced in maternally separated animals.

Because our results demonstrate that LI is indeed a ubiquitous phenomenon and can be measured with the three para-

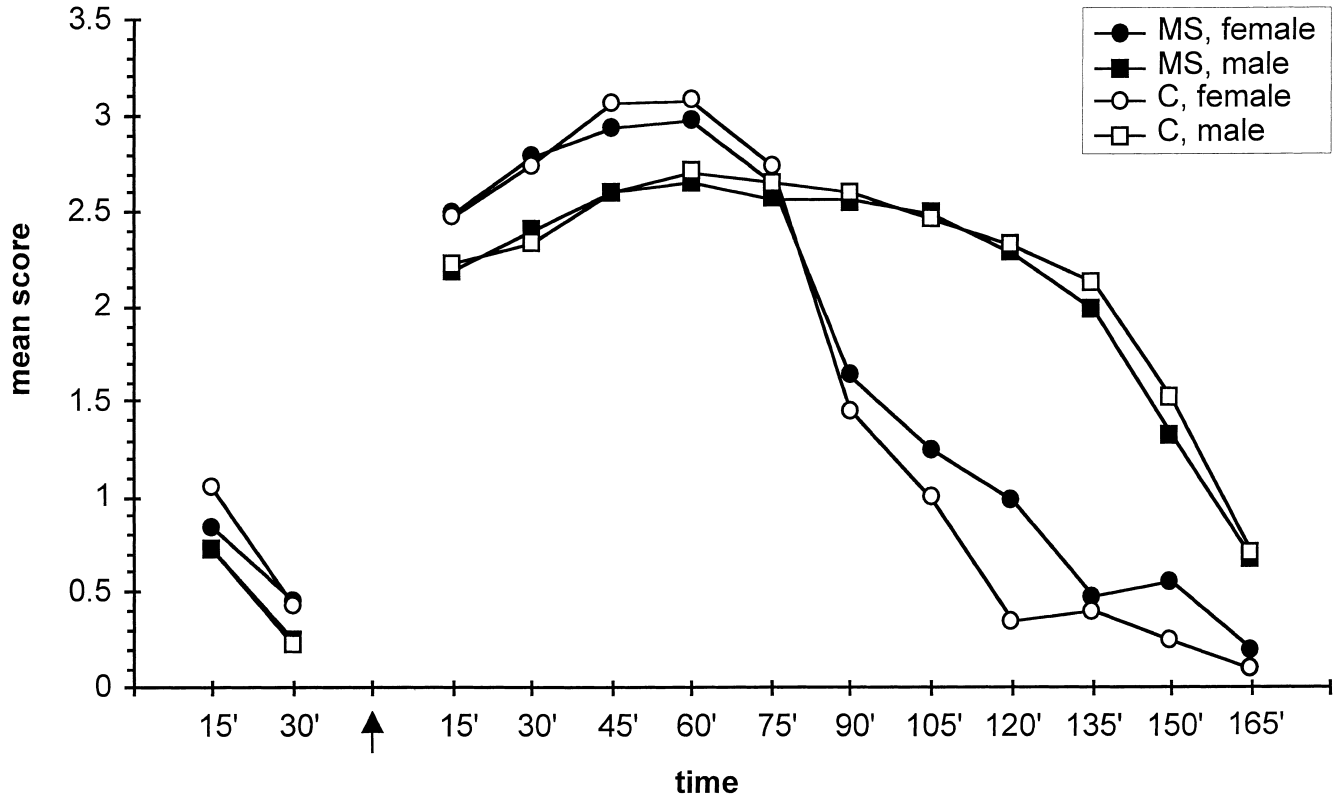

FIG. 7. Time course of apomorphine-induced stereotypy (mean score) for male and female animals of the two experimental groups (MS = maternally separated, C = control). Apomorphine injection is denoted by ↑.

digms used in the present studies, the differences between the present results and those reported by Ellenbroek and Cools may be interpreted to be a consequence of using a different separation procedure, i.e., a repeated, but shorter separation (4×6 h) in our studies compared to that employed by Ellenbroek and Cools (1×24 h). In addition, the delay by an average of 5 days in carrying out the manipulation (between day 12 and 18 as opposed to day 10 in the Ellenbroek and Cools study) may also have affected the results. That different manipulation procedures lead to varying behavioral outcomes may be due to different underlying endocrinological and neurochemical effects consequent to a specific manipulation.

Our results, in contrast to those of Ellenbroek and Cools, are more in line with results of early handling procedures, i.e., a brief (between 3 and 30 min) daily separation of the experimental subjects from the mother and littermates, in which there was enhanced LI in the active avoidance procedure (43) as well as in the CER paradigm (13,14,30,42).

In reference to the literature, it is interesting to note that early handling and maternal separation are often reported to lead to opposite effects in some neuroendocrinological and behavioral measures: early handling is reported to reduce corticosterone release in response to stress in juvenile [e.g., (2,4)] and adult rats (28,29), to decrease emotionality [e.g., (1,8,21)], and to enhance learning in adulthood as a consequence of improved stress-coping mechanisms (19,43,44). In contrast, early maternal separation is reported to have either no effect on behavior and on the endocrinological response in juvenile rats (15,35), or to have effects opposite to those of early handling, i.e., increased and prolonged corticosterone release in response to stress in juvenile and adult rats (31,33,38). Increased emotionality and impaired learning performance as a consequence of deficits in coping with stressful situations are also reported (39). Although corticosteroid levels as a measure for the endocrine response to stress were not measured in the present study, results from the active avoidance task as well as reduced suppression in CER would seem to suggest that our maternally separated subjects show improved behavioral strategies in coping with stress, which is in opposition to the reports cited above.

Data from endocrinological studies reveal that "the outcomes for hypothalamic–pituitary–adrenal axis activity and corticosterone receptor properties in adult life depend upon the duration, frequency, and age of the pups at the time of separation" (33). Consequently, comparisons of endocrinological and behavioral effects of maternal separation are problematic due to the large variety of different procedures used. Kuhn et al. (18) reported that two of the endocrinological consequences of maternal separation (decrease in growth hormone and increase in corticosterone) could be differentiated depending on the age when separation occurred and its duration. The critical effect of these two parameters is further supported by other researchers (9,16,20,22,27,31,33,35,36,38). Thus, it is not surprising that maternal separation procedures that start early in life may also lead, on the behavioral level, to different outcomes than those that start later in development. Such methodological variation may even give rise to opposite effects on the behavioral and neuroendocrine characteristics of the adult offspring (5).

As previously stated, our results indicate that maternal separation improves learning performance in general, a find-

ing that is also in line with the early handling studies, where early handled animals showed improved learning performance in both spatial memory tasks (26) and avoidance performance (19,43). In the present study improved learning performance in the maternally separated group could be found throughout all three LI experiments, as indicated by better avoidance learning within the NPE animals, decreased contextual conditioning in the CER paradigm, shorter latencies during the rebaseline session, and faster recovery following CTA (mainly in females).

Gender differences for neurodevelopmental disorders with a predominance in males are often found (also in humans), and it has been suggested that females have a greater flexibility than males in responding to novel or aversive stimuli (40). This, considered together with the fact that males are more affected by postnatal manipulations, may explain the reason why enhancement of LI in the CTA paradigm could only be found in maternally separated males in our study. For LI in the active avoidance paradigm and the CER experiments no gender differences were observed, probably reflecting differences in "sensitivity" of the three different procedures. In the CER paradigm maternally separated animals displayed a significant gender difference in that the males of this group were less suppressed. Such gender differences could not be found within the controls. These findings are in line with the hypothesis that maternal separation improves responding to stressful situations and that males are more affected by postnatal manipulations than females. Nevertheless, the faster recovery of maternally separated female rats remains unexplained.

In response to drug challenge, maternally separated rats have previously been reported to show reduced sensitivity to dopamine (DA) agonists such as amphetamine (25,45) and enhanced sensitivity to the DA agonist apomorphine (12,33). These findings are not in line with our own results, which show an enhanced response in maternally separated females to the higher dose of amphetamine tested and failed to demonstrate a treatment effect after an injection of apomorphine. Clearly, differences in the separation procedure employed in each of the aforementioned studies may also have marked consequences for sensitivity to subsequent drug challenge. With respect to the apomorphine results, a further explanation for the different results obtained may relate to a methodological issue. In our study a stereotypy rating scale to score all behavioral components (sniffing, chewing, gnawing) of the apomorphine-induced response was used as opposed to only a measurement of gnawing (12,33). It is, therefore, possible that an effect on a specific behavioral component of the apomorphine response could have been masked. The lack of relationship between the enhanced LI seen following maternal separation and the results obtained following DA challange may reflect the dependence of LI on phasic, rather than on tonic DA transmission, as has also been noted by Peters et al. (30). LI has been assumed to be mediated by the mesolimbic DA system [for a recent review, see (41)].

In all of the previously cited studies maternal separation was performed earlier than in our study. It must also be considered that the mesolimbic DA system may be more sensitive to environmental alterations occurring during the first postnatal week than to those occurring later, providing yet further explanations for the differences in the results obtained.

Nevertheless, the results obtained in our own experiments again show similarities to effects reported from early handling studies, in which early handled males were reported to show increased sensitivity to amphetamine (14,34); females, however, were not investigated in these studies. Gender differences in response to drugs are a well-known phenomenon and frequently reported in the literature (6,10). Females usually show higher sensitivity to DA agonists (6), and gender differences in drug response are suggested to be due to the direct or indirect modulation of mesostriatal DA activity by gonadal steroid hormones (6). It may therefore be possible that early maternal separation also alters DA activity by modulating steroid hormone release (24) and that females, due to the influences of estrogen, are more sensitive to these alterations.

In summary, our results suggest that a maternal separation procedure consisting of 4 $\times$ 6 h on days 12, 14, 16, and 18 leads to long-term effects on learning performance and alterations in selective attention. The impact of this procedure on drug sensitivity is less clear. The results obtained in all three LI experiments are consistent and do not support the hypothesis that CTA may be measuring behavior different to that of active avoidance and conditioned emotional response. Further, our results do not support the suggestion that maternal separation leads to disruption of LI. At the behavioral level our results resemble most closely those in which early handling was used as the preweaning manipulation. This fact suggests that the parameters chosen in the present study, whereby maternal separation started on day 12 and, therefore, at the end of the sensitive period for HPA axis alterations, lead to effects that are clearly different from those where the manipulation procedure is carried out earlier in development. Indeed, Rots et al. (33) report that "paradoxical effects on later HPA axis activity are obtained with 24-h deprivation on postnatal days 11–12 compared to postnatal days 3–4," a statement that is supported, on the behavioral level, by our own findings.

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