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Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum

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Abstract

In order to determine the effect of postnatal environments on some behavioral and neurochemical depressive-like parameters, male Sprague–Dawley rats were reared from weaning in either social isolation, standard laboratory conditions, or environmental enrichment. Open-field activity was assessed at postnatal days 37, 65, 93 and 107 and 1 h before the last open-field test, a forced-swimming test was carried out. After behavioral tests, the monoamines concentrations were analyzed in prefrontal cortex and ventral striatum. Relative to control and isolation rearing, the environmental enrichment reduced open-field activity, led to antidepressive-like effects and increased serotonin concentrations in the prefrontal cortex. Social isolation, on the other hand, did not affect open-field activity, but increased depressive-like behavior and reduced the amount of norepinephrine in the ventral striatum. Those neurochemical changes induced by rearing conditions correlated with the behavioral performance in the forced-swimming test. Also, immobility behavior could be predicted by locomotor activity even from the first week of housing. Overall, specific variations in physical and social environment during early rearing lead to some behavioral and neurochemical alterations which might be relevant for understanding the role that neurodevelopmental and experiential factors could have in human depression.

Keywords: Depression; Environmental enrichment; Social isolation; Monoamines; Prefrontal cortex; Striatum; Open-field behavior; Forced-swimming behavior; Stress; Brain development; Model; Rats

1. Introduction

Enriched environments, i.e. housing conditions providing a combination of enhanced social relations, physical exercise and non-social stimuli, are used to study relationships between behavior and experience-dependent changes in the brain (for review see Rosenzweig and Bennett, 1996 and Van Praag et al., 2000). At the behavioral level, it has been shown that rearing animals in a complex environment reduces anxiety (Fernández-Teruel et al., 2002), accelerates habituation (Zimmermann et al., 2001), enhances learning and memory (Larsson et al., 2002; Schrijver et al., 2002) and improves stress-coping abilities (for review see Fernández-Teruel et al., 2002). Social isolation, conversely, might produce several negative long-term consequences for the animal. It has been demonstrated that isolated rats exhibit a well characterized behavioral pattern called social isolation syndrome, that includes locomotor hyperactivity in a novel environment (Hall, 1998; Heidbreder et al., 2000; Robbins et al., 1996), increased anxiety in several paradigms (Weiss et al., 2004), impairments in learning and memory tasks (Larsson et al., 2002; Schrijver et al., 2002) as well as in the pre-pulse inhibition test (PPI) (Weiss et al., 2000). Additionally, isolation can alter the dopaminergic mesolimbic system and the response to several dopaminergic compounds (Hall, 1998; Robbins et al., 1996). Commonly, behavioral

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studies have focused either on learning/memory in enriched animals, or on locomotor activity and PPI in isolated rodents, and less on the emotional repertory of both conditions.

On the other hand, there is a growing body of epidemiological evidence strongly suggesting that early adverse experiences in the form of neglect and/or abuse are preeminent factors in the development of depressive disorder (Kendler et al., 2002; Parker et al., 1995). Basic and clinical studies also indicate a complex interplay between genetic susceptibility, environmental experience, and maturation-aging factors (for review see Levine, 2005; Plotsky et al., 1998). However, the neural mechanisms underlying emotional abnormalities stemming from early life adversity remain poorly understood. Although this developmental–experiential process could be a risk factor in human depression, most of the widely used rodent models that are sensitive to the effects of antidepressant agents, do not include postnatal manipulations (for review see Cryan et al., 2002). Even so, there is evidence suggesting that specific variations of postnatal environment in rats and monkeys, such as, maternal separation, social defeat, or acute and chronic stress might produce long lasting effects that resemble some behavioral and neurobiological phenotypes of depression (for review see Pryce et al., 2005).

Pharmacological, neuroimaging, lesion and postmortem studies have pointed out that several major depression symptoms can derive from dysfunction in prefrontal cortex, striatal and brain stem systems (Anisman and Zacharko, 1992; Drevets et al., 2004; Hayley et al., 2005; Maes and Meltzer, 1995). Among diverse neurochemical alterations, the depletion of serotonin (5-HT) and norepinephrine (NE) are the most widely reported, although there are some inconsistencies, principally on their metabolites levels and turnovers (Hayley et al., 2005; Maes and Meltzer, 1995). Furthermore, it is known that different forms of early social and physical deprivation also produce various neurochemical alterations in the cortico-striatal monoamines pathways, which have been related with the behavioral changes they induced (Hall, 1998; Pryce et al., 2005). However, the association between brain monoamines concentrations in prefrontal cortex, ventral striatum or hippocampus and depressive-like behaviors is not yet clear, and even less in enriched or isolated animals. Actually, there are no studies where both monoamines contents, and forced-swimming behavior have concurrently been measured in enriched animals. Regarding isolation rearing, despite monoamines contents and forced-swimming behavior have been measured, the contradictory evidence does not allow to establish a clear relationship between them (Hall et al., 1998; Hall et al., 2001; Heritch et al., 1990; Koh et al., 2007).

We previously reported that behavioral despair in rats could be buffered or increased according to the early social and physical stimulation received (Brenes-Sáenz et al., 2006). Hence, to support this developmental hypothesis that early environmental life events could predispose adult depressive-like response, we aimed to investigate the effect of rearing conditions on forced-swimming behavior and its relationship with the 5-HT and NE concentrations in prefrontal cortex and in ventral striatum, respectively. On the other hand, knowing that spontaneous open-field activity is usually affected by enrichment or isolation rearing (Hall, 1998; Pietropaolo et al., 2004; Zimmermann et al., 2001), we included it to analyze how, along the housing period, the differential rearing do exert lasting effects on behavior, aside from the observed in the forced-swimming behavior. Moreover, since spontaneous locomotor activity can be used as an index of simple information-processing or learning which reflects the organism's ability to adapt effectively to its environment (Elliot and Grunberg, 2005), we addressed the question whether a faster adaptation to a novel environment during early development would predict successful coping to a new stressful situation during adulthood.

2. Method

2.1. Animals and housing conditions

Forty-five male Sprague–Dawley rats obtained at postnatal day (PND) 22 (LEBi Laboratories, University of Costa Rica, San José) were housed in groups of six to keep the housing conditions of pre-weaning. To facilitate habituation to our colony room and to minimize influence of stress following transport from animal supplier, a one-week acclimatization period was included. At PND30, the animals were randomly distributed into three experimental groups ($n=15$ each). Two groups were kept in standard cages (top 26.5 cm × 42 cm, lower 22 cm × 37.5 cm, height 18 cm and bottom 825 cm^2; Alphete, Germany) under either isolation (SI) or group housing (three rats by cage) (SC). The other group was housed in an enriched environment (EE) in a specially designed box (120 cm length × 70 cm width × 100 cm height) containing non-chewable plastic objects and two PVC tubes, five food dispensers and two water bottles (Brenes-Sáenz et al., 2006). These objects were rearranged after a maximum of two days, to create a novel environment and to promote foraging behavior. In the other housing conditions, bed changing, food and water supply were done three times per week during the whole experiment. As it is shown in Table 1, the rats were maintained in their respective home environments until PND114 under 12:12 h light–dark schedule (light on at 06:00–18:00 h), room temperature at 20.5 °C ± 1.20 °C, 78–87% of relative humidity, 10 air cycles per hour and free access to water and food. One hour before behavioral tests, the animals were placed in an adjacent dimmed room (one 25 W red bulb) and 10 min prior to test, they were individualized in a clean cage and transported to the testing

Table 1
Experimental design

Tests[a]	W	HC	OFT	OFT	OFT	FST/OFT	NA
Weeks	0	1	2	6	10	12	13
PND	21	30	37	65	93	106–107	114

[a] W: Weaning. HC: housing conditions. OFT: Open Field Test. FST: Forced Swimming Test. NA: Neurochemical analysis. PND: postnatal day.

room. All animals were tested in a pre-determined sequence (one rat of each group in this order: EE-SC-SI), which was kept constant between tests. All behavioral tests (Table 1) were videotaped (Allview OPCOM BR29 Kit Close Circuit, USA) during the dark phase (19:00–24:00 h) under infrared light and the same environmental conditions of the colony room. All experimental procedures were done in accordance to the guidelines of the Costa Rican Ministry of Science and Technology for the Care and Use of Laboratory Animals and were approved by the Institutional Committee for Animal Care and Use of the University of Costa Rica. Particular care was taken in order to minimize the number of animals used and to reduce their suffering.

2.2. Open-field test (OFT)

Briefly, the apparatus consisted of a gray square arena (70×70×40 cm divided into four equal squares) illuminated with one 25 W red bulb located 130 cm above the center of the field. After 30 s of adaptation, locomotion (the number of squares crossed with the four paws), the number of rearing (posture sustained with hind — paws on the floor) and the time spent on grooming (including washing or mouthing of forelimbs, hind-paws, face, body and genitals) were manually counted for 10 min from the videotape recording. After each test the arena was cleaned with 90% alcohol solution. As it is shown in Table 1, the OFT was done during PNDs 37, 65, 93 and 107. In the PND107, the test started at least 1 h after the forced-swimming test.

2.3. Forced-swimming test (FST)

At PNDs 106 and 107, the FST was carried out. The whole test procedure consisted of two days with sessions lasting 15 min and 5 min, respectively. A dark Plexiglas cylinder (35 cm tall, 30 cm diameter) was used; it was filled to 21.5 cm±1.5 with water (25 °C±0.5 °C) guarantying that the animals' hind-paws did not touch the cylinder's bottom. After each session the rats were removed from water, dried with a towel and placed in a warmed enclosure. The duration in seconds of immobility (the lack of motion of the whole body, except for small movements necessary to keep the animal's head above the water), swimming (the movement, usually horizontal throughout the swim chamber that also includes crossing into another quadrant) and climbing (vigorous movements with the forepaws in and out of the water, usually directed against the wall of the cylinder) and the frequency and duration of diving (when the whole body of the animal, including the head, was submersed towards to the cylinder's bottom and then returned to the surface) were manually scored from the video of the 5-min session (day 2).

2.4. Monoamines concentrations

After behavioral tests (PND114), rats were decapitated and brains were extracted and immersed in a 0.9% saline solution. After cleaning and drying, the prefrontal cortex (PFC: one coronal cut of 1 mm thick from frontal pole) and ventral striatum (VS: one coronal cut of 2 mm thick anterior from optical chiasm) of both hemispheres were dissected and weighted. Thereafter, the PFC and VS samples were analyzed for their contents of norepinephrine (NE), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), using high-performance liquid chromatography coupled with electrochemical detection (HPLC-EC) with some minor modifications as we previously reported (Fornaguera and Schwarting, 2002). The mobile phase was delivered by a 515 HPLC pump (Waters Corporation, MA, USA) at 1.0 mL/min into a Keystone Catecholamine column (C18, 100×4.6 mm, 3 μm, Waters Corporation, MA, USA). The column eluate was monitored by a pulsed electrochemical detector (464 Waters Corporation, MA, USA) equipped with a glassy carbon electrode operating at a potential of 700 mV with the full scales range from 20 nA to 100 nA. Data were acquired and integrated using Data Apex software (CSW32-Chromatography Station for Windows, Hungary). The substrate concentration was expressed as nanograms per milligram of wet tissue weight. The 5-HT turnover was computed following this formula: $5\text{-HT}_t = [5\text{-HIAA}/5\text{-HT}]$.

2.5. Data analysis

Results are expressed as means±standard error of the mean (SEM). Behavior and monoamines concentration were analyzed among groups using one-way multivariate variance analysis (MANOVA) followed by Tukey's HSD test. In order to compare behavioral data over PNDs, a MANOVA for repeated measures followed by Bonferroni pairwise comparison test was used. We also performed a one-way multivariate covariance analysis (MANCOVA) in order to remove the likely variability brought by body weight over FST behavior. Pearson correlation coefficients (r) were calculated between behavioral and neurochemical parameters. Multiple linear regression analysis was run between immobility and OFT behaviors. Differences between means or coefficients were considered statistically significant when P was less than 0.05.

3. Results

3.1. Open-field activity

OFT data preceding (PNDs 37, 65, 93) or following FST (PND107) were analyzed separately due to its possible effect on OFT behaviors (Table 1). With respect to grooming behavior (Fig. 1A), MANOVA revealed that environmental enriched animals (EE) showed significantly higher levels than the standard control (SC) and social isolated (SI) groups at PND37 [$F(2,42)=23.83$, $p<0.0001$], PND65 [$F(2,42)=16.26$, $p<0.0001$] and PND93 [$F(2,42)=8.1$, $p<0.001$]. At PND107 EE group still showed the highest grooming (Fig. 1A) but significant differences were not observed [$F(2,42)=1.36$, $p=0.3$]. Grooming duration did not differ significantly at any PND between SC and SI groups. When this behavior was compared within each group over PNDs, the MANOVA for repeated measures showed a progressive reduction of grooming duration in EE rats which was significant [$F(1,14)=36.24$,

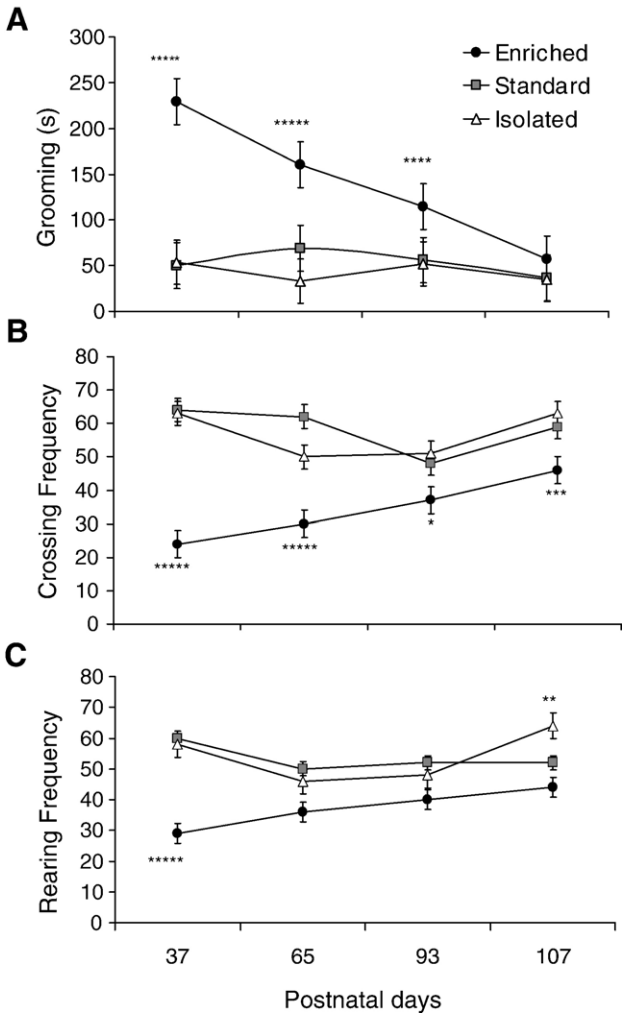

Fig. 1. Effects of rearing conditions on open-field activity. Grooming (A), locomotion (B) and rearing (C) were measured at postnatal days 37, 65, 93 and 107. The fourth open-field test (day 107) was carried out after the forced-swimming test. Results are expressed as means ± SEM of 15 rats per group. *$P<0.02$, **$P<0.002$, ***$P<0.006$, ****$P<0.001$, *****$P<0.0001$. All P values correspond to overall between-groups comparisons.

$p<0.0001$] between PND37 compared with PND93 and PND107 (Bonferroni, $p<0.05$), and between PND65 compared with PND107 (Bonferroni, $p<0.05$). The SC group also showed a grooming reduction over time, but it was significant [$F(1,14)=72.14$, $p<0.0001$] only between PND65 and PND107 (Bonferroni, $p<0.05$). Into SI group no significant tendency over PNDs was observed.

As it is shown in Fig. 1B, EE rats presented significant less locomotion compared with the SC and SI groups, at PND37 [$F(2,42)=63.03$, $p<0.0001$], PND65 [$F(2,42)=12.83$, $p<0.0001$], PND93 [$F(2,42)=4.34$, $p<0.02$] and PND107 [$F(2,42)=5.7$, $p<0.006$]. Similar to grooming, locomotion between SC and SI groups was not significantly different at any time point assessed. According to the MANOVA for repeated measures, the EE group showed a significant increase in this behavior, whereas the SC group showed a significant reduction [$F(1,14)=586.49$, $p<0.0001$] between PND37 and PND93 (Bonferroni, $p<0.05$). At PND107, EE animals showed more locomotion than during the previous three tests (Bonferroni, $p<0.05$). Even so, the EE group never reached the levels of the other groups. The SI rats only showed a small reduction in locomotion in the PND65 compared with PND37 [$F(1,14)=0.02$, $p<0.89$].

Similar to locomotion, the EE rats showed the lowest rearing frequencies over time (Fig. 1C), but significant differences between groups appeared only at PND37 [$F(2,42)=29.4$, $p<0.0001$] (HSD, $p<0.05$). Rearing in SC and SI groups did not differ at PNDs 37, 65 and 93. Conversely, at PND107, SI rats showed significantly more rearing behavior [$F(2,42)=7.47$, $p<0.002$] (HSD, $p<0.05$) than EE and SC rats, which did not differ between one another. The repeated measures analysis revealed that EE rats showed a progressive increase in rearing over tests, with a significant difference [$F(1,14)=11.81$, $p<0.004$] between PND37 and PND107 (Bonferroni, $p<0.05$). In SI animals, there was an increase at PND107 compared with PND93 [$F(1,14)=10.22$, $p<0.006$] and PND65 [$F(1,14)=7.0$, $p<0.02$]. In fact, this increases in PND107 overreached the levels observed at PND37 (Fig. 1C). The SC did not show any significant differences among PNDs and the highest frequency appeared at PND37.

3.2. Forced swimming

The MANOVA analysis revealed that housing conditions affected behavior in the FST (Fig. 2), since groups differed with respect to immobility [$F(2,42)=16.81$, $p<0.0001$], swimming [$F(2,42)=14.48$, $p<0.0001$], and climbing [$F(2,42)=14.48$, $p<0.0001$] behaviors. Specifically, EE animals showed the lowest level of immobility and the highest level of swimming and climbing; conversely, the SI animals had the highest level of immobility and the lowest level of swimming and climbing. The SC group showed intermediate values in such behaviors. Diving behavior was significantly more expressed [$F(2,42)=13.61$, $p<0.0001$] in EE animals (6.27 ± 1.39, mean frequency ± SEM) compared with SC (0.93 ± 0.64) and SI rats (0.27 ± 0.15) (HSD, $p<0.05$); whereas these latter groups did not differ significantly from each other.

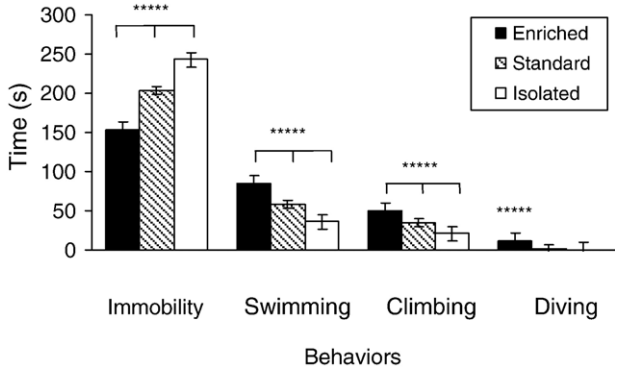

Fig. 2. Effects of rearing conditions on forced-swimming behavior. The test was carried out during postnatal days 106 and 107. The data correspond to session test (day 107). Results are expressed as means ± SEM of 15 rats per group. The lines upon the bars indicate significant differences among groups. The lines were omitted when only one group differs from the others. *****$P<0.0001$.

On the other hand, body weight was different among groups throughout the whole experiment, with SI as the heaviest and EE as the lightest group (data not shown). In PND107 body weight not only significantly differed among the three groups [$F(2,42)=32.07, p<0.0001$], but also correlated positively with immobility behavior ($r=0.57$, $p<0.0001$). Therefore, we conducted a MANCOVA analysis to subtract statistically the likely effect of body weight over FST behavior. The analysis showed that regardless there were significant body weight differences among groups at PND107, the immobility [$F(2,41)=4.63$, $p<0.01$], swimming [$F(2,41)=3.47$, $p<0.04$], climbing [$F(2,41)=3.47$, $p<0.04$] and diving behaviors [$F(2,41)=42.46$, $p<0.001$] kept the same significant tendencies and between-groups differences previously detected by MANOVA analysis (see above).

3.3. Correlations between open-field and forced-swimming behaviors

The aim of this analysis was to determine whether open-field activity could predict the differential performance showed by each group in the FST. Each OFT behavior corresponding to the PNDs 37, 65 and 93 (Fig. 1) was averaged to be included into the analysis. Afterward, we selected immobility as a core behavior of FST (Fig. 2), and we used it as dependent variable into the multiple regression analysis. We included all OFT behaviors as predictors. Despite these behaviors correlated significantly with immobility (grooming: $r=-0.41$, $p<0.003$; rearing: $r=0.39$, $p<0.004$; and locomotion: $r=0.45$, $p<0.001$), the stepwise multiple regression analysis showed that the unique OFT behavior able to predict immobility was locomotion ($R=0.45$, $R^2=0.20$; $p<0.002$). The other OFT behaviors were removed from the equation in the first step of the analysis due to their lower predictive power. Moreover, supposing that locomotion of the first OFT (Fig. 1B, PND37) was not affected by repeated measures, we also used it to predict immobility (Fig. 2). The analysis showed that the prediction capacity of locomotion at PND37 was slightly higher than those obtained using the average of locomotion previous to FST ($R=0.46$, $R^2=0.21$; $p<0.002$). When regression analysis was run within each group it failed to reach the significance. Even so, there was a tendency towards higher regression coefficient in SI ($R=0.30$, $R^2=0.09$; $p<0.27$) compared with SC ($R=0.25$, $R^2=0.06$; $p<0.37$) and EE groups ($R=0.09$, $R^2=0.008$; $p<0.74$).

3.4. Monoamines concentration in prefrontal cortex (PFC) and ventral striatum (VS)

After 84 days of housing conditions (see Table 2), rats of the EE group showed the highest levels of PFC 5-HT [$F(2,42)=4.07$, $p<0.02$], whereas there was no difference between the SC and SI groups. The concentrations of 5-HIAA and NE in this brain region did not differ significantly among groups, but SI animals showed higher levels of 5-HIAA and 5-HT turnover (5-HT_t) than the other groups. In the VS, the NE concentration was significantly [$F(2,42)=6.30$, $p<0.004$] lower in SI group than in the others (HSD, $p<0.05$), showing EE the highest amount. The concentration of 5-HT and 5-HIAA was not different among groups. In VS, the unique suggestive tendency appeared over 5-HT_t, which was lower in EE and higher in SC rats.

Table 2
Effects of rearing conditions on norepinephrine and serotonin concentration

	Enriched	Standard	Isolated
Prefrontal cortex [a]			
NE	0.05±0.02	0.08±0.04	0.06±0.01
5-HT	0.02±0.01	$1\times10^{-5}\pm1\times10^{-5}$	$1\times10^{-5}\pm1\times10^{-5}$
5-HIAA	0.01±0.01	$1\times10^{-5}\pm1\times10^{-5}$	0.04±0.04
5-HIAA/5-HT*	0.50±0.5	1±0.5	40±4
Ventral striatum [a]			
NE	0.11±0.02	0.08±0.02	0.03±0.07
5-HT	0.53±0.09	0.50±0.13	0.54±0.11
5-HIAA	0.03±0.01	0.09±0.03	0.06±0.03
5-HIAA/5-HT*	0.14±0.30	0.83±0.30	0.64±0.30

[a] Monoamines concentrations are expressed in nanograms per milligram (ng/mg) of wet tissue weight as means±SEM of 15 rats per group. *5-HT turnover (5-HT_t).

3.5. Correlations between forced-swimming behavior and neurochemistry

Pearson correlation analysis was performed in order to examine a possible relationship between behavioral parameters and neurotransmitter concentration. According to our goals, we analyzed the relationship between FST behaviors and 5-HT and NE in both PFC and VS, respectively.

Over all subjects, relationships were found among PFC 5-HT concentrations and behavior in the FST, namely immobility ($r=-0.56$, $p<0.004$), swimming ($r=0.51$, $p<0.02$), climbing ($r=0.51$, $p<0.02$) and diving ($r=0.65$, $p<0.0001$). In addition, correlations were also detected among the NE level in VS and immobility ($r=-0.42$, $p<0.002$), swimming ($r=0.42$, $p<0.004$), and climbing ($r=0.42$, $p<0.004$), whereas diving failed to reach significance ($r=0.26$, $p>0.08$). Overall, the rats that spent more time in active behaviors (swimming, climbing and diving) had higher levels of 5-HT and NE in PFC and VS, respectively. Conversely, the rats with higher levels of immobility had lower concentrations of such neurotransmitters in these brain regions.

When the correlation coefficients were computed separately for each housing group, only the EE animals showed significant correlations between 5-HT and immobility ($r=-0.53$, $p<0.05$) and diving ($r=0.56$, $p<0.03$). The NE in the VS did not show any significant correlation within any group, although it showed the same tendencies described by all subjects (data not shown).

4. Discussion

Rats reared from weaning on environmental enrichment, standard and social isolation conditions differed on several behavioral and neurochemical parameters, which can be summarized in five major findings. First of all, enriched rats showed the lowest locomotion and the highest grooming at each time point measured. Second, in FST the differential

rearing modified the coping-stress behaviors according to the social and physical stimulation received. Third, rearing conditions altered the concentration of 5-HT and NE in PFC and VS, respectively. Fourth, differences in neurotransmitters concentration could account for the behavioral changes we observed in FST, as it was pointed out by the correlation analysis. Fifth, the locomotor activity in OFT was the best immobility' predictor, despite the other OFT behaviors also correlated highly with FST performance. Details regarding possible effects of social and structural housing during critical developmental periods on behavior and neurochemistry will be discussed in the following.

4.1. Open-field activity

Rats housed in the enriched environment showed the lowest levels of locomotion and rearing, and the highest levels of grooming over all PNDs tested; whereas between standard and isolated animals no differences were observed in these parameters. In agreement with previous reports (Pietropaolo et al., 2004; Schrijver et al., 2002; Zimmermann et al., 2001) we found that the combination of social and physical enrichment, instead of only social housing had the strongest effect on open-field behaviors. Likewise, other studies using Sprague–Dawley rats have reported similar levels of open-field activity when isolated and grouped reared rats were compared, regardless the isolation period (Weiss et al., 2000; Weiss et al., 2004).

The effect of enrichment was already detected after seven days of housing conditions (PND37) and persisted until the PND107. As far as we know, under our experimental conditions (namely, continuous enrichment) this is the earliest effect reported on open-field behavior. In the current experiment, even when the enrichment effect was noted from pre-pubertal age until adulthood, it was different not only along PNDs but also according to the behavior analyzed. In fact, we found striking differences on locomotion activity throughout the whole experiment, whereas on rearing behavior the significance was only reached at PND37. Furthermore, enrichment induced the highest levels of grooming at each time point analyzed, making evident a long lasting effect, which was independent of a tendency towards reduction along PNDs. Interestingly, grooming is seldom analyzed when open-field activity is assessed in enriched or isolated animals. Indeed, only in few papers grooming has been measured in enriched rodents (Jolles et al., 1979; Pietropaolo et al., 2004). Moreover, studies only describe a reduction in spontaneous activity but they do not mention what animals do in the time they were not rearing or moving into an open field. Here we showed that enriched rats spent a lot of time on grooming while they were disengaged on exploratory behaviors. This increment in grooming was evident even from the first minutes of each OFT (data not shown). Therefore, we suppose that a fast increase in this behavior and a subsequent reduction in locomotion and rearing, would indicate that enriched animals habituated to the open-field environment earlier than the other groups.

In order to analyze the possible effect of forced-swimming stress over open-field activity we repeated the OFT immediately after the FST. At PND107 we found an increase in locomotion and a simultaneous decrease in grooming compared with preceding PNDs. The changes in locomotion were more evident in control and isolated rats than in the enriched rats, because in these animals all open-field behaviors seemed to follow their previous tendencies. Thus, changes in spontaneous activity observed at PND107 could not be uniquely attributed to the effect of swimming stress. Nonetheless, isolated rats did show significant differences on rearing at this PND. In fact, this behavior in isolated rats was not only significantly higher than in the other groups in PND107, but also compared with its own preceding values (PND65 and PND93), including the PND37 where the significance was not reached. It is possible that isolated rats sensitized to further stress, as it was the re-exposition to OFT immediately after FST (for details see Method and Table 1). Likewise, others have reported that rats pre-exposed to acute stress show an intensity-dependent effect evident on rearing rather than on other OFT behaviors (Larsson et al., 2002).

4.2. Forced swimming

In our experiment, housing conditions affected not only immobility but also swimming, climbing and diving behaviors. Since both enriched and isolated rats differed not only from control animals but from each other, the differences among groups, therefore, could be explained by the extent of physical and social stimulation provided by rearing conditions.

Our data clearly support the idea that environmental enrichment promotes appropriate coping behaviors (swimming, climbing and diving) when faced with an unavoidable stress situation as well as preventing the development of behavioral despair. Based on our results as well as on previous observations of higher diving in enriched animals (Magalhaes et al., 2004), we consider this behavior not only as an emergent one promoted by enrichment as we had pointed out (Brenes-Sáenz et al., 2006), but as the most effective escape–attempt behavior that rats could ever display in this test.

Environmental enrichment produces an antidepressive-like effect in the FST, as it had already been suggested elsewhere (Brenes-Sáenz et al., 2006; Magalhaes et al., 2004) This effect could be somehow attributed to enhanced neural plasticity and neurogenesis that usually is underwent throughout environmental enrichment (Hattori et al., 2007; Koh et al., 2007). This suggestion arises first, from the evidence that enrichment or exercise increase neurogenesis and the brain levels of several neurotrophins (Russo-Neustadt et al., 2001; Van Praag et al., 2000; Zheng et al., 2006), such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which have also shown antidepressive-like effects when they are infused into several brain regions (Siuciak et al., 1997); and second, from the fact that neurotrophins and neurogenesis are strongly implied in the molecular mechanisms underlying the effects of antidepressant drugs in human and animal trails (i.e., FST) (for review see, Duman and Montaggia, 2006).

On the other hand, in our experiment the social isolation seemed to be a predisposition factor that not only diminished

the drive to attempt the escape, but also impaired the active behaviors to cope with an uncontrollable stress situation, like it was the swimming session. The fact that immobility in the pretest session was already higher in isolated animals highlights this viewpoint (data not shown). Thus, the stress produced by isolation could be equitable to the effects produced by other methods (inescapable footshock, restraint and pretest swimming), at least in its ability to increase immobility in the test session (Cryan et al., 2002; Rodríguez-Landa and Contreras, 2000), indicating as well that isolation rearing was enough to induce a depressive-like effect in the FST.

Finally, the differences among groups observed in FST behavior cannot be attributed to the differences on body weight. The MANCOVA analysis of FST with body weight as covariate (for details see results) led us to reject the assumption that the heaviest animals (i.e. isolated rats) had the highest immobility due to a diminished motor and physical performance (i.e. lower exercise capacity) rather than by a reduced drive to attempt the escape.

4.3. Correlations between open-field and forced-swimming behaviors

We found that regardless of the housing conditions, the rats that showed higher levels of locomotor activity stilled immobile longer and consequently, displayed lower levels of swimming, climbing and diving behaviors. Moreover, immobility (PND107) could be already predicted even from the PND37. As we expected, locomotor activity was related with depressive-like behaviors, but we cannot detect a differential pattern influenced by housing. This could be explained by the fact that isolated animals did not differ from grouped animals in their open-field activity. Even so, the highest regression coefficient between locomotion and immobility behavior was detected into isolation group. On the other hand, enriched animals did show differences in open-field activity along the housing period which were consistent with that observed in the forced-swimming behavior, suggesting that both behavioral patterns were somehow related. However, this could not be confirmed by the regression analysis with the enriched rats.

Overall, those animals that adapted later to the stress produced by an open and novelty space, disengaged earlier from active forms of coping (i.e. swimming or climbing) and then express longer immobility in the FST. Here, coping-stress style seemed to be a behavioral trait relatively stable during development which differed between rats possibly, as a part of their intrinsic biological variability.

4.4. Monoamines concentration in PFC and VS

In the current experiment different postnatal experiences did have an effect on adult neurochemistry, although the effects varied among neurotransmitters and brain areas examined. In the PFC, the enrichment increased the 5-HT levels compared with the other two conditions. One might suppose that it is the interaction of social and physical factors, rather than either element by itself, which explains higher 5-HT levels in enriched rats, since control and isolated groups did not differ between one another. At the neural level, it is difficult to attribute to one specific regulatory mechanism the higher 5-HT amount observed in enriched rats. Interestingly, PFC in the enriched group was significantly heavier than in the other groups (data not shown). Given that 5-HT amount is normalized by tissue weight (5-HTng/PFCmg) it is possible that there was not only more 5-HT by axon terminals, but more 5-HT fibers innervating PFC in enriched rats. It is known that 5-HT and neurotrophins (i.e., BDNF) can facilitate the formation and maintenance of synapses in the central nervous system (for review see Duman and Montaggia, 2006; Van Praag et al., 2000). In addition, 5-HT and BDNF influence one another, such that 5-HT enhances BDNF expression, and BDNF ensures 5-HT neuron survival (Hayley et al., 2005). Since environmental enrichment improves BNDF (Van Praag et al., 2000), therefore, one can infer that higher 5-HT levels and heavier PFC found here might be the result of a complex interplay between such molecular mechanisms, which would ultimately lead to the behavioral outcomes of environmental enrichment. This idea is underscored by our correlation analysis, since 5-HT correlated negatively with immobility and positively with swimming, climbing and diving behaviors. These correlation patterns were observed over all subjects as well as within enriched group, suggesting that 5-HT in PFC is somehow involved in the behavioral changes observed in the FST, especially in the antidepressive-like effect of environmental enrichment. Besides, these data provide further evidence about that endogenous 5-HT tone would underlie the active coping-response (swimming, climbing and diving) to swimming stress in undrugged animals. Pharmacological and microdialysis studies on FST (for review see Cryan et al., 2002) had already demonstrated that higher levels of 5-HT are associated with a reduction in immobility and an increase in the time spent on swimming (Page et al., 1999). Nevertheless, both increased 5-HT contents in PFC as well as correlations between 5-HT and FST behaviors, have not yet been reported in enriched animals.

Although isolated animals showed the highest levels of immobility, in this group there was no association between this FST behavior and 5-HT concentration. The stress produced by isolation was not evident in 5-HT content but the possible impairment in 5-HT systems may be deduced from higher 5-HT_t detected. This finding is consistent with the idea that increased utilization of neurotransmitter during severe stress, such as chronic isolation and FST, cannot be compensated by an increase in synthesis, leading to depressive-like behaviors (Anisman and Zacharko, 1992). Indeed, higher 5-HIAA and/or reduced 5-HT release or postmortem amount have also been observed in cortex and hippocampus after different isolation periods (Bickerdike et al., 1993; Jaffe et al., 1993; Miura et al., 2002a; Miura et al., 2005; Rilke et al., 2001), suggesting that social isolation could impair not only the 5-HT turnover but also its biosynthesis. In our experiment the significance of 5-HT_t could not be reached due to the within-group variability in 5-HT and 5-HIAA amount. Interestingly, however, the 5-HT_t in each group followed a tendency that matches with that observed in immobility behavior: environmental enrichment with the lowest immobility time (153.33 ± 14.74, mean ± S.E.M) and 50% of

5-HT_t; standard housing with middle immobility time (204.33 ± 10.24) and 100% of 5-HT_t; and social isolation with the highest immobility time (242.46 ± 5.90) and 4000% of 5-HT_t. Given these tendencies, thus, one might suppose that 5-HT metabolism in PFC was altered according to the extent of stimulation allowed by housing conditions, and ultimately it could be related with the differential performance of each group in the FST.

In the current study, NE in the VS was significantly lower in isolated animals than in the other groups, having the enriched rats the highest levels. Furthermore, NE concentration correlated negatively with immobility and positively with swimming, climbing and less with diving behavior. Given that enriched animals showed the highest levels of active behaviors, it is possible that higher NE in VS would be also implicated in antidepressive-like effect of enrichment, even synergized with 5-HT in PFC. The role of NE in FST is supported by the fact that antidepressant drugs which inhibit norepinephrine reuptake (desipramine or reboxetine) reduce immobility increasing climbing and the simultaneous NE release in frontal cortex (Cryan et al., 2002; Page et al., 2003). Higher NE levels have been found in several brain regions of enriched mice but unfortunately, no behavioral test was added to establish an association (Naka et al., 2002). Conversely and in agreement with our findings, both lower basal levels as well as stress-induced reduction of NE even in VS, have also been found in isolated animals (Anisman and Sklar, 1981; Bakshi et al., 1996; Fulford and Marsden, 1997; Gavrilovic et al., 2005). The evidence of supersensitive α-1 and decreased number of α-2 adrenoreceptors in striatum of isolated rats (Lopez de Ceballos et al., 1983) supports the idea that striatal NE tone could be reduced by isolation stress. Thus, in the current experiment the NE depletion could account for the depressive-like effect observed in isolated rats. This is in keeping with the evidence that treatments that depleted or inactivated central NE produce sedation or depressive-like effects, whereas treatments or drugs which increased brain NE concentration are associated with behavioral stimulation or antidepressive-like effects (for review see Cryan et al., 2002; Maes and Meltzer, 1995).

5. Conclusion

In summary, environmental enrichment improves animals' information-processing ability and hence, reduced the continuous arousal when rats are facing to a novel stressful environment. This effect was already evident only seven days after the onset of enrichment and persisted until PND107. Environmental enrichment reduced depressive-like behavior, meanwhile social isolation increased it. Relative to control or isolation group, environmental enrichment augmented the 5-HT levels in prefrontal cortex. The 5-HT turnover in this brain region was not significantly different among groups, but the enriched rats showed the lowest and isolated rats the highest ratios. Furthermore, isolation rearing diminished the NE amount in ventral striatum. Both, the 5-HT and NE concentrations correlated negatively with immobility and positively with active behaviors in the forced-swimming test. Moreover, open-field and forced-swimming behaviors correlated between them, but the unique open-field parameter able to predict immobility was locomotion. Taken together, the present data support previous findings regarding the effects of early life events on adult response to uncontrollable stress, and also provide further evidence of how early environmental manipulations can modify the later expression of some behavioral and neurochemical parameters associated with depression.

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