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Postnatal Aversive Experience Impairs Sensitivity to Natural Rewards and Increases Susceptibility to Negative Events in Adult Life

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Evidence shows that maternal care and postnatal traumatic events can exert powerful effects on brain circuitry development but little is known about the impact of early postnatal experiences on processing of rewarding and aversive stimuli related to the medial prefrontal cortex (mpFC) function in adult life. In this study, the unstable maternal environment induced by repeated cross-fostering (RCF) impaired palatable food conditioned place preference and disrupted the natural preference for sweetened fluids in the saccharin preference test. By contrast, RCF increased sensitivity to conditioned place aversion (CPA) and enhanced immobility in the forced swimming test. Intracerebral microdialysis data showed that the RCF prevents mpFC dopamine (DA) outflow regardless of exposure to rewarding or aversive stimuli, whereas it induces a strong and sustained prefrontal norepinephrine (NE) release in response to different aversive experiences. Moreover, the selective mpFC NE depletion abolished CPA, thus indicating that prefrontal NE is required for motivational salience attribution to aversion-related stimuli. These findings demonstrate that an unstable maternal environment impairs the natural propensity to seek pleasurable sources of reward, enhances sensitivity to negative events in adult life, blunts prefrontal DA outflow, and modulates NE release in the reverse manner depending on the exposure to rewarding or aversive stimuli.

Keywords: cross-fostering, dopamine, norepinephrine, prefrontal cortex, salience attribution

Introduction

A growing body of evidence suggests there is an association between exposure to aversive events during the postnatal period and increased vulnerability to a variety of neuropsychiatric and psychosocial disorders, such as depression, anxiety, psychosis, and vulnerability to substance abuse (McEwen 2000; Meaney 2001; Heim and Nemeroff 2002; Matthews and Robbins 2003; Nemeroff 2004). The prefrontal cortex is involved in goal-directed behavior and affective processing and regulation of motivational salience or intensity of behavioral responses (Jentsch and Taylor 1999; Matsumoto et al. 2003; O'Doherty 2004). In rodents, maternal separation from pups may produce anxiety- and depressive-like behaviors (Boccia et al. 2007; Kikusui and Mori 2009) and alter prefrontal cortex neural plasticity (Pascual and Zamora-León 2007). Accumulating evidence also suggests that synaptic connections within the prefrontal cortex are dramatically shaped by early experience (Poeggel et al. 2003; Bock et al. 2008) and direct effects of the alteration of maternal care received by the offspring on mesocorticolimbic dopamine (DA) function, vulnerability to psychostimulants, and prefrontal sensorimotor gating in adult animals have been recently reported (Meaney et al. 2002; Brake et al. 2004; Zhang et al. 2005).

Monoamines in the medial prefrontal cortex (mpFC) play a major role in emotional, motivational, and cognitive functions (Goldman-Rakic 1999; Arnsten and Robbins 2002; Aston-Jones and Cohen 2005; Lapiz and Morilak 2006). In particular, prefrontal norepinephrine (NE) and DA are critically involved in the processing of rewarding or aversive stimuli and attribution of motivational salience (Feenstra 2000; Mingote et al. 2004; Fallon et al. 2007; Ventura et al. 2007, 2008; Volkow et al. 2009). The propensity of animals and humans to seek out rewards and to avoid punishments entails the ability to form an internal representation of the value of rewarding and punishing stimuli and make predictions about their occurrence to guide behavior (O'Doherty 2004). Because this is a clearly adaptive behavior, investigating how this ability is affected by environmental experiences during early development may be relevant to understanding several emotional and motivational deficits in adult humans. Although several studies have been reported the effects of postnatal adverse experiences on susceptibility to stress exposure (Pryce et al. 2005; Arborelius and Eklund 2007; Jezierski et al. 2007; Lupien et al. 2009; Rivarola and Suárez 2009), the impact produced by the early perturbation of the mother–infant bond on catecholamine modulation of mpFC function and processing of rewarding and aversive stimuli in adult life remains unknown.

Previously, we showed that recurrent adoptions experienced by pups can help to disclose some mechanisms underlying the genetical–environmental interplay that links the early perturbation of the mother–infant bond to heightened CO_2 sensitivity and anxiety (D'Amato et al. 2011). Therefore, to represent early postnatal aversive experience, we used the same repeated cross-fostering (RCF) procedure. Interestingly, pups undergoing RCF were not neglected by adoptive mothers (e.g. nursing, grooming/licking) but, in contrast to controls, they emitted a higher number of separation-induced

ultrasonic vocalizations regardless of the type of olfactory cues that provide information about proximity to mother (clean vs. own-cage bedding). Here, we hypothesized that RCF in NMRI outbred female mice may alter the propensity to orient behavior toward rewarding experiences and affect sensitivity to aversive conditions in adult life. To verify this hypothesis, female mice exposed to RCF were tested for chocolate-induced conditioned place preference (CPP), the saccharin preference test (SPT), lithium chloride (LiCl)-induced conditioned place aversion (CPA), and the forced swimming test (FST). Moreover, because prefrontal catecholamines are involved in processing the affective value of positive and negative stimuli processing, we investigated by in vivo intracerebral microdialysis whether this early adverse experience produces enduring effects on the prefrontal DA and NE outflow induced by rewarding (chocolate) or aversive (restraint and LiCl) stimuli. Finally, to assess the possible association between RCF-induced behavioral changes and prefrontal NE transmission, we investigated the effects of selective prefrontal NE depletion on LiCl-induced CPA.

Materials and Methods

Animals

NMRI outbred mice (Harlan, Italy) were used in all experiments. At 12 weeks of age, mice were mated by housing 2 females with 1 male in transparent polysulfone cages (26.7 × 20.7 × 14.0 cm) with water and food ad libitum. Room temperature (21 ± 1°C) and a 12:12 h light–dark cycle (lights on at 07.00 AM) were kept constant. After 15 days, males were removed and pregnant females were isolated, left in clean cages, and inspected twice a day for live pups. All experiments were conducted in accordance with and under license from the Italian Department of Health legislation (DL 116/92) and regulations on the use of animals for research, and in accordance with the NIH guidelines on animal care. Females were chosen as subjects for this study because of the significant incidence of gender differences in stress-related disorders and their greater stress sensitivity (Bale 2006). To prevent potential estrous cycle group synchronization, experimental subjects for cross-fostered and control female groups were sorted by collecting not >2 individuals per cage/litter for both behavioral and microdialysis experiments.

Drugs

Chloral hydrate, 6-hydroxydopamine (6-OHDA), GBR 12909 (GBR), and LiCl (63.5, 127, 190.5 mg/kg) were purchased from Sigma (Sigma Aldrich, Milan, Italy). Chloral hydrate (450 mg/kg) and GBR (15 mg/kg) were dissolved in saline (0.9% NaCl) and injected intraperitoneally (i.p.) in a volume of 10 mL/kg. 6-OHDA was dissolved in saline containing Na-metabisulfite (0.1 M).

Repeated Cross-Fostering

The procedure for RCF has been previously described (D'Amato et al. 2011) and is summarized briefly. Pups from the same litter spent the first postnatal day (PND0) with their biological mother. On PND1, litters were randomly selected and assigned to experimental (RCF) or control treatment. Each experimental litter was fostered by replacing the mother with a novel lactating female caring for pups of the same age; this procedure was repeated daily (4 times, from PND1 up to PND4) until the fourth adoptive mother was reached. Pups were left with the last adoptive mother until weaning. Procedurally, fostering consisted of first removing the dam from the cage, then removing the entire litter, and immediately introducing it into the home cage of a different dam, whose pups had just been removed. RCF pups were placed in the nest and semi-covered with the home cage bedding of the adoptive mother; the dam was then reintroduced. The procedure of shifting between litters lasted about 30 s and took place everyday (PND1 to PND4) between 10.30 and 11.00 AM. Control mothers and pups were left together from PND0 until weaning. Control litters were only picked up daily and reintroduced in their home cage, were covered with home cage bedding and had their mothers returned within 30 s from PND1 to PND4. Animals were weaned at PND28, separated by sex and housed in groups of 4 littermates. All of the experiments were performed on 5-month-old adult female mice.

Place Conditioning

Behavioral experiments were performed using a place conditioning apparatus (Ventura et al. 2007, 2008). The apparatus comprised 2 gray Plexiglas chambers (15 × 15 × 20 cm) and a central alley (15 × 5 × 20 cm). Two sliding doors (4 × 20 cm) connected the alley to the chambers. In each chamber 2 triangular parallelepipeds (5 × 5 × 20 cm) made of black Plexiglas and arranged in different patterns (always covering the surface of the chamber) were used as conditioned stimuli. The training procedure for place conditioning has been described previously (Ventura et al. 2007, 2008). Briefly, on day 1 (pretest), mice were free to explore the entire apparatus for 20 min. During the following 8 days (conditioning phase), mice were confined daily for 40 min alternately in 1 of the 2 chambers. For CPP, one of the patterns was consistently paired with palatable food (milk chocolate, 1 g, paired chamber) and the other with standard food (mouse standard diet, 1 g, unpaired chamber). For CPA, one of the patterns was consistently paired with LiCl (63.5, 127, 190.5 mg/kg i.p.) and the other with saline. This schedule lasted throughout conditioning. Both groups (controls and RC) were balanced as for the pairing between 1 of the 2 patterns and the unconditioned stimulus (chocolate and different doses of LiCl).

For both groups (control and RCF) pairings were balanced so that for half of each group the unconditioned stimulus (chocolate, LiCl 63.5, 127, 190.5 mg/kg i.p.) was paired with one of the patterns and for half with the other (standard food, saline). Testing for the expression of CPP or CPA was conducted on day 10 using the pretest procedure. Behavioral data were collected and analyzed by the "EthoVision" (Noldus, The Netherlands) fully automated video tracking system (Spink et al. 2001). Briefly, the experimental system is recorded by a CCD video camera. The signal is then digitized (by a hardware device called "frame grabber") and transmitted on the computer's memory. Later, the digital data were analyzed by means of the EthoVision software to obtain "time spent" (s), which was used as raw data for preference scores in each sector of the apparatus of each subject. Chocolate consumption was assessed by weighing the amount remaining at the end of each conditioning session (chocolate: Day 1, 3, 5, and 7).

Saccharin Preference Test

Familiarization

Saccharin solution was prepared using distilled water and pure saccharin (Hermes Susstoff AG, Zurich). The solutions were freshly prepared immediately before use and offered at room temperature. Animals living in pairs were singly housed in a test cage for 1 h. Here, animals were exposed to a double choice (either saccharin solution [0.25% or 0.5%] or drinking water) from graduated tubes (10 mL volume). Intake was measured to the nearest 0.1 mL. The test cage was the same throughout the experiment.

Experimental Phase

After familiarization, every other day (day 1 up to 6), animals were moved into their own test cages and their 1-h saccharin intake was measured. Thereafter, animals were placed back in their home cages. Only animals drinking at least 0.1 mL on day 1 were included in the study.

Forced Swim Test

Mice were gently placed in individual glass cylinders (height 40 cm and diameter 18 cm) containing 20 cm water at 28 ± 2°C. Mice were

exposed to the apparatus for 10 min for the first experience (pretest) and for 5 min 24 h later for the test session (test); they were then removed from the cylinder, allowed to dry in a small cage placed under a heat source for 10 min, and returned to their home cages. The behavior exhibited by each animal during the course of the test session was videotaped using a remote-controlled video camera placed frontally inside the cubicle. Later, an observer blinded to the treatment received by each animal analyzed the videotape with the aid of the Observer program (Version 3.0, System for Macintosh, Noldus, Wageningen, The Netherlands, 1997). The duration (s) of each of the following behavioral items was taken as the dependent variable: Immobility = total absence of movement; struggling = vigorous attempts at climbing the walls of the cylinder; swimming = active swimming; paddling = small movements of one of the posterior paws not producing displacement.

Microdialysis

Animals were anesthetized with chloral hydrate (450 mg/kg), mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, United States of America) equipped with a mouse adapter and unilaterally implanted with a guide cannula (stainless steel, shaft outer diameter (OD) 0.38 mm, Metalant AB, Stockholm, Sweden) counterbalanced in either the right or left hemisphere of the mpFC (Ventura et al. 2003, 2005, 2007). A 1-mm long guide cannula was fixed with epoxy glue; dental cement was added for greater stability. According to the Franklin and Paxinos atlas, coordinates from bregma were: +2.52 anteroposterior (AP); ±0.6 L for both control and RCF animals (Franklin and Paxinos 1997). The probe (dialysis membrane length 2 mm, OD 0.24 mm, MAB 4 cuprophane microdialysis probe, Metalant AB) was introduced 24 h before microdialysis experiments. The location of the microdialysis probe in the mpFC is shown in Figure 1. Animals were lightly anesthetized with chloral hydrate (350 mg/kg) to facilitate the manual insertion of the microdialysis probe into the guide cannula and then returned to their home cages. The outlet and inlet probes tubing were protected by locally applied parafilm. The membranes were tested for in vitro recovery of NE and DA (relative recovery (%): NE = 12.2 ± 0.72; DA = 10.5 ± 0.83; $n = 15$) on the day before use in order to verify recovery. The microdialysis probe was connected to a CMA microdialysis (CMA) 100 syringe pump (Carnegie Medicine Stockholm, Sweden) through PE-20 tubing and an ultra-low torque dual channel liquid swivel (Model 375/D/22QM, Instech Laboratories, Inc., Plymouth Meeting, PA, United States of America) to allow free movement. Artificial cerebrospinal fluid (147 mM NaCl, 1 mM MgCl, 1.2 mM $CaCl_2$, and 4 mM KCl) was pumped through the dialysis probe at a constant flow rate of 2 µL/min. Experiments were carried out 22–24 h after probe placement. Each animal was placed in a circular cage furnished with microdialysis equipment (Instech Laboratories, Inc.) and home cage bedding on the floor. Dialysis perfusion was started 1 h later, at which time the mice were left undisturbed for approximately 2 h before baseline samples were collected. The mean concentration of the 3 samples collected immediately before treatment (<10% variation) was taken as basal concentration. Before microdialysis experiments, mice from both control and RCF groups were assigned to either a rewarding (chocolate) or an aversive experience (restraint, LiCl). For the rewarding condition, a single piece (1 g) of milk chocolate (Milka, Kraft) was used for microdialysis. Consumption was assessed by weighing the amount of chocolate left at the end of the experiment. Finally, latency to eat was recorded and mice were weighed on the day of the experiment. Animals subjected to restraint were placed for 180 min on restraint apparatus formed by an adjustable neck-blocking support mounted on a Plexiglas base and a movable U-shaped metal piece that could be fixed to the base at the level of the animal's hips, thus preventing the mouse from turning on its back (Cabib and Puglisi-Allegra 1991). For all treatments (chocolate, restraint, and LiCl), dialysate was collected every 20 min for 180 (chocolate and restraint) or 120 min (LiCl). Twenty microliters of the dialysate samples were analyzed by high performance liquid chromatography (HPLC). The remaining 20 µL were kept for possible subsequent analysis. Concentrations (pg/20 µL) were not corrected for probe recovery. The HPLC system consisted of an Alliance (Waters Corporation, Milford, MA, United States of America) system and a coulometric detector (ESA Model 5200A Coulochem II) provided with a conditioning cell (M 5021) and an analytical cell (M 5011). The conditioning cell was set at 400 mV, electrode 1 at 200 mV, and electrode 2 at −250 mV. A Nova-Pack C18 column (3.9 × 150 mm, Waters) maintained at 30°C was used. The flow rate was 1.1 mL/min. The mobile phase was as previously described (Ventura et al. 2005, 2007). The assay detection limit was 0.1 pg.

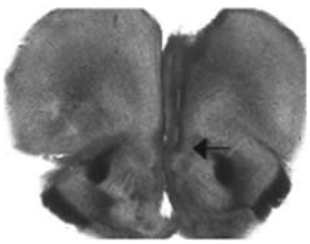

Figure 1. The location of microdialysis probes in the mpFC. Photomicrograph of coronal sections (approximately +2.50 mm anterior to bregma [Franklin and Paxinos 1997]) showing the microdialysis probe trace in the mpFC. Arrowhead indicates the tip of the probe.

Medial Prefrontal Cortex NE Depletion

Anesthesia and surgery were carried out as previously described (Ventura et al. 2003, 2005, 2007, 2008). Animals were injected with GBR (15 mg/kg) 30 min before the 6-OHDA microinjections in order to protect dopaminergic neurons. The bilateral injection of 6-OHDA (1.5 µg/0.1 µL/2 min for each side) was made into the mpFC (coordinates: +2.52 AP; ±0.6 L; −2.0 V) (Franklin and Paxinos 1997) through a stainless steel cannula (0.15 mm outer diameter, UNIMED, Switzerland) connected to a 1 µL syringe by a polyethylene tube and driven by a CMA/100 pump (NE-depleted group). The cannula was left in place for an additional 2 min after the end of the infusion. Sham animals were subjected to the same treatment, but received an intracerebral vehicle after GBR administration. Animals were used for behavioral experiments 7 days after surgery. NE and DA tissue levels in the mpFC were assessed by HPLC-electrochemical detection analysis to evaluate the extent of depletion, as previously described (Ventura et al. 2003, 2005, 2007).

Prefrontal NE Depletion and LiCl-Induced CPA

CPA was used to evaluate the effects of selective mpFC NE depletion on LiCl-induced aversion. Two groups of RCF and 2 groups of control animals (Sham and NE-depleted mice) were used. The experiment was carried out as described in the previous paragraph. Because RCF and controls showed a different dose-dependent aversion to the LiCl-paired compartment, the doses of LiCl used for the conditioning phase were 127 and 190.5 mg/kg for RCF and control animals, respectively. Animals performed this experiment 7 days after surgery.

Statistical Analyses

Place Conditioning

For place conditioning experiments, statistical analyses were performed on preference scores assessed by calculating the time spent in chocolate or LiCl (paired) and standard food/saline (unpaired) compartments on the test day minus the time spent in the same compartments on the pretest session. For the CPP experiment, data were analyzed using 2-way repeated-measures analysis of variance (ANOVA) with 1 between factor (early experience, 2 levels: Control, cross-fostering) and 1 within factor (pairing, 2 levels: Paired, unpaired), followed by post hoc comparisons (Tukey honestly significant difference

(HSD) test). Data for mean chocolate consumption collected during the conditioning phase of CPP were analyzed by 1-way ANOVA. To rule out basal differences between control and RCF groups, a 1-way ANOVA was performed to analyze locomotor activity during the pretest session.

For CPA experiments, data were analyzed using 2-way repeated-measures ANOVA with 2 between factors (early experience, 2 levels: Control, cross-fostering; dose, 3 levels: 63.5, 127, 190.5 mg/kg i.p.) and 1 within factor (pairing, 2 levels: Paired, unpaired), followed by post hoc comparisons (Tukey HSD test). Locomotor activity during the conditioning phase of LiCl-induced CPA was analyzed by repeated-measures ANOVA with 2 between factors (early experience, 2 levels: Control, cross-fostering; dose, 3 levels: 63.5, 127, 190.5 mg/kg i.p.) and 1 within factor (day, 4 levels: d1, d3, d5, and d7). A repeated-measures ANOVA with 1 between factor (early experience, 2 levels: Control, cross-fostering) and 1 within factor (day, 4 levels: d1, d3, d5, and d7) was performed for each dose. Finally, to rule out any spontaneous preference for 1 particular chamber, mean time spent in paired and unpaired chambers during the pretest session was analyzed by repeated-measures ANOVA for each group.

Saccharin Preference Test

For the SPT, statistical analyses were performed by 2-way repeated-measures ANOVA with 2 between factors (early experience, 2 levels: Control and RCF; dose, 2 levels: 0.25% and 0.5%) and 1 within factor (time, 5 levels: From day 1 up to 5). Separate repeated-measures ANOVA (early experimental condition, 2 levels: Control or RCF; time, 5 levels: From day 1 up to 5) was also performed for each dose (0.25% and 0.5%). Finally, percentage preference of saccharin intake was evaluated.

Forced Swim Test

Statistical analyses were carried out on the total duration in seconds of each behavioral response on pretest or test days. Data were analyzed by 1-way ANOVA for each behavioral item: Immobility, paddling, struggling, and swimming.

Microdialysis

Data obtained from microdialysis experiments were analyzed on raw data. The effects of chocolate exposure, restraint experience, or LiCl on NE and DA release in the mpFC were analyzed by separate repeated-measures ANOVA with 1 between factor (early experience, 2 levels: Control, cross-fostering) and 1 within factor (time, 10 levels: 0, 20, 40, 60, 80, 100, 120, 140, 160, 180 for chocolate and restraint, and 7 levels: 0, 20, 40, 60, 80, 100, 120 for LiCl). Simple effects were assessed by 1-way ANOVA for each time point. Individual between-group comparisons were carried out, when appropriate, by post hoc test (Duncan Multiple Range Test). Finally, to assess whether the hemisphere used for sampling collection could affect microdialysis data, the effect of lateralization (right side vs. left side) was analyzed by repeated-measures ANOVA with 1 between factor (side: 2 levels: Right and left) and 1 within factor (time, 10 levels: 0, 20, 40, 60, 80, 100, 120, 140, 160, and 180) within each group (control, cross-fostering) for chocolate and restraint experiments. Data from mean chocolate consumption, latency to eating, and animals' body weight, were analyzed by 1-way ANOVA.

Medial Prefrontal Cortex NE Depletion

The effects of prefrontal NE depletion on DA and NE tissue levels were analyzed by 2-way ANOVA (early experience, 2 levels: Control, cross-fostering; pre-treatment, 2 levels: Sham, NE-depleted), followed by post hoc comparisons (Tukey HSD test).

Prefrontal NE Depletion and LiCl-Induced CPA

Effects of selective mpFC NE depletion on LiCl-induced CPA (127 or 190.5 mg/kg i.p.) were analyzed by repeated-measures ANOVA, with 1 between factor (pre-treatment: Sham, NE-depleted) and 1 within factor (pairing, 2 levels: Paired, unpaired), followed by post hoc comparisons (Tukey HSD test).

Results

Place Conditioning

The effects of RCF experience on CPP are shown in Figure 2. There was no statistically significant difference in preference for 1 chamber of the apparatus in control or RCF groups during the pretest session (Supplementary Table S1). For test session, the repeated-measure ANOVA revealed a significant early experience × pairing interaction ($F_{1,18} = 4.51$; $P < 0.05$). Post hoc testing revealed that whereas control animals showed a significant preference for the chocolate-paired compartment ($P < 0.05$). RCF animals showed no preference for the chocolate-paired compartment. ANOVA analyses revealed no significant effect on mean chocolate consumption during the conditioning phase of CPP (Supplementary Table S1). Finally, there were no significant differences in locomotor activity between control and RCF during the pretest session (Supplementary Table S1).

The effects of RCF experience on CPA are shown in Figure 3. Neither the control nor the RCF group showed significant chamber preference during the pretest session (Supplementary Table S1). For CPA test, the repeated-measures

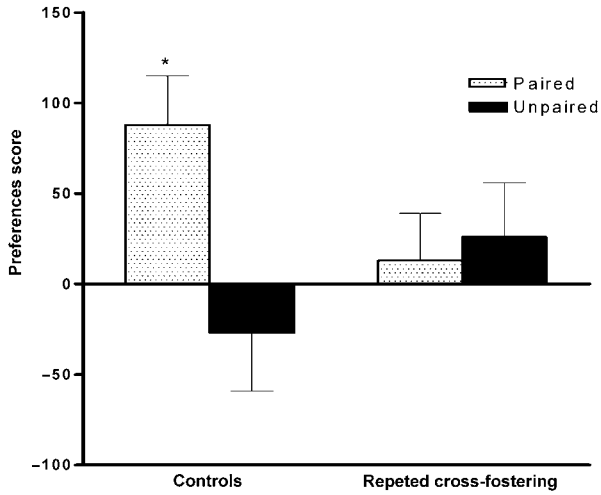

Figure 2. Effects of RCF on chocolate-induced CPP. All data are expressed as preference scores as follows: Mean time spent (±SE; s) in paired and unpaired compartments on the test day minus the time spent in the same compartments during the pretest session ($n = 10$ per group). *$P < 0.005$ compared with the unpaired chamber.

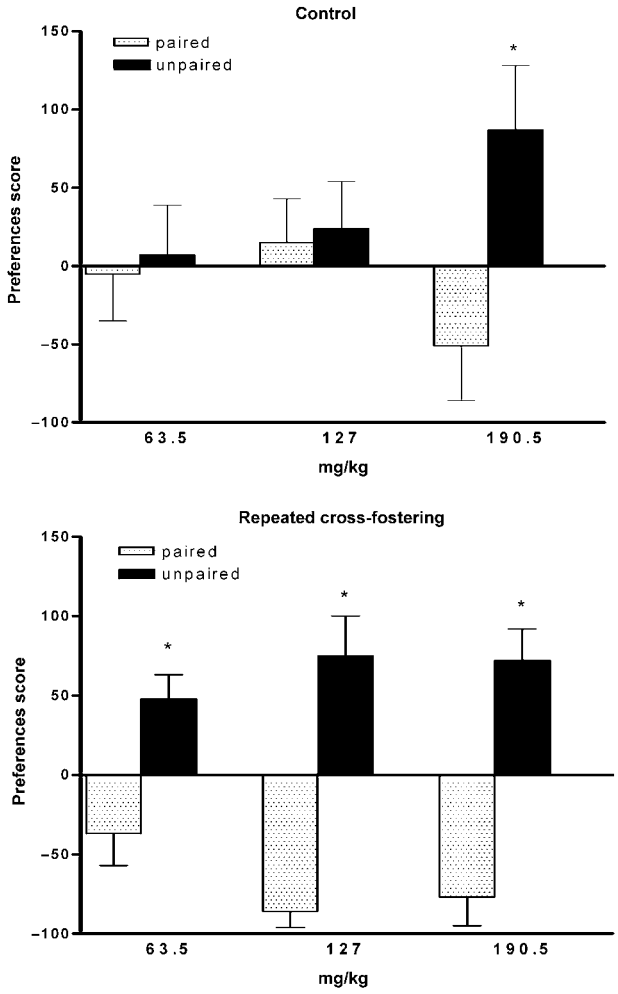

Figure 3. Effects of RCF on LiCl-induced (63.5, 127, 190.5 mg/kg i.p.) CPA. All data are expressed as preference scores as follows: Mean time spent (±SE; s) in paired and unpaired compartments on the test day minus the time spent in the same compartments during the pretest session ($n = 8$ per group). *$P < 0.05$ compared with the paired chamber.

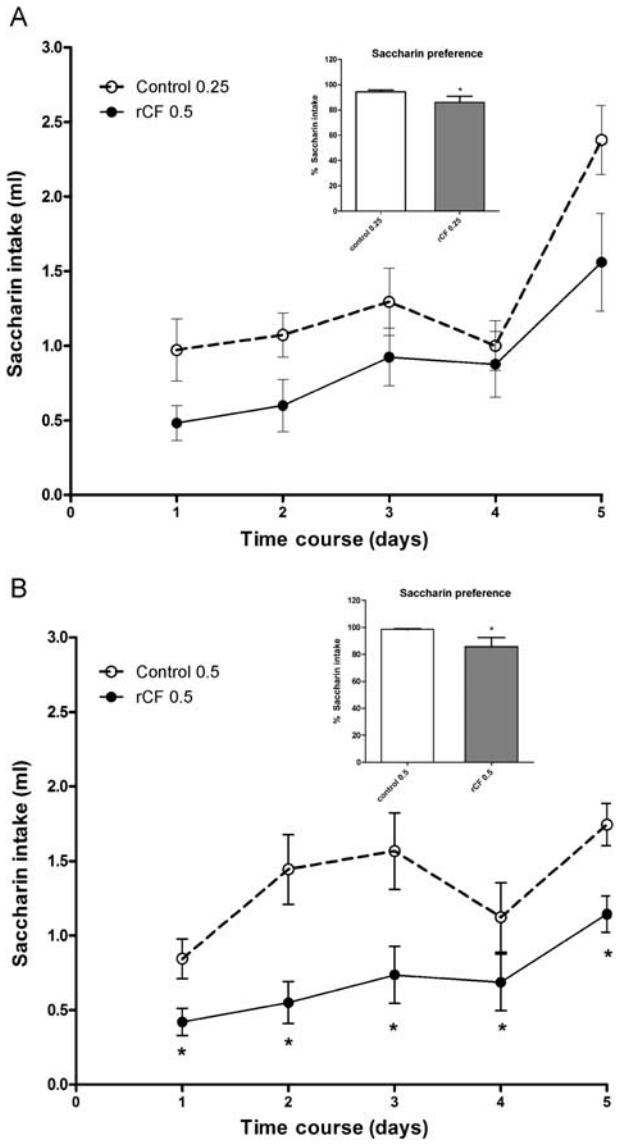

Figure 4. Effects of RCF under the SPT. The figure depicts saccharin intake (mL) of cross-fostered (RCF) and control animals at 2 different concentrations (0.25% in *A* and 0.5% in *B*) during 5 days (d1–d5) of the SPT. All data are expressed as the mean of saccharin intake (±SE; mL). The small inserts in *A* and *B* depict % of preference changes between control and RCF animals. *$P < 0.05$ compared with control animals.

ANOVA revealed a significant early experience effect ($F_{1,42} = 14.34$: $P < 0.001$). Post hoc comparisons indicated that whereas RCF animals showed an aversion to the LiCl-paired compartment at all doses (63.5 mg/kg: $P < 0.05$; 127 mg/kg: $P < 0.001$; 190.5 mg/kg: $P < 0.001$), control animals showed a significant aversion to the LiCl-paired compartment only at the higher dose (190.5 mg/kg: $P < 0.001$). This suggests that early adverse experience may increase sensitivity to aversive experience in adult life. The repeated-measures ANOVA did not reveal a significant interaction between early experience, doses, and day with regard to locomotor activity during the conditioning phase of CPA. Moreover, the repeated measures ANOVA for each dose revealed no significant early experience × day interaction, regardless the dose administered (Supplementary Table S1).

Saccharin Preference Test

In the SPT, ANOVA analysis revealed a significant day × dose interaction ($F_{4,20} = 2.66$; $P < 0.05$) and a significant early experience effect ($F_{1,200} = 8.034$; $P < 0.01$). The repeated measures ANOVA for each dose revealed a significant day effect ($F_{4,120} = 18.603$; $P < 0.0005$) for the 0.25% dose and significant day effects ($F_{4,80} = 11.082$; $P < 0.0005$) and early experience ($F_{1,80} = 7.548$; $P < 0.05$) for the 0.5% dose. Thus, although both groups showed a significant increase in saccharin intake over time, the RCF group drank less sweetened solution than control mice (Fig. 4).

Finally, preference data showed that the percentage of saccharin drank over the total fluid intake was higher in control mice than in RCF mice (0.25%: [$F_{1,30} = 4.834$; $P < 0.05$]; 0.5%: [$F_{1,20} = 11.096$; $P < 0.005$]).

Forced Swim Test

One-way ANOVA revealed a significant effect for swimming, struggling, and immobility on both pretest and test days (pretest: Swimming [$F_{1,22} = 5.18$; $P < 0.05$]; struggling [$F_{1,22} = 4.61$; $P < 0.05$]; immobility [$F_{1,22} = 9.21$; $P < 0.05$]). Test:

Swimming ($F_{1,22} = 4.45$; $P < 0.05$); struggling ($F_{1,22} = 6.75$; $P < 0.05$); immobility ($F_{1,22} = 5.62$; $P < 0.05$). RCF experience significantly decreased swimming and struggling and increased immobility on both pretest and test days (Table 1), but had no significant effect on paddling.

Microdialysis

Data analyses for the effects of the rewarding stimulus (chocolate) on the prefrontal NE and DA outflow showed a significant early experience × time interaction (NE: $F_{9,129} = 2.53$; $P < 0.05$; DA: $F_{9,129} = 2.026$; $P < 0.05$). Simple effect analyses revealed a significant time effect for the control group only and a significant difference between the two groups (control vs. RCF) attributable to chocolate exposure. In control animals, chocolate produced a time-dependent increase of mpFC in the NE and DA outflow, reaching a maximal increase of ~50% at 100 and 60 min for NE and DA, respectively (Fig. 5). By contrast, in the RCF group, chocolate exposure produced no significant NE or DA increase (Fig. 5). The absolute (average) basal levels of NE or DA for each group did not differ significantly (NE: Control = 1.39 ± 0.23, RCF = 1.37 ± 0.21; DA: Control = 0.69 ± 0.19, RCF = 0.65 ± 0.19 pg/20 µL). Finally, statistical analyses for the effects of brain side on the prefrontal NE and DA outflow revealed no significant side × time interaction in control (Supplementary Table S1) animals. ANOVA analysis of latency to eat and animals' body weight revealed no significant effect on mean chocolate consumption during microdialysis (Supplementary Table S1). Statistical analyses for the effects of restraint on the prefrontal NE and DA outflow revealed a significant early experience × time interaction (NE: $F_{9,129} = 2.17$; $P < 0.05$; DA: $F_{9,129} = 2.26$; $P < 0.05$). Simple effect analyses revealed a significant time effect for both NE and DA outflow in the control group, and a significant time effect only for the NE outflow in the RCF group. Moreover, a significant difference in the NE or DA outflow between control mice and RCF mice subjected to restraint was shown (Fig. 6). Restraint produced a more prolonged NE increase in the RCF group compared with the control group and no significant DA augmentation. Control animals showed a time-dependent increase in the NE and DA outflow reaching a maximal increase of ~100% at 20 min and of ~65% at 100 min, respectively. Restraint induced longer and more sustained NE increases in RCF animals than in the control group and no significant DA increase. The absolute (average) basal levels of NE or DA for each group did not differ significantly (NE: Control = 1.42 ± 0.2, RCF = 1.38 ± 0.22; DA: Control = 0.65 ± 0.21, RCF = 0.61 ± 0.17 pg/20 µL). Finally, statistical analyses for the effects of brain side on the prefrontal NE and DA outflow revealed no significant side × time interaction in control or RCF mice (NE: $F_{9,54} = 1.39$, ns; DA: $F_{9,54} = 0.29$, ns) animals (Supplementary Table S1).

Statistical analyses for the effects of LiCl on the prefrontal NE and DA outflow revealed a significant early experience × time interaction for NE ($F_{6,90} = 2.304$; $P < 0.05$) and a significant early experience ($F_{1,90} = 4.76$; $P < 0.05$), and time effect ($F_{6,90} = 5.83$; $P < 0.005$) for DA (Fig. 7). Moreover, a significant difference in the NE or DA outflow between control and RCF mice injected with LiCl was shown. LiCl administration induced more sustained the NE outflow and lower DA in RCF mice compared with control animals.

Table 1
Effects of RCF in the FST

	Pretest			Test		
	Control	RCF	$F_{1,22}$	Control	RCF	$F_{1,22}$
Immobility	1941 ± 267	3009 ± 220	9.21*	1206 ± 170	1779 ± 156	5.62*
Swimming	944 ± 86	675 ± 75	5.18*	354 ± 35	220 ± 44	4.45*
Struggling	1513 ± 152	1025 ± 578	4.61*	741 ± 123	405 ± 67	6.75*
Paddling	1642 ± 137	1294 ± 104		701 ± 69	598 ± 76	

Note: Overall immobility, swimming, and struggling scores are reported for pretest (10 min) and test session (5 min), respectively. All data are expressed as mean time spent (±SE; s) (n = 9–15 per group).
*$P < 0.05$ compared with the control group.

Medial Prefrontal Cortex NE Depletion

Two-way ANOVA for the effects of prefrontal NE depletion on DA and NE tissue levels showed a significant pre-treatment

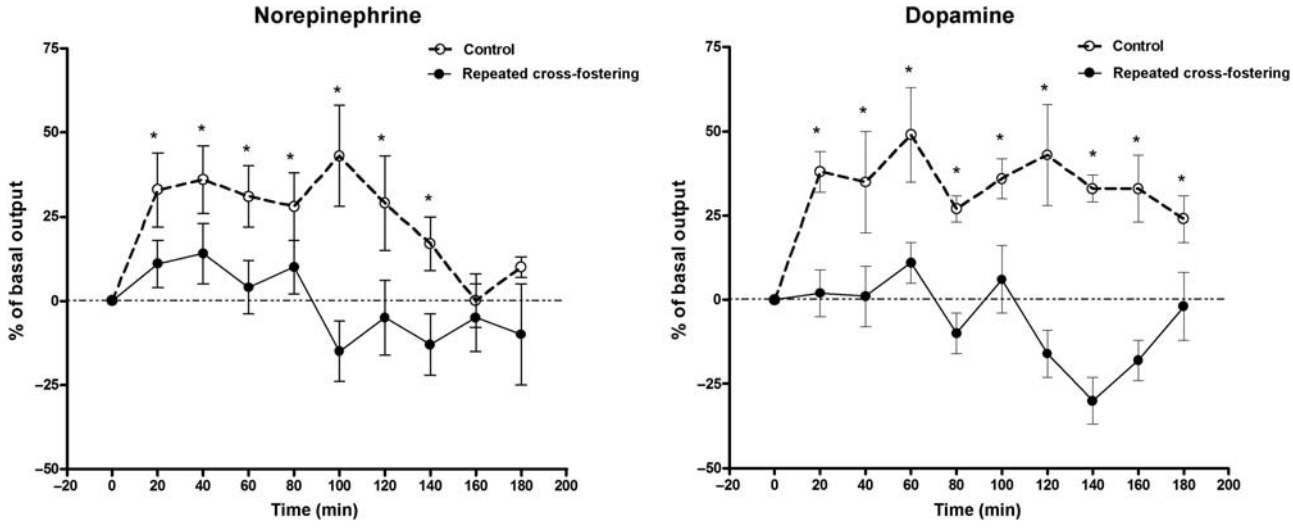

Figure 5. Effects of RCF on NE and DA outflow in the mpFC induced by rewarding stimulus exposure (chocolate). Extracellular NE and DA in the mpFC of control and cross-fostered (RCF) animals exposed to 1 g milk chocolate. Results are expressed as percent changes from the base levels of each experimental group (n = 8 per group). Chocolate pellets were presented at time 0. All data are expressed as mean ± SE. *$P < 0.05$ compared with the other group.

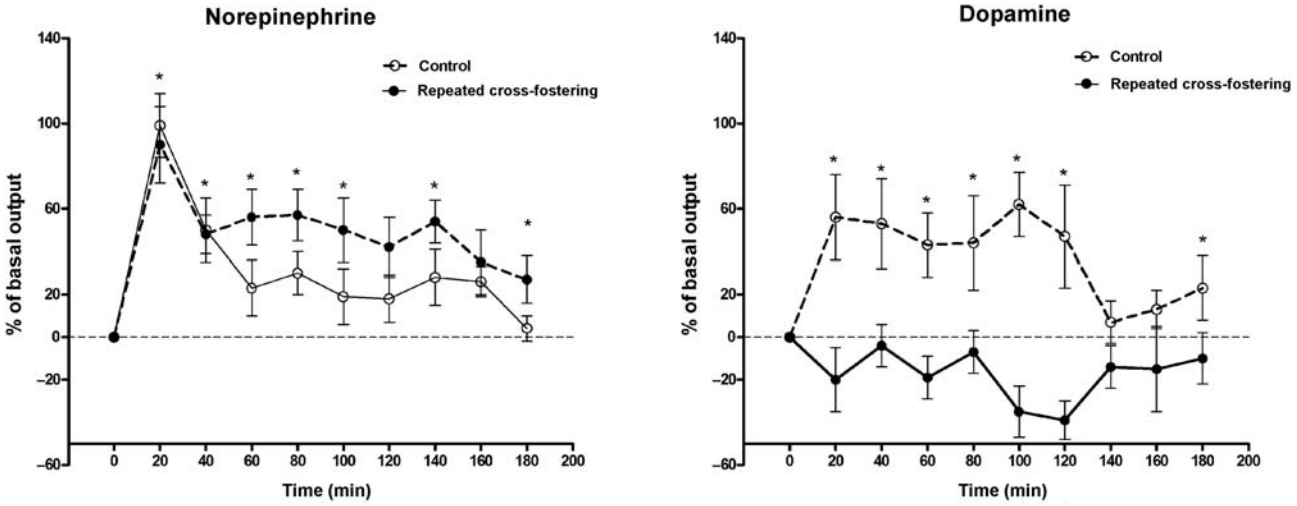

Figure 6. Effects of RCF on NE and DA outflow in the mpFC induced by aversive stimulus exposure (restraint). Extracellular NE and DA in the mpFC of control and cross-fostered (RCF) animals exposed to restraint. Results are expressed as percent changes from the base levels of each experimental group ($n = 8$ per group). Restraint was initiated at time 0. All data are expressed as mean ± SE. *$P < 0.05$ compared with the other group.

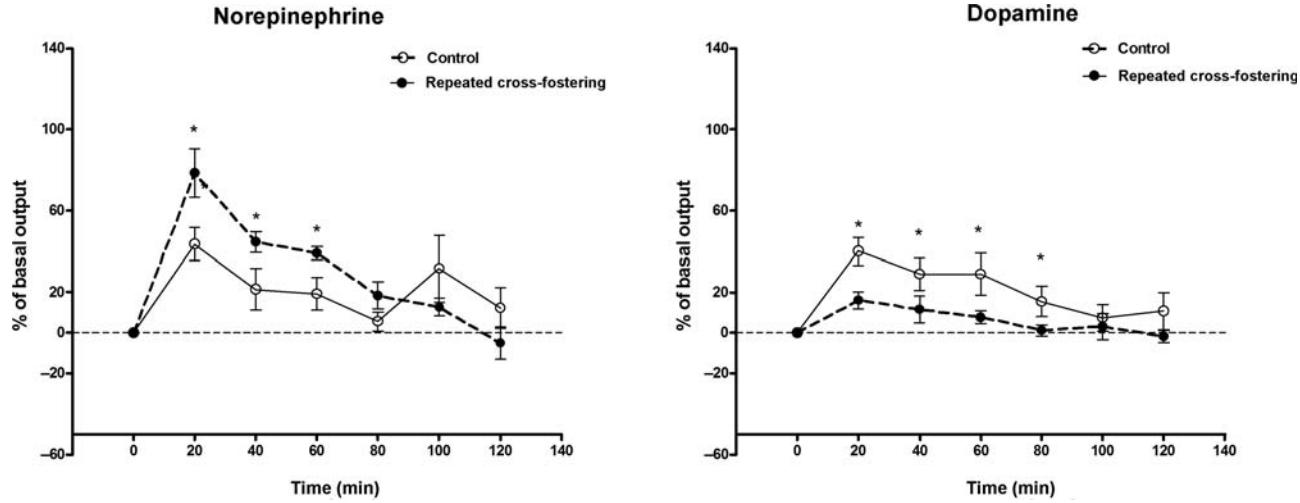

Figure 7. Effects of RCF on NE and DA outflow in the mpFC induced by LiCl treatment. Extracellular NE and DA in the mpFC of control and cross-fostered (RCF) animals injected with LiCl (190.5 mg/kg i.p). Results are expressed as percent changes from the base levels of each experimental group ($n = 8$ per group). LiCl was administered at time 0. All data are expressed as mean ± SE. *$P < 0.05$ compared with the other group.

effect for NE only ($F_{1,28} = 7.75$; $P < 0.005$). Selective depletion of NE prefrontal cortical afferents yielded approximately a 90% decrease in NE tissue levels (Sham = 435 ± 29; NE-depleted = 49 ± 13 ng/g wet tissue), whereas DA tissue levels were spared (Sham = 233 ± 35; NE-depleted = 217 ± 36 ng/g wet tissue). Post hoc comparisons showed no significant difference between Sham control and Sham RCF groups (NE: control = 419.34 ± 22; RCF = 451.3 ± 12; DA: control = 243 ± 16; RCF = 224 ± 13).

Effects of Prefrontal NE Depletion on LiCl-Induced CPA

The effects of NE depletion in the mpFC on LiCl-induced CPA are shown in Figure 8. No significant chamber preference was observed in control or RCF mice during the pretest session (Supplementary Table S1). The repeated-measures ANOVA revealed no significant pre-treatment × pairing interaction for both control and RCF groups (for testing session). However, post hoc comparison revealed that while both sham groups showed a significant aversion to the LiCl-paired compartment (RCF: $P < 0.005$; control: $P < 0.005$), NE-depleted groups showed no aversion for either compartment (RCF: ns; control: ns), indicating that prefrontal NE depletion abolished LiCl-induced CPA in both control and RCF animals.

Discussion

The experiments described here suggest that postnatal RCF experience blunts the natural tendency to seek pleasurable sources of natural rewards and increases sensitivity to negative events in adult life. Thus, in spite of the fact that RCF and control pups do not differ in terms of maternal care received (D'Amato et al. 2011), events that occurred during the early postnatal days (the replacement of the biological mother and the repeated shifting of pups to different adoptive dams) are early life events capable of producing enduring consequences in adult life.

This study demonstrates several long-lasting changes in the adult offspring of cross-fostered pups, particularly: 1) an

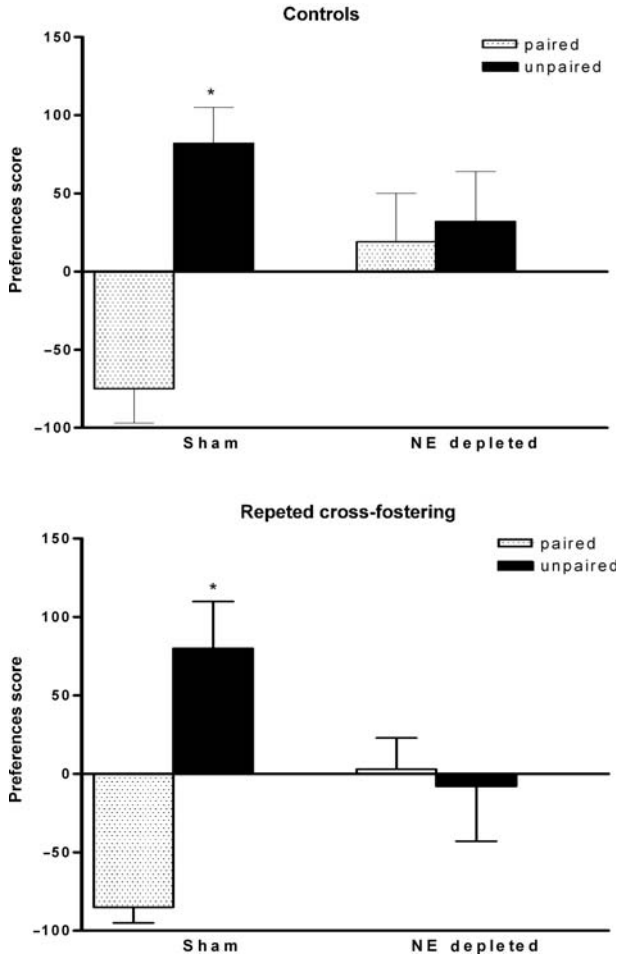

Figure 8. Effects of prefrontal cortical NE depletion on CPA induced by LiCl. Preference scores shown by Sham and NE-depleted groups in the CPA test ($n = 8$ per group; control LiCl 190.5 mg/kg i.p.; RCF LiCl 127 mg/kg i.p.). All data are expressed as mean ± SE. *$P < 0.005$ compared with the paired chamber.

impairment of the CPP induced by palatable food (chocolate); 2) a decrease in the natural preference for sweetened fluids in the SPT; 3) increased sensitivity to aversive experience (CPA); 4) greater immobility in the FST (compared with controls); 5) suppression of place aversion in animals that underwent mpFC NE depletion; 6) prevention of the prefrontal cortical DA and NE outflow during exposure to a rewarding stimulus; and 7) a different pattern of prefrontal cortical DA and NE release in response to aversive stimuli, with prolonged NE elevation and no significant DA increase. These findings are also reported in Supplementary Table S2.

During the neonatal period, the brain is particularly vulnerable to environmental stimuli. Exposure of the developing nervous system to adversity early in life can therefore shape synaptic plasticity and neurogenesis (Aisa et al. 2009; Lupien et al. 2009; Korosi et al. 2011). Thus, perinatal stress in rodents and primates may permanently change brain functioning, increasing the probability of dysfunctional behavior and physiology in adulthood, including cognitive impairment, HPA axis dysfunction, energy homeostasis imbalance, and alterations in sociability and reward processing (Mintz et al. 2005; Pryce et al. 2005; Coccurello et al. 2009; McClelland et al. 2011). Early life adverse experiences have been shown to increase the risk of developing depression or anxiety disorders later in life (Heim and Nemeroff 2001, 2002; Meaney 2001; Sánchez et al. 2001) and can produce persistent alterations in aminergic transmission in different areas of the brain (Arborelius and Eklund 2007; Jezierski et al. 2007). The mesolimbic dopaminergic system is a target of early life adverse experience; repeated events of maternal separation may exacerbate the rewarding effects of psychostimulants and DA response to stress (Meaney et al. 2002; Brake et al. 2004). The pFC is particularly susceptible to the early perturbation of social attachment during the maturational period (Teicher et al. 2003; Spinelli et al. 2009) and neonatal exposure to psychosocial stressors may significantly alter the morphology and neurochemical balance of prefrontal catecholamine afferences (Benes et al. 2000; Braun et al. 2000; Arborelius and Eklund 2007; Pascual and Zamora-León 2007). Our data demonstrate that RCF experience can disrupt the prefrontal processing of rewarding salient stimuli, thereby blunting prefrontal DA response to reinforcing events.

Repeated Cross-Fostering Impairs Sensitivity to Natural Reward and Exacerbates Responses to Adverse Experiences

Our data demonstrate that RCF experience early in life produces enduring effects in adult life. Adult RCF animals appear to be less sensitive to the rewarding value of positive stimuli. By contrast to controls, which showed a clear preference for the chocolate-paired chamber in the CPP test, the lack of preference in RCF animals indicates that early postnatal experience prevents in these animals the attribution of motivational salience to conditioned stimuli paired with primary natural rewarding events. This appears to be consistent with recent data reporting that neonatally handled rats showed a less CPP to sweet food (Silveira et al. 2010). Moreover, because chocolate ingestion did not differ between groups and because there was a strong tendency toward increased chocolate intake (control: 2.125 ± 0.466; RCF: 3.225 ± 0.362), it is difficult to ascribe the impairment of CPP shown by cross-fostered animals to reduced chocolate consumption during the conditioning phase.

Reduced preference for sweetened fluids in the SPT further supports the idea that the perturbation of mother–infant bonding induced by the RCF procedure alters the individual tendency to orient behavior toward pleasurable and rewarding natural experiences in adult life. It is noteworthy that a decreased sensitivity to pleasurable events or a reduced ability to experience pleasure is regarded as one hallmark symptom of depression-like syndrome (i.e. anhedonic state). However, the literature on anhedonia-like behavior in experimental models of early deprivation or maternal separation is inconsistent, showing either no effects on sucrose preference (Shalev and Kafkafi 2002) or a minor impact of the incentive value of sucrose consumption in rats (Rüedi-Bettschen et al. 2005; Leventopoulos et al. 2009). Furthermore, the disruption of early rearing conditions may alter the reward sensitivity to psychostimulants during adulthood (Der-Avakian and Markou 2010). Moreover, genetic background and complex gene–environment interplay may determine either susceptibility or resilience to cocaine self-administration in different inbred mouse strains fostered by non-strain-related mothers (van der Veen et al. 2008). Our results support the hypothesis that early life experience can shape the response toward natural

appetitive stimuli (chocolate and saccharine), although we cannot extend our results to different pharmacological rewarding stimuli such as drugs of abuse.

It has been shown that the amount of the maternal care received at birth can influence prefrontal DA responsivity to stress in adult life (Zhang et al. 2005; Kaffman and Meaney 2007). In particular, when subjected to a stress challenge, adult animals raised by "low caring" mothers showed blunted DA signaling in the right medial pFC and increased DA response in the left hemisphere (Zhang et al. 2005). The discrepancies between blunted pFC DA outflow in RCF mice and the enhanced DA response found in the left mpFC of neglected animals may be reconciled considering that RCF adoptive mothers did not provide a lesser degree of maternal care to RCF pups (D'Amato et al. 2011). A functional hemispheric asymmetry occurs in the medial pFC specifically in stressful conditions involving mesocorticolimbic DA input into this brain region (Sullivan and Gratton 1998; Brake et al. 2000). However, lateralization of stress-induced variation in pFC DA function appears to be modulated by the nature and duration of the stressor (Carlson et al. 1991).

Taken together, our data support the idea that the removal of stable and predictable social bonds may severely disrupt the propensity to seek pleasurable sources of natural rewards later in life. The responses observed from place aversion behavior further suggest that the impact produced by early RCF on adult life is not restricted to the ability to seek rewarding experiences. As shown by the strong aversion of cross-fostered mice to doses of LiCl lower than those used in control animals, this early experience can also exacerbate the sensitivity of animals to aversive stimuli. Because there was no significant difference in motor activity between control and RCF groups during the conditioning phase of CPA, we can rule out the occurrence of unspecific locomotor effects upon sensitivity to LiCl treatment observed in the CPA experiment. The possible link between enhanced susceptibility to LiCl and RCF is supported by the total suppression of CPA in prefrontal NE-depleted mice. The results from prefrontal NE depletion indicate that NE transmission is required for LiCl-induced CPA in both control and cross-fostered animals. This is consistent with the previously reported LiCl-induced increase of prefrontal NE and the demonstration that the lack of NE release induced by exposure to aversive stimulus prevents motivational salience attribution to conditioned stimuli (Ventura et al. 2007). When submitted to the pretest phase of FST, cross-fostered animals showed a higher immobility and lower active behaviors than controls, thus indicating a higher perceived motivational impact of stressful experience. This different behavioral pattern was also evident during the test phase of FST, which is classically used to screen antidepressant drugs. Moreover, as corroborated by the lack of motor impairment during the pretest session of CPP, it is difficult to ascribe the higher immobility of RCF animals in the FST to a deficit in locomotor activity.

Repeated Cross-Fostering and Prefrontal NE and DA Imbalance

The behavior of cross-fostered mice appears to be associated with the prefrontal processing of salient stimuli. In fact, mice that underwent RCF exhibit long-lasting effects on the prefrontal DA and NE transmission evoked by rewarding and aversive events. In particular, microdialysis experiments showed that, depending on the valence of the stimuli, postnatal RCF affects prefrontal NE release in an opposite manner and prevents the mpFC DA outflow regardless of the exposure to rewarding or aversive stimuli. Whereas this is consistent with previous reports (Bassareo et al. 2002; Fallon et al. 2007; Ventura et al. 2007, 2008) in which a time-dependent catecholamine increase is observed in control animals, RCF mice showed no DA or NE increase in response to chocolate exposure.

Although prefrontal catecholamine outflow induced by chocolate exposure has been related to the motivational impact of palatable food or salient stimulus (Ventura et al. 2007, 2008), this increase could also be interpreted as the mere exposure to a novel stimulus, regardless of its rewarding value. However, because novelty has been shown to have a rewarding value (Bevins and Bardo 1999; Bevins et al. 2002; Reichel and Bevins 2008), chocolate is a stimulus that simultaneously involves salient, rewarding and novel features. Consistent with our data showing suppression of prefrontal DA efflux in response to a rewarding stimulus, separation from the mother has been shown to alter the balance of dopaminergic innervation in prefrontal and limbic areas and result in reduced dopaminergic innervation within the mpFC (Braun et al. 2000).

In the second set of experiments, we further investigated whether RCF experience affected prefrontal catecholamine transmission in response to aversive stimuli in adult life. Stressful experiences, such as restraint, are known to promote an increase of mpFC amine release, a response that is considered relevant for cognitive, emotional, and behavioral coping (Finlay et al. 1995; McQuade et al. 1999; Feenstra et al. 2000; Pascucci et al. 2007, 2009). Consistent with previous reports, control mice responded with enhanced prefrontal DA and NE release to the experience of being held in the restraining apparatus (Pascucci et al. 2009). By contrast, the exposure to the restraining condition produced a different response pattern of prefrontal DA and NE release in RCF mice. When compared with controls, RCF animals showed no significant DA release but more prolonged NE outflow in response to restraint, thus suggesting a dissociation between NE transmission and selective impairment of cortical DA outflow. Remarkably, compared with control animals, LiCl administration produced a more sustained NE outflow and a lower DA outflow in RCF mice, thus confirming the higher sensitivity to aversive events induced by the cross-fostering experience.

Hence, microdialysis data support the view that the alteration of prefrontal catecholamine response may underlie both reduced sensitivity to pleasure and overreaction to negative events.

Early Adverse Events and Functional Dissociation between Prefrontal DA and NE Encoding of Positive and Negative Motivational Signals

By contrast with the pattern of NE release, RCF produced a severe deficit in DA-dependent functional encoding of both pleasant and aversive stimuli. In particular, the lack of parallel changes in stress-induced DA and NE outflow supports the idea of the partial disconnection between the two catecholamines within the mpFC (Ventura et al. 2003, 2005; Pascucci

et al. 2007). Two non-mutually exclusive interpretations could be suggested to explain the different DA and NE responses and, in particular, the sustained prefrontal NE outflow in cross-fostered animals.

One explanation could be related to the role of prefrontal NE transmission in "resetting" the ongoing activities in the face of relevant environmental changes. Both rapid orientation of attentive resources to novel events and increased NE release have been reported in response to all known experimental stressors (Dayan and Yu 2006; Doya 2008). Thus, in spite of blunted prefrontal DA response to both rewarding (chocolate) and aversive (restraint and LiCl) stimuli, increased prefrontal NE could be needed to redirect attention toward particularly arousing events in order to process potentially dangerous stimuli. Another possible interpretation could be related to the role of prefrontal NE in encoding motivationally salient stimuli (Ventura et al. 2008). Prefrontal NE has been proposed to increase in a graded manner depending on the salience of the stimulus (Feenstra et al. 2000; Ventura et al. 2008). Accordingly, the sustained prefrontal NE increase shown by cross-fostered animals could represent an index of higher perceived salience of aversive conditions. This interpretation is strengthened by the comparison of behavioral and microdialysis data. In fact, the CPA experiment indicates that this early emotional experience exacerbates sensitivity to aversive stimuli, as shown by the stronger aversion to LiCl and by the higher LiCl-induced NE prefrontal increase in RCF compared with control mice.

The possible correlation between heightened aversion to LiCl and prolonged prefrontal NE release to aversive stimuli appears to be further supported by the total suppression of CPA in prefrontal NE-depleted cross-fostered mice. This is consistent with the previously reported LiCl-induced increase of prefrontal NE and the demonstration that the lack of NE release induced by exposure to an aversive stimulus prevents motivational salience attribution to conditioned stimuli in a place-conditioning procedure (Ventura et al. 2007). Thus, the strong elevation of prefrontal NE in response to both the restraint experience and the LiCl treatment parallels the higher sensitivity of cross-fostered animals to the lower dose of LiCl in the place conditioning procedure. In other words, it seems that the RCF experience lowered the threshold that triggers prefrontal NE outflow, as mirrored by LiCl-induced CPA at lower doses than in control mice and by the enhanced NE release during LiCl exposure and restraining experience. Together, we provide evidence that perturbation of the early rearing environment can induce a broad spectrum of behavioral alterations reminiscent of depressive symptoms and permanently change mpFC DA and NE transmission in adult life. To the best of our knowledge, this is the first study to report a functional dissociation between suppressed DA and NE outflow in response to rewarding natural stimuli and heightened NE release in response to aversive stimuli. On these grounds, we show that postnatal formation/refinement of catecholaminergic transmission of mpFC is shaped by the instability of the maternal environment and that these changes can severely affect behavioral coping strategies and efficient adaptation to opposite environmental events in adult animals. The dichotomy between underestimation of positive stimuli and overestimation of negative events observed in RCF animals is reminiscent of Beck's cognitive formulation of depression as biased processing of incoming information and genesis of dysfunctional beliefs (Beck 2008). Thus, the behavioral/neurochemical profile shown by RCF animals raises the possibility that different prefrontal DA/NE response to salient stimuli may be relevant for the pathogenesis of depressive-like behaviors.

In conclusion, our results shed light on the relationship between traumatic early attachment, prefrontal catecholamine responses and vulnerability to psychiatric disorders later in life. Understanding how the ability to process motivationally salient stimuli is affected by environmental experiences during early brain development might help to identify different emotional/motivational deficits in adult humans and to understand how prefrontal alterations of neural processing of both rewarding and aversive stimuli can induce depression-like behavioral outcomes.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes

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