Behavioural Brain Research 197 (2009) 125–137

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats

Juan C. Brenes[a,*], Michael Padilla[a], Jaime Fornaguera[a,b]

[a] Neuroscience Research Program, University of Costa Rica, Costa Rica
[b] Biochemistry Department, School of Medicine, University of Costa Rica, Costa Rica

ARTICLE INFO

Article history:
Received 30 April 2008
Received in revised form 9 August 2008
Accepted 14 August 2008
Available online 22 August 2008

Keywords:
Environmental enrichment
Social isolation
Depression
Anxiety
Open field
Elevated plus-maze
Forced swimming
Hippocampus
Serotonin
Norepinephrine

ABSTRACT

Our previous work has shown that male Sprague–Dawley rats reared in social isolation, standard housing and environmental enrichment differ in their spontaneous open-field activity and in some neurobehavioral depressive-like parameters. Here, we extended this evidence by using a shorter postweaning rearing period (1 month) and including additional evaluations. First, in order to obtain a better characterization of the exploratory strategies among rearing conditions we analyzed in detail the spontaneous activity at the first minute and during the 10-min session. Second, we asked whether the changes in open-field activity were related with basal anxiety levels in the elevated plus-maze. Third, behavior in the forced-swimming test was analyzed and afterward, the tissue levels of hippocampal norepinephrine and serotonin were assessed. The possible relationship between neurotransmitters and forced-swimming behavior were explored through correlation analyses. We found that rearing conditions (i) differed on locomotor habituation and on sensory-motor exploration at the first minute and during the 10-min session without modifying the plus-maze behavior; (ii) affected differentially the grooming time, its sequential components, and the relationship between grooming and locomotor parameters; (iii) modified forced-swimming behavior and the hippocampal concentration of norepinephrine, serotonin, and its turnover; and (iv) produced different correlation patterns between both neurotransmitters and forced-swimming behaviors. Overall, environmental enrichment accelerated open-field habituation and led to behavioral and neurochemical antidepressant-like effects. In contract, isolation rearing strongly impaired habituation and simple information processing, but showed marginal effects on depressive-like behavior and on hippocampal neurochemistry. The current results suggest that differential rearing is not only a useful procedure to study behavioral plasticity or rigidity in response to early experience, but also to modeling some developmental protective or risk factors underlying depressive disorders.

1. Introduction

There is overwhelming evidence indicating that long-lasting behavioral consequences of early postnatal experience may result from neurochemical and neuroanatomical adaptations of hippocampal and cortico-striatal neural circuits in response to the housing environment in which animals are raised [22,31,37,39]. Rearing rats under environmental enrichment and social isolation leads to profound behavioral and neurochemical alterations [19,20,49,59] which have been used for modeling developmental-related alterations underlying some neuropsychiatric disorders [11,20,31,49,61]. In a recent study [10] we found that approximately 80 days of housing did induce differential effects on behavior and brain neurochemistry between enriched and isolated rats. In the forced-swimming test (FST), a widely accepted predictive model of the efficacy of antidepressant drugs [13], the environmental enrichment significantly reduced immobility behavior whereas social isolation increased it. Along postnatal days environmental enrichment consistently diminished locomotor activity and increased grooming time compared with standard housing and social isolation conditions. At the neurochemical level, we found that environmental enrichment augmented the serotonin (5-HT) concentration in the prefrontal cortex, whereas social isolation reduced the levels of ventral striatum norepinephrine (NE). Furthermore, rearing conditions affected differentially the association among FST behaviors and the concentration of 5-HT and NE in such brain regions [10].

In the current experiment we aimed to replicate some of these findings adding several specific variations. First, in order to identify whether these rearing effects can be detected sooner, a shorter

* Corresponding author at: Programa de Investigación en Neurociencias, Universidad de Costa Rica, ZIP code 2060, San Pedro, Costa Rica. Tel.: +506 2207 49 03; fax: +506 2207 58 27.
E-mail address: brenesaenz@gmail.com (J.C. Brenes).

0166-4328/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2008.08.014

housing period was used. The peri-adolescence and adolescence are considered critical developmental windows to shaping the emotional behavior in rodents through altering their social and structural housing [46,47]. However, depending on the behavioral parameter selected, minimum rearing periods are required to observe the outcome of environmental enrichment and social isolation [20,49]. For example, periods of isolation up to 2 or 4 weeks after weaning are not sufficient to alter spontaneous locomotor activity and sensory-motor gating in the pre-pulse inhibition task, respectively [4,20,43,60]. Meanwhile at the first week of rearing enriched rats already differ from standard and isolated ones in the open-field test (OFT) [10], but in the FST a clear effect of enrichment appeared at 3rd and 4th weeks after weaning [10,31,35]. Therefore, and considering the evidence from isolation and enrichment studies, 1 month of rearing was considered as a suitable period to observe behavioral and neurochemical differences among enriched, standard, and isolated housed rats.

Second, instead of analyzing repeatedly the open-field activity throughout the rearing period [10,11], the likely habituation process undergone during one unique OFT session was investigated. In rats, habituation to an open field is usually measured during long test sessions and data are presented in blocks of 5 or 10 min, where rapid changes in spontaneous activity promoted by rearing conditions cannot be detected [32,61,62]. Therefore, in the current experiment we analyzed in detail the spontaneous activity during a 10-min session paying special attention to the first minute. As it has been claimed elsewhere [10,19], open-field habituation must be typified by using more specific exploratory-related parameters, which describe what animals do while they are not rearing or moving. Accordingly, in order to obtain a better characterization of the exploratory strategies that animals use, the relationship among locomotor parameters, thigmotactic scanning, and grooming behavior was examined. Previous evidence indicating that environmental enrichment increases grooming and reduces locomotion [10,11,46] allows us to suppose that whole grooming or at least some of their structural and sequential components may be involve in the habituation process. Furthermore, since anxiety-like behavior in the elevated plus-maze (EPM) has been found reduced by environmental enrichment [21,26] and increased by social isolation [57,62], this test was assessed prior to OFT in an attempt to clarify if likely changes in open-field activity were related with differences in fear/avoidance motivated behavior among rearing conditions.

Third, in order to replicate preceding findings the FST was carried out. The use of different housing periods has suggested that the effect of rearing conditions on this test may be time-dependent [10,27,31,35]. Unfortunately, some studies compared either enriched with isolated animals or each one separately with grouped animals but not all groups among each other; thus the magnitude of these differences cannot be clearly estimated. Since in the current experiment the rearing period is half shorter than we previously used, we expected to find that the differences among enriched, grouped, and isolated groups were less robust than those observed after longer housing periods [10,27].

Fourth, since the hippocampus is one of the most susceptible brain regions to early environmental stimulation or stress [22,39,40,59], it was chosen here as the target brain region to detect the effect of rearing. Differences in hippocampus function have been widely implicated in the pathophysiology of stress-related mood disorders, and in the therapeutic actions of antidepressant drugs [16,34,51,52,55]. However, hippocampal monoamines are seldom investigated in traditional or postnatal rearing models of depression. In the present study we measured the postmortem concentration of hippocampal 5-HT and NE. Relative to standard group we expected to find increased levels of NE and 5-HT in enriched rats and diminished ones in the isolated animals. On the basis of previous findings [10], we also hypothesized that FST performance is differentially associated with the amount of both neurotransmitters. It is well-known that antidepressant drugs which act on NE system reduce immobility through a selective increase in climbing behavior, whereas drugs which act on 5-HT system also reduce immobility, but increasing selectively swimming behavior [13,41,42]. Hence, if the basal tone of these neurotransmitters is associated with FST behavior even in absence of pharmacological treatment, we expected to find positive correlations between NE and climbing, and between 5-HT and swimming, and negative ones between both neurotransmitters and immobility behavior. Since correlations depend on the within-groups variability and on the specific behavioral and neurochemical profile expressed by each group, we suppose that correlation coefficients between neurotransmitters and FST behavior would be different among rearing conditions. Overall, following a 32-day period of differential rearing, behavior in the EPM, OFT, and FST were assessed and afterward, the tissue levels of hippocampal 5-HT and NE were examined.

2. Materials and methods

2.1. Animals and housing conditions

The housing conditions were identical to those reported in our previous studies [10,11]. Briefly, 36 male Sprague–Dawley rats (22 days old) obtained from LEBi Laboratories (University of Costa Rica, San José) were habituated to our colony room during 1 week keeping with the pre-weaning housing conditions ($n = 6$). At postnatal day (PND) 28 they were randomly distributed into three experimental groups ($n = 12$ each). Two groups were kept in polycarbonate rat cages (top 26.5 cm × 42 cm, lower 22 cm × 37.5 cm, height 18 cm and bottom 825 cm^2) under either isolation (SI) or group housing (three rats per cage) (SC). The other group was housed in a physically enriched cage (120 cm length × 70 cm width × 100 cm height) containing non-chewable plastic objects, two PVC tubes, five food dispensers and two water bottles, which were rearranged after 1 or 2 days. The enrichment cage was cleaned once or twice per week. In the other housing conditions, bedding, food, and water supply (provided ad libitum) were changed three times per week throughout the whole experiment. Rats were maintained under 12:12 h light–dark schedule (light on at 06:00–18:00 h), room temperature at 22 °C ± 2.8 °C, 68–91% of relative humidity, and 10 air cycles per hour. Behavioral tests were conducted and videotaped during the night cycle (19:00–24:00 h) and were carried out in the following order: the elevated plus-maze at PND60, the open field at PND62, and the forced swimming at PNDs 64–65. One hour before each test, the animals were placed in an adjacent dimmed room (one 25 W red bulb) and 10 min prior to test, they were placed individually in separate clean cages and transported to the testing room. All animals were tested in a pre-determined sequence (one rat of each group), which was kept constant between tests. At PND70 the animals were sacrificed to perform the neurochemical analysis. 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.

2.2. Elevated plus-maze test (PND60)

The wood-made apparatus consisted of four arms of equal dimensions (50 cm × 10 cm) and was raised 50 cm above the floor. Two arms, enclosed by walls 40 cm high, were perpendicular to two opposed open arms. To avoid falls, the open arms were surrounded by a Formica rim 0.5 cm high. Two rats were simultaneously tested in two mazes located in separate rooms, each one dimly illuminated with two 25 W red bulb located 150 cm above the maze. The rats were placed in the center of the maze, facing one of the closed arms. The following parameters were manually scored during 5 min: the numbers of entries (when four paws being placed on any arm or on the central area), total arms crossings, the time percentage spent on each arm [(seconds in each arm/300 s) × 100], rearing behavior, head-dipping, and stretch-attempt-posture (SAP). Maze was cleaned between sessions with a 90% alcohol solution.

2.3. Open-field test (PND62)

Two gray square wood-made chambers (70 cm × 70 cm × 40 cm divided in four equal squares) were used. Each room test was dimly illuminated with one 25 W red bulb located 130 cm above the open-field floor. A single rat was placed in the center of the arena and during 10 min, locomotion (the number of squares crossed with the four paws), the frequency of rearing (posture sustained with hind – paws on the floor), the time (in seconds) spent on grooming (including washing or mouthing of forelimbs, hind – paws, face, body and genitals) and on thigmotac-

tic scanning (including touching the vibrissae along the walls of the square while animal was moving) were manually counted. Grooming behavior was also separated in its sequential components similarly as it has been carried out elsewhere [6]. Phase I includes rapid elliptical forepaw strokes around the nose and vibrissae, Phase II includes small unilateral and/or large bilateral strokes over face and head by one or both paws, and Phase III includes body licking of the ventrolateral torso and genitals. All these parameters were expressed as percentage of time from total grooming. Moreover, the distance traveled (cm) and the time (in seconds) spent on the whole arena as well as in the central area were automatically measured using the video tracking system ANY-maze™ version 4.30 (Stoelting Co., USA). All parameters regarding central area were corrected to eliminate from the measures the fact that all animals started the test therein. The time and distance traveled in the center of the arena were counted once the animal left the zone (with the four paws). The arena was cleaned between tests with a 90% alcohol solution.

2.4. Forced-swimming test (PND64-65)

Rats were individually placed into two identical Plexiglas cylinders (45 cm height, 31 cm diameter) containing water (25 °C ± 0.5 °C) to a depth of 30 cm (the animals' hind-paws did not touch the cylinder's bottom). The swimming sessions consisted of a 15-min pretest (day 1) followed 24 h later by a 5-min test (day 2). After each session the rats were removed from water, dried with a towel, and placed in a warmed enclosure, and the cylinders were cleaned and refilled. 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 cylinder that also includes crossing into another quadrant), 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 during the first 5 min of both sessions.

2.5. Monoamines detection (PND70)

Rats were decapitated, brains were removed and quickly dissected on ice, and then the right and left hippocampus were extracted. Since we found within-group asymmetries and between-group weight differences especially in the left hippocampus (data not shown) only the right hippocampus was used for monoamines detection. In these samples we measured the contents of norepinephrine (NE), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), using high-performance liquid chromatography coupled with electrochemical detection (HPLC-EC) as we previously reported [10]. The mobile phase was a buffer solution containing EDTA-$Na_2{\cdot}2H_2O$ (0.02 M, Sigma, USA), KH_2PO_4 (0.14 M, Sigma, USA) and $C_8H_{17}NaO_3S$ (0.74 mM, Sigma, USA). It was adjusted to pH 3.5 with H_3PO_4 (1.0 M, Aldrich, USA) and mixed with CH_3CN (Aldrich, USA) and CH_3OH (Spectrum, USA) in the ratio of 90:50:50. 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 mm × 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. The substance concentration was expressed as nanograms per milligram of wet tissue weight. The 5-HT turnover (5-HIAA/5-HT) was also reported.

2.6. Data analysis

Results are expressed as mean ± S.E.M. Behavioral and neurochemical data were analyzed between-groups using one-way ANOVA followed by Fisher-protected LSD post hoc test. For OFT data, a two-way ANOVA with time as repeated measure factor (10 blocks of 1 min each) and housing as between-subject factor (EE, SC, SI) was also used. Likewise, the FST sessions (days 1 and 2) were compared with each other through an ANOVA for repeated measures. In order to estimate the size of the effect of rearing conditions on FST behaviors from days 1 to 2 the partial eta square (η^2) was used. One-way ANOVA for repeated measures followed by Bonferroni's pairwise comparisons test was run within-groups in order to evaluate the locus of significant main effects and/or interactions, when appropriate. Linear regression analysis (R) within OFT behaviors was performed. Pearson correlation coefficients (r) were computed between climbing and NE, swimming and 5-HT, and between both neurotransmitters and immobility behavior. For all tests, the level of significance was defined as $p < 0.05$.

3. Results

3.1. Elevated plus-maze

Neither the time spent in the arms (closed and open) nor the frequency of open-arms entries were different among groups (Fig. 1A and B). Only the closed-arm entries ($F_{(2,33)} = 6.65$, $p < 0.04$) and the total crossing frequency ($F_{(2,33)} = 6.30$, $p < 0.005$) were significantly higher in EE than in the other groups (Fig. 1B), which did not differ between one another. Rearing behavior on the arms, and the ethological parameters head-dipping and stretch-attempt-posture (SAP) appeared slightly increased in EE rats, but they did not differ significantly from those observed in SC and SI littermates (data not shown). Between these latter groups, no differences were observed at all.

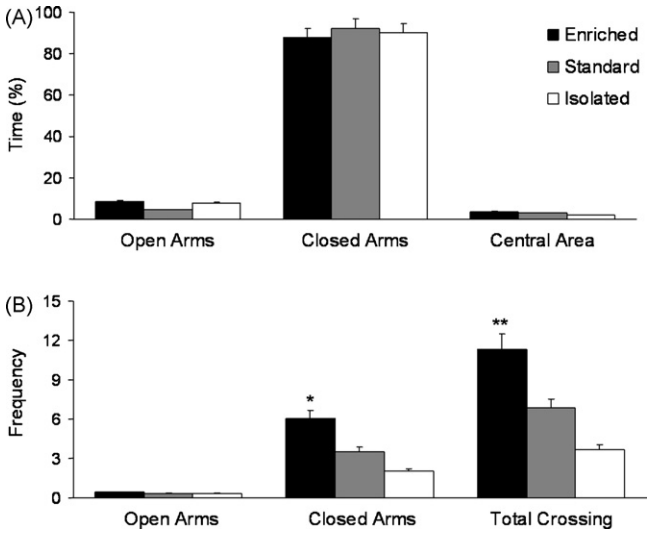

Fig. 1. Effect of rearing conditions on elevated plus-maze behavior (postnatal day 60). (A) Time spent on the arms and on the center of the maze expressed as percentages from the 5-min session. (B) The number of arms entries and total arms crossed. Results are expressed as means ± S.E.M. ($n = 12$ each). The enriched group differed from the others: $^{*}p < 0.04$, $^{**}p < 0.005$.

3.2. Open-field activity during the 10-min session

3.2.1. Locomotor activity

The open-field activity along the 10-min session is shown in Fig. 2. For crossing behavior (Fig. 2A) there was a significant main effect of time ($F_{(1,33)} = 124.26$, $p < 0.0001$) and housing ($F_{(2,33)} = 44.40$, $p < 0.0001$) and a time × housing interaction ($F_{(2,33)} = 6.19$, $p < 0.005$). The ANOVA revealed first, that all animals habituated to the open-field environment (Fig. 2A) as reflected by a gradual and significant decrease in their locomotor activity (crossing frequencies) throughout the test session (EE: $F_{(1,11)} = 147.53$, $p < 0.0001$; SC: $F_{(1,11)} = 23.99$, $p < 0.0001$; SI: $F_{(1,11)} = 15.22$, $p < 0.002$), and second, that there were different profiles of locomotor activity among all groups (Fig. 2A). Indeed, only in three time points (1, 4 and 6 min) SI and SC animals behaved similarly, in the remaining minutes SI rats always showed the highest crossing frequencies. In contrast, EE group never reached the levels of SC group. These tendencies were confirmed by the analysis of total crossing frequencies (Fig. 2A), in which SI animals showed higher crossings than their SC and EE littermates, and SC rats also showed higher frequencies than their EE congeners ($F_{(2,33)} = 44.40$, $p < 0.0001$). The analysis of total distance traveled into the arena showed the same significant differences among the three housing conditions ($F_{(2,33)} = 47.19$, $p < 0.0001$) already detected by the observation of crossing frequency (data not shown). Moreover, this analysis also revealed significant differences in the distance traveled into the central area ($F_{(2,33)} = 5.37$, $p < 0.01$), showing EE the lowest (254.7 ± 21.8 cm) and SI the highest values (428.7 ± 42.1 cm) without differing from SC (328 ± 45.4 cm) group. No significant differences ($F_{(2,33)} = 0.55$, $p > 0.6$) were observed for

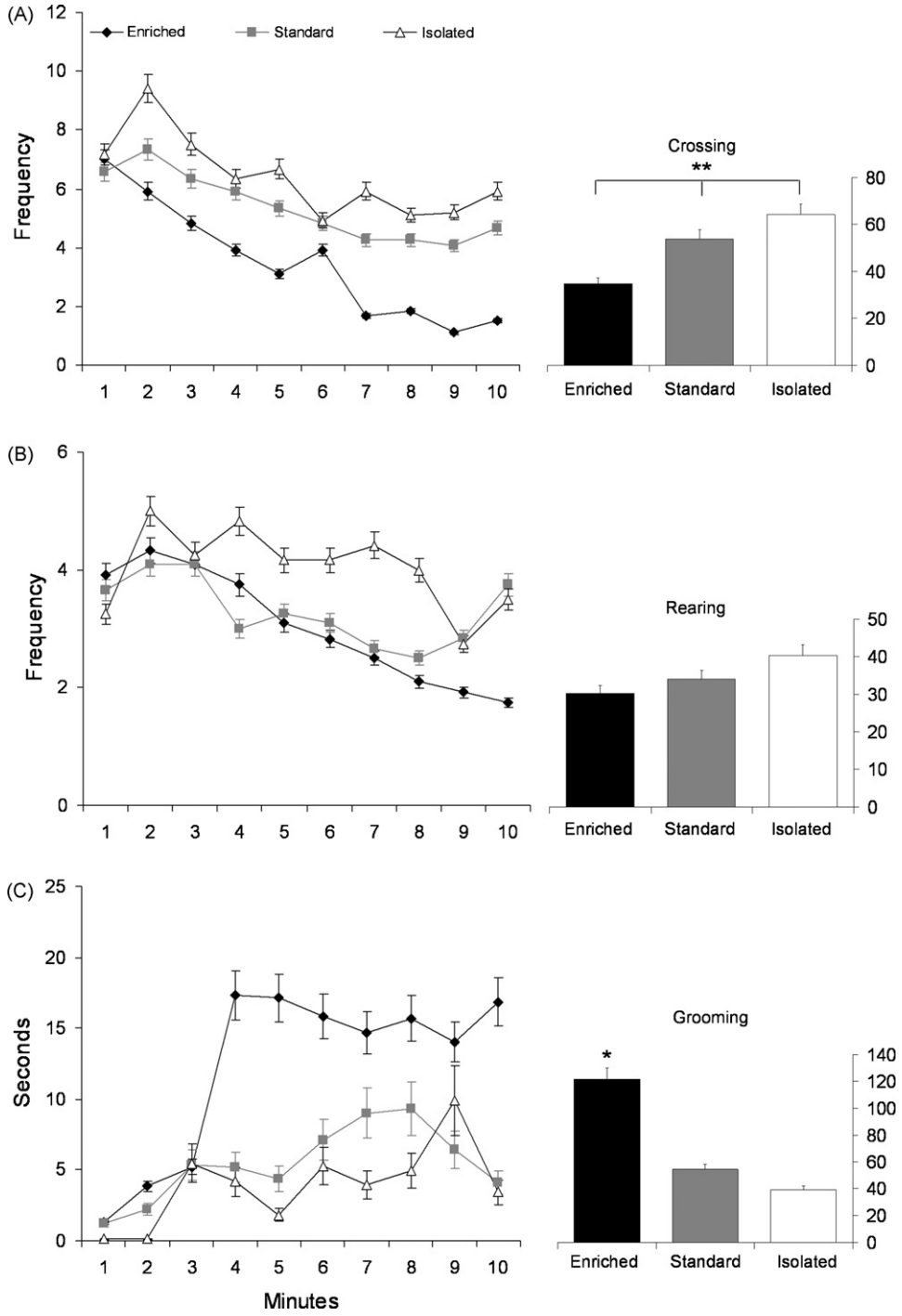

Fig. 2. Effect of rearing conditions on open-field behavior (postnatal day 62). (A) Crossing, (B) Rearing, and (C) Grooming behaviors. Left panels show activity throughout the 10-min session. Right panels show the sum of total activity corresponding to each behavior. Results are expressed as means ± S.E.M. ($n = 12$ each). The enriched group differed from the others: $^{*}p < 0.001$. The lines above the bars indicate significant differences among all groups: $^{**}p < 0.0001$.

the time spent on this area (data not shown). Regarding the latter, the speed of locomotion (m/s) was significantly different ($F_{(2,33)} = 46.79$, $p < 0.0001$) among the three rearing conditions with EE as the slowest and SI as the fastest groups (EE: 0.074 ± 0.003; SC: 0.085 ± 0.004; and SI: 0.091 ± 0.003). From the activity in the periphery of the arena, the EE rats (45.9 ± 4.6) spent significantly ($F_{(2,33)} = 17.60$, $p < 0.0001$) lower time scanning than SC (78.3 ± 4.4) and SI (80 ± 4.4) littermates, which did not differ between one another.

3.2.2. Rearing behavior

For this behavior (Fig. 2B) there was a main effect of time ($F_{(1,33)} = 20.97$, $p < 0.0001$) but not a main effect of housing ($F_{(2,33)} = 2.85$, $p > 0.07$). Moreover, a time × housing interaction was also detected ($F_{(2,33)} = 3.46$, $p < 0.04$). A detailed analysis of the interaction and the main effect of time indicated that only in EE rats there was a significant gradual reduction in this behavior ($F_{(1,11)} = 21.79$, $p < 0.001$). Although in SC ($F_{(1,11)} = 2.54$, $p > 0.14$) and SI rats ($F_{(1,11)} = 2.55$, $p > 0.14$) rearing also tended to decrease, this

was not consistent during all open-field minutes. For total rearing (Fig. 2B) there were no significant differences among groups ($F_{(2,33)} = 2.85$, $p > 0.07$), but SI showed the highest and EE the lowest frequencies.

3.2.3. Grooming behavior

In regard to grooming behavior (Fig. 2C), a main effect of time ($F_{(1,33)} = 16.24$, $p < 0.0001$) and housing ($F_{(2,33)} = 9.42$, $p < 0.001$), but not a time × housing interaction ($F_{(2,33)} = 1.92$, $p > 0.16$) was detected. A detailed within-groups analysis of the time effect revealed that EE showed a significant increase in grooming ($F_{(1,11)} = 8.40$, $p < 0.01$), mainly during the first 3 min, and from the third minute to the fourth (Fig. 2C). In the following minutes, grooming in EE rats not only remained almost invariable, but it was also always higher than in SC and in SI groups. In SC ($F_{(1,11)} = 3.29$, $p > 0.1$) and SI ($F_{(1,11)} = 4.90$, $p > 0.06$) animals, grooming was increasing throughout the OFT session but it failed to reach the significance due to its high variability (Fig. 2C). In respect to the total grooming time (Fig. 2C) ($F_{(2,33)} = 9.42$, $p < 0.001$), EE rats groomed longer than SC and SI animals, which did not differ between one another (Fig. 2C).

A detail analysis of the structural and sequential components of grooming (Fig. 3A) showed that from the total grooming duration the EE rats spent significantly lower time percentage on elliptical strokes (Phase I) than ($F_{(2,33)} = 5.53$, $p < 0.008$) SC and SI animals, which did not differ between one another even though SI animals did approximately 18% more elliptical strokes than the SC littermates. The elliptical strokes episodes ($F_{(2,33)} = 5.43$, $p < 0.009$) were less frequent in EE than in SI rats, without differing from SC congeners (Fig. 3B). Interestingly, the mean time for each episode was lower in SI and higher in EE rats (Fig. 3C), but it lacked the significance level ($F_{(2,33)} = 0.28$, $p > 0.75$). No significant differences among groups were found on time percentage, frequency and episode rate for unilateral and bilateral strokes (Phase II). Indeed, Phase II of grooming was the shortest and least frequent in all groups (Fig. 3A–C). In contrast, body licking (Phase III) was significantly higher in EE than in SC and SI littermates ($F_{(2,33)} = 7.46$, $p < 0.002$), which did not differ between each other, despite that body licking in SI group was 17% lower than in SC group (Fig. 3A). The body licking episodes were more frequent in EE than in SI rats ($F_{(2,33)} = 5.43$, $p < 0.009$), which did not differ from SC littermates (Fig. 3B). The episode rate was significantly different among all conditions ($F_{(2,33)} = 8.02$, $p < 0.001$) with EE as the highest and SI as the lowest groups (Fig. 3C).

In order to describe if the highest grooming time observed in EE animals was due to the fact that there were more animals progressively engaged in this behavior throughout the OFT session, further analyses with grooming were conducted. The first MANOVA analysis compared between-groups the number of animals that groomed per minute (Fig. 3D), revealing that there were significant effects on minutes 2, ($F_{(2,33)} = 3.81$, $p < 0.03$), 9 ($F_{(2,33)} = 3.92$, $p < 0.03$) and 10 ($F_{(2,33)} = 4.90$, $p < 0.01$). Overall, there were more EE

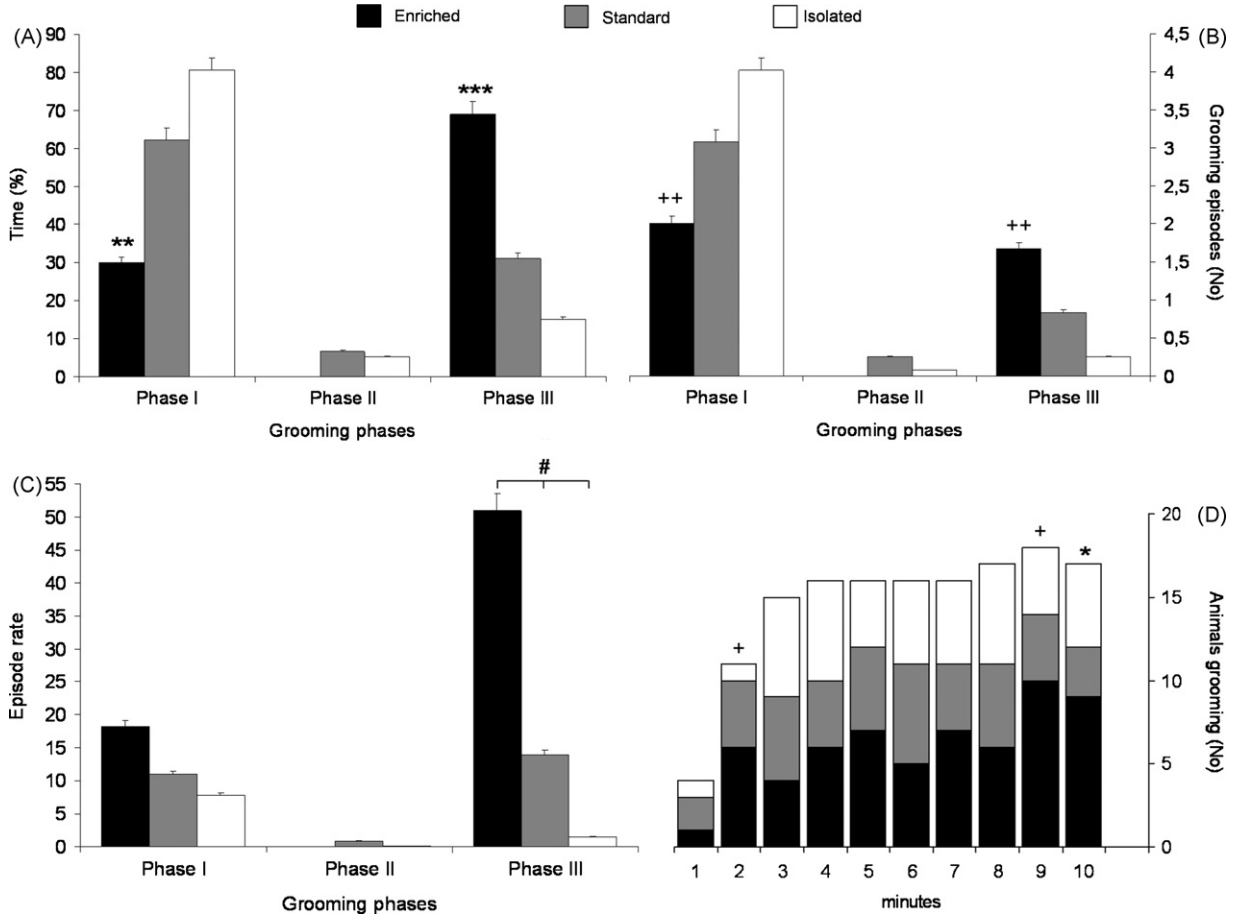

Fig. 3. Structural and sequential analysis of grooming behavior. (A) The percentage of time spent on elliptical strokes (Phase I), unilateral and bilateral strokes (Phases II), and body licking (Phase III). (B) Number of episodes for each grooming phase. (C) The episode rate (seconds/frequency) according with the grooming phases. This measure represents the mean duration for one single grooming episode. Results are expressed as means ± S.E.M. ($n = 12$ each). (D) Number of animals grooming minute by minute along the session. The enriched group differed from the others: $^{*}p < 0.01$, $^{**}p < 0.008$, $^{***}p < 0.002$. The enriched group differed from the isolated one: $^{+}p < 0.03$, $^{++}p < 0.009$. The lines above the bars indicate significant differences among all groups: $^{\#}p < 0.001$.

animals engaged in this behavior compared with SI (minutes 2, 9 and 10) and SC littermates (minute 9). However, the ANOVA for repeated measures showed that there was no main effect of housing ($F_{(2,33)} = 2.67, p > 0.08$), indicating that the sum of animals finally engaged in grooming did not differ among groups. Even so, we found a significant main effect of time ($F_{(1,33)} = 15.37, p < 0.0001$) and a time × housing interaction ($F_{(2,33)} = 19.55, p < 0.001$). A detailed analysis of these effects revealed that although in all groups there were slightly more animals doing grooming (Fig. 3D) progressively throughout the minutes (namely from minute 1 to 4 and from minute 7 to 10), this tendency was only significant in the EE group ($F_{(1,11)} = 3.81, p < 0.03$). The lineal regression analysis confirmed this trend showing that in EE ($R^2 = 0.48$, $F_{(1,10)} = 7.41$, $p < 0.03$) but not in SC ($R^2 = 0.0001$, $F_{(1,10)} = 0.002$, $p > 0.97$) nor SI animals ($R^2 = 0.12$, $F_{(1,10)} = 2.24$, $p > 0.3$) there was a positive significant association for the number of animals grooming per minute (Fig. 3D). In regards to the latter, the averaged latency (in seconds) for the first grooming was much lower in EE rats (145.6 ± 32.1) than in SC (192.2 ± 42.2) and SI (207.2 ± 39.4) littermates, but the mean-group comparison failed to reach the significance ($F_{(2,33)} = 0.71, p > 0.5$) due to the high within-group variability observed in all conditions. Even so, the latency for the grooming Phase III was significantly ($F_{(2,33)} = 3.96$, $p < 0.03$) lower in EE rats than in SI congeners (EE: 364.4 ± 47.2; SC: 485.9 ± 45.2; and SI: 535.1 ± 39.7).

3.2.4. Regression analysis between locomotion and grooming behavior

In order to determine whether locomotor activity and grooming behavior were mutually involved in open-field habituation, a regression analysis was performed. Since higher grooming levels appeared in the second half of the OFT session (Fig. 2C), the total grooming time was selected as dependent variable and the total crossing frequency as independent. The regression analysis (Fig. 4A) showed that in EE animals a decrease in crossing frequency predicted a subsequent increase in grooming time in 40% ($R^2 = -0.40$, $F_{(1,10)} = 6.53$, $p < 0.03$). In SC rats, a negative coefficient between both behavior also appeared (Fig. 4A), but the extent of the association was much lower than that detected for the EE animals ($R^2 = -0.14$, $F_{(1,10)} = 1.67$, $p > 0.23$). In contrast, no association between crossing and grooming ($R^2 = 0.04$, $F_{(1,10)} = 0.38$, $p > 0.55$) was observed in SI group (Fig. 4A). Since body licking percentage and its episode rate were much higher in EE rats than in the other groups (Fig. 3A and C), a regression analysis between body licking time and crossing was carried out. Again, a negative regression coefficient in EE group ($R^2 = -0.41$, $F_{(1,10)} = 7.11$, $p < 0.02$) was detected (Fig. 4B). In SC ($R^2 = 0.01$, $F_{(1,10)} = 0.09$, $p > 0.78$) and SI groups ($R^2 = 0.02$, $F_{(1,10)} = 0.24$, $p > 0.64$) no tendency appeared (Fig. 4B).

3.2.5. Locomotor activity during the first minute

Analyzing the locomotor activity during the first minute of open-field activity could allow differentiating the animals according to their information-processing ability, namely how rapidly the animals start to adjust their behavior in response to a novel open-field environment. We selected the locomotor activity because it was significantly different among rearing conditions and also because we considered it as the most important parameter of open-field habituation in this experiment. In order to detect changes in locomotor activity while animals were moving within the same square, the distance traveled instead of crossings, was analyzed. As is shown in Fig. 5, EE rats significantly traveled lesser distances ($F_{(2,33)} = 3.42$, $p < 0.04$) and at slower speed (m/s) ($F_{(2,33)} = 3.38$, $p < 0.05$) than SI littermates in the whole arena (Fig. 5B and C, respectively). In spite of that, the EE rats traveled greater distances ($F_{(2,33)} = 4.50$, $p < 0.02$) into the center than SC and SI animals (Fig. 4B). Furthermore, EE rats stayed longer ($F_{(2,33)} = 3.31$, $p < 0.05$) and did more entries ($F_{(2,33)} = 3.93, p < 0.03$) into this area than SI animals (Fig. 4D and E, respectively). For these two parameters SC animals showed intermediate values without differing significantly from EE and SI congeners. Moreover, the scanning time (Fig. 5D) was already lower in EE rats than in SC and SI littermates ($F_{(2,33)} = 10.33, p < 0.0001$) as it was detected during the entire test. The lineal regression analysis carried out including all subjects, revealed that the distances traveled during the first minute predicted the distances traveled during the entire session ($R^2 = 0.12$, $F_{(1,10)} = 4.48$, $p < 0.04$). Within-groups, there was found a significant regression coefficient in SI group ($R^2 = 0.41$, $F_{(1,10)} = 7.02$, $p < 0.02$) but not in SC ($R^2 = 0.12$, $F_{(1,10)} = 1.40$, $p > 0.26$) nor in EE ($R^2 = 0.03$, $F_{(1,10)} = 0.26$, $p > 0.62$) littermates. Likewise, the locomotion speed at minute 1 predicted the locomotion speed during the whole test including all subjects ($R^2 = 0.12$, $F_{(1,10)} = 4.45$, $p < 0.04$). Within-groups, a significant regression coefficient was detected again in SI group ($R^2 = 0.32$, $F_{(1,10)} = 4.73$, $p < 0.02$) but not in SC ($R^2 = -0.12$, $F_{(1,10)} = 1.32$, $p > 0.28$) nor in EE ($R^2 = -0.03$, $F_{(1,10)} = 0.34$, $p > 0.57$) groups.

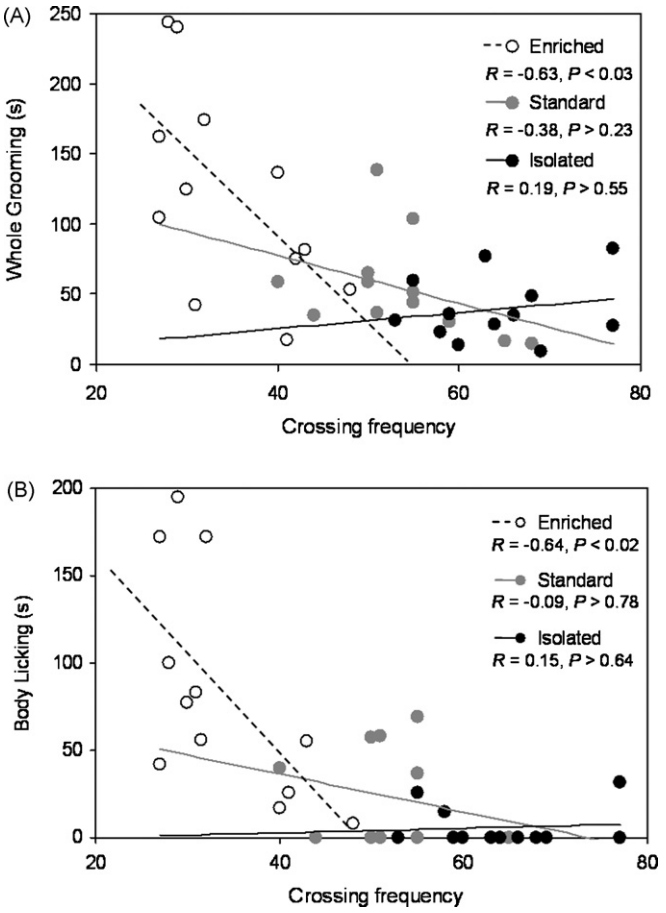

Fig. 4. Scatter plots show the regression coefficients between crossing frequency and whole grooming time (A), and between crossing frequency and body licking (B). Data correspond to the 10-min session. Symbols indicate the rearing conditions ($n = 12$ each).

3.3. Forced-swimming behavior

Including all subjects, a main effect of session was detected. That is, the time of immobility during the first 5-min period increased ($F_{(1,35)} = 18.94$, $p < 0.0001$), whereas the time of swimming ($F_{(1,35)} = 7.29, p < 0.01$) and climbing ($F_{(1,35)} = 19.65, p < 0.0001$) decreased from days 1 to 2. A detailed within-group analysis

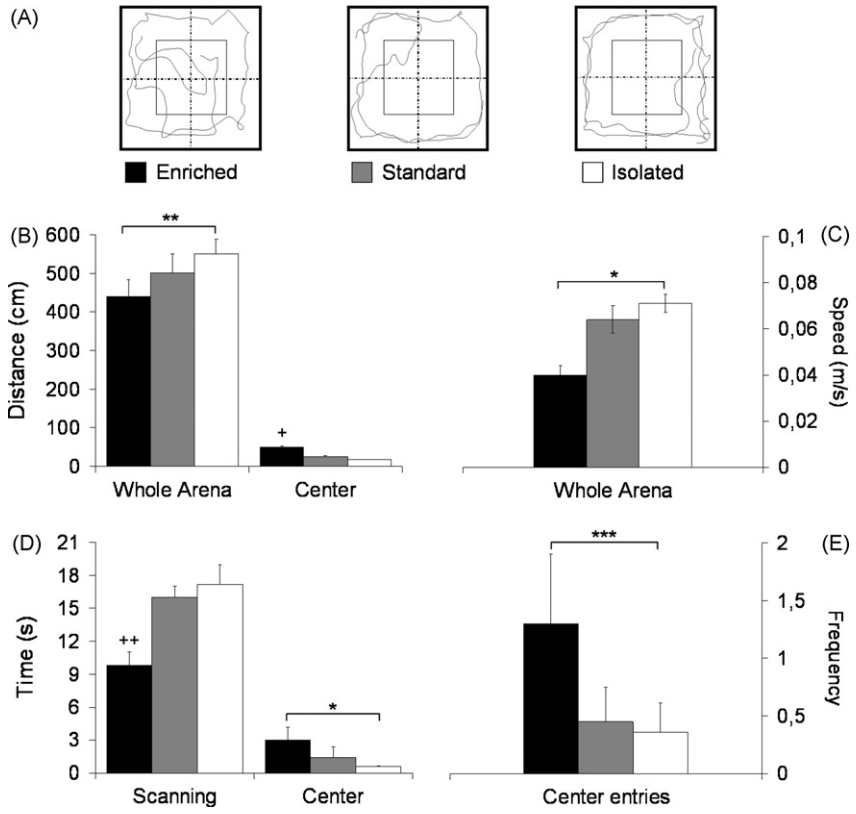

Fig. 5. Open-field activity during the first minute. (A) Path of locomotion illustrating the averaged activity into each group. (B) Distance traveled (cm) in the whole arena and in the center. (C) Locomotion speed (m/s) in the whole arena. (D) Time (s) spent on thigmotactic scanning and on staying in the center. (E) Frequency of entries into the central area. The lines above the bars indicate that the enriched group differed from the isolated one: $^{*}p < 0.05$, $^{**}p < 0.04$, $^{***}p < 0.03$. The enriched group differed from the others: $^{+}p < 0.02$, $^{++}p < 0.0001$.

revealed that the increase in immobility behavior observed from one test to another was significant in SC ($F_{(1,11)} = 7.64$, $p < 0.02$, $\eta^2 = 0.41$) and SI ($F_{(1,11)} = 30.98$, $p < 0.0001$, $\eta^2 = 0.74$) rats, but not in the EE littermates ($F_{(1,11)} = 1.66$, $p > 0.23$, $\eta^2 = 0.13$). In respect to swimming, it did not increased significantly in EE rats ($F_{(1,11)} = 0.34$, $p > 0.57$, $\eta^2 = 0.03$) as it did in SC ($F_{(1,11)} = 5.89$, $p < 0.05$, $\eta^2 = 0.30$) and in SI congeners ($F_{(1,11)} = 14.16$, $p < 0.003$, $\eta^2 = 0.56$). On climbing behavior there was found a significant decrease from days 1 to 2 in all groups (EE: $F_{(1,11)} = 5.57$, $p < 0.04$, $\eta^2 = 0.34$; SC: $F_{(1,11)} = 4.95$, $p < 0.05$, $\eta^2 = 0.31$; and SI: $F_{(1,11)} = 10.54$, $p < 0.008$, $\eta^2 = 0.49$).

The between-groups comparison of the pretest (day 1) revealed that EE rats showed a significant higher swimming time ($F_{(2,33)} = 3.57$, $p < 0.04$, $\eta^2 = 0.18$) than SC and SI littermates, which in fact did not differ between one another. Regarding immobility behavior, EE rats showed a trend towards lower levels than SC and SI congeners ($F_{(2,33)} = 2.91$, $p > 0.07$, $\eta^2 = 0.15$). On climbing neither significant differences nor any tendency among groups were observed ($F_{(2,33)} = 0.03$, $p > 0.97$, $\eta^2 = 0.002$). The ANOVA analysis of the test session (day 2) revealed that, relative to SC and SI (Fig. 6) groups, the EE condition reduced immobility ($F_{(2,33)} = 5.75$, $p < 0.007$, $\eta^2 = 0.26$) (Fig. 6) by significantly increasing swimming ($F_{(2,33)} = 7.76$, $p < 0.001$, $\eta^2 = 0.33$) and marginally increasing climbing behaviors ($F_{(2,33)} = 0.61$, $p > 0.55$, $\eta^2 = 0.04$). Between the SC and SI groups no significant differences were found on any FST behavior, but SI rats showed the highest immobility and the lowest swimming and climbing levels (Fig. 6). Diving behavior in both FST sessions was practically unrepresented among rearing conditions, and therefore the time spent on it was added to the swimming behavior score. For example, in the second session there was counted only 5 divings in the EE group with a mean frequency of 0.42 ± 0.33 (data not shown).

3.4. Hippocampal NE and 5-HT concentration

The EE group showed significantly higher tissue levels of NE ($F_{(2,33)} = 15.47$, $p < 0.0001$) and 5-HT ($F_{(2,33)} = 11.14$, $p < 0.0001$) than SC and SI groups (Fig. 7), which did not differ between each other. For both neurotransmitters, SI animals showed the lowest amount (Fig. 7). Regarding 5-HIAA, the concentration in SI animals was notably higher than in SC rats and in EE littermates (Fig. 7). Even so, the between-group comparison failed to reach the significance ($F_{(2,33)} = 2.85$, $p > 0.07$). On 5-HT turnover (Fig. 7), SI animals showed a very higher ratio than SC and EE congeners ($F_{(2,33)} = 7.55$, $p < 0.002$). Although the turnover in EE was almost twofold lower than in SC (Fig. 7), this difference was not significant.

3.5. Correlations between FST behavior and NE and 5-HT concentration

The Pearson correlation analysis between FST behaviors (on day 2) and hippocampal neurochemistry revealed that climbing behavior correlated positively with NE concentration in EE rats (Fig. 8A) ($r = 0.77$, $p < 0.002$). In SC animals a positive and moderate coefficient was also found (Fig. 8A), but this differed in magnitude and significance relative to that detected in EE littermates ($r = 0.42$, $p > 0.18$). In SI rats no association between either variable was observed ($r = -0.05$, $p > 0.87$). In addition, immobility behavior correlated negatively with the NE amount in EE ($r = -0.76$, $p < 0.002$) and SC rats ($r = -0.59$, $p < 0.04$) but not in the SI littermates ($r = -0.01$, $p > 0.76$). In respect to swimming (Fig. 8B), this behavior correlated positively with 5-HT concentration in EE ($r = 0.84$, $p < 0.0001$) and SI rats ($r = 0.65$, $p < 0.01$), but not in SC congeners ($r = 0.14$, $p > 0.67$). Likewise, immobility behavior and 5-HT also cor-

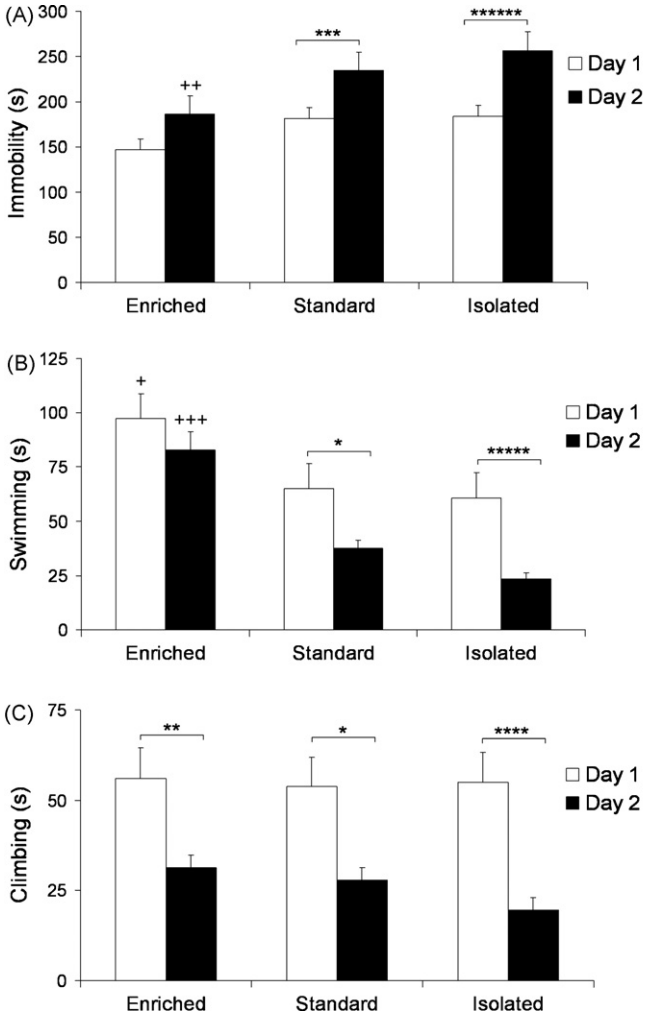

Fig. 6. Effect of rearing conditions on immobility (A), swimming (B), and climbing (C) behaviors in the forced-swimming test. The data correspond to the first 5 min of both sessions (postnatal days 64 and 65). Results are expressed as means ± S.E.M. ($n = 12$ rats each). The significant differences between sessions (days 1–2) are indicated with the lines above the bars: $^{*}p < 0.05$, $^{**}p < 0.04$, $^{***}p < 0.02$, $^{****}p < 0.008$, $^{*****}p < 0.003$, $^{******}p < 0.0001$. The between-groups comparisons show that the enriched group differed from the others in the swimming time in both sessions (day 1: $^{+}p < 0.04$, day 2: $^{++}p < 0.007$) and in the immobility time in day 2 ($^{+++}p < 0.002$).

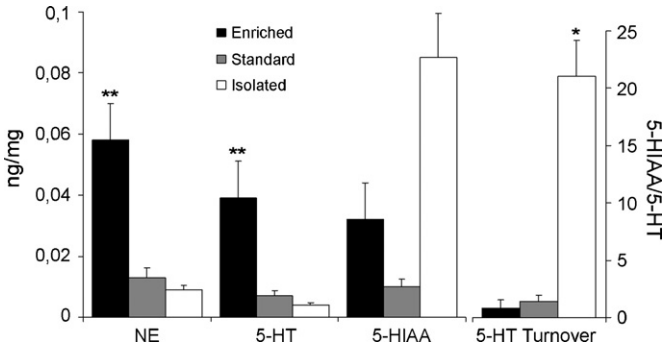

Fig. 7. Effect of rearing conditions on NE, 5-HT, and 5-HIAA concentration, and on 5-HT turnover in hippocampus (postnatal day 70). Results are expressed in nanograms per milligram (ng/mg) of wet tissue weight as means ± S.E.M. ($n = 12$ rats each). 5-HT turnover = (5-HIAA/5-HT). The isolated group differed from the others, $^{*}p < 0.002$. The enriched group differed from the others, $^{**}p < 0.0001$.

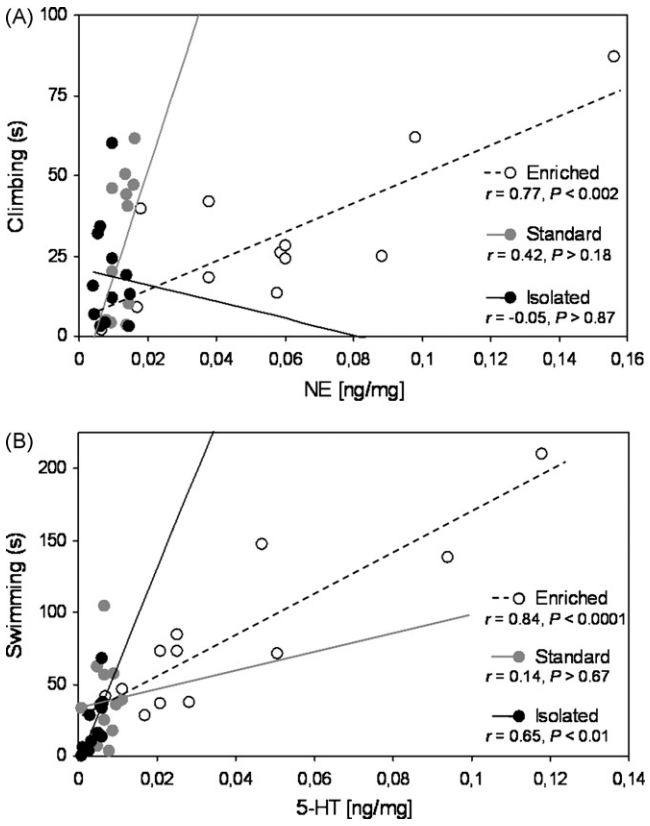

Fig. 8. Scatter plots show the Pearson correlation coefficients between climbing and NE (A), and between swimming and 5-HT (B). Symbols indicate the rearing conditions ($n = 12$ each). Neurotransmitters concentration was expressed as nanograms per milligram (ng/mg) of wet tissue weight. Forced-swimming behaviors (s) correspond to the 5-min session in day 2 (PND65).

related in EE ($r = -0.80$, $p < 0.0001$) and SI ($r = -0.58$, $p < 0.05$) but not in SC rats ($r = -0.06$, $p > 0.86$), with the difference that coefficients regarding immobility were all negatives.

4. Discussion

The present study investigated whether a short postweaning period under environmental enrichment and social isolation was enough to produce differential effects upon spontaneous open-field activity, plus-maze and forced-swimming behavior, and on tissue levels of NE and 5-HT in hippocampus. We also analyzed in detail the open-field activity during the first minute and along the 10-min session paying special attention to the possible role of grooming on locomotor habituation. Furthermore, we explored the relationship between neurotransmitters concentration and FST behaviors. As we expected, a strong effect of enrichment was detected on behavioral and neurochemical parameters, except for the performance in the EPM where no relevant differences among groups were found. Relative to control group, the effect of isolation rearing was largely observed on open-field activity and in a lesser extent on FST behavior and hippocampal neurochemistry. The likely contribution of social, structural and developmental factors upon shaping behavior and brain neurochemistry will be discussed in the following.

4.1. Anxiety-like behavior in the EPM

Rearing conditions did not affect EPM behavior, except for the crossing frequency and closed-arm entries, which were significantly higher in enriched rats. In this test either longer enrichment

or isolation periods have produced anxiolytic or anxiogenic-like effects, respectively [21,26,57,62] which suggest that the lack of the expected performance of isolated and enriched rats in the EPM may be in principle attributed to the duration of housing. Nevertheless, in the current experiment all animals irrespective of their housing conditions spent so much time in the closed arms that a ceiling effect was produced (see Fig. 1A). Even though it is still unclear for us the reason why all animals behaved in that way, a methodological issue could be addressed as plausible explanation for these unexpected results. The first factor taken into account is the light. In a previous study the use of two 40-W white bulbs (around 250-lx) produced that both enriched and grouped mice spent no more than 20 s in the open arms [50], values which are very close to those we found in the current experiment using two 25-W red bulbs (around 200-lx). In contrast, an extremely low illumination (around 12-lx) not only increased the general activity in the EPM, but also allowed differentiating the grouped from the isolated rats [62]. Since high illumination levels have been found to reduce significantly the entries into and the time spent in the open arms [5], a mild anxiogenic condition induced by light could explain our results. Alternatively, tail-marking with permanent marker for individual identification has been recently reported that produces strong differences in EPM behavior [12]. Marked rats had lower latencies to leave the closed arms as well as to enter the open arms, and spent a significantly higher time in the open arms than unmarked rats [12]. In the current experiment we marked the rats on the tail with one ring for the standard, two rings for the isolated, and no rings for the enriched rats. So, if the tail-marking led to an anxiolytic-like effect which was proportional with the amount of marks, tail-marking could damp the supposed differences between standard and isolated rats, and between both and enriched ones. The latter could be supported by the fact that enriched rats (5 ± 0.67) differed significantly from standard (2.17 ± 0.55) and isolated littermates (0.83 ± 0.51) in the amount of fecal boli deposited in the EPM ($F_{(2,33)} = 12.98$, $p < 0.0001$). Even though isolated rats had almost three fold lower boli than standard littermates, this difference did not reach the significance level (LSD, $p > 0.11$). Interestingly, it seems to be an association between the amount of fecal boli and the number of tail marks, that is the more the marks the lower the fecal boli ($r = -0.65$, $p < 0.0001$). Since defecation is an autonomous reaction proposed to characterize the level of emotionality [15], it may be thought that standard and isolated rats were less reactive to the EPM environment than enriched ones, as consequence of tail-marking. Finally, strain differences have been found on EPM behavior [54,57], where the Sprague–Dawley rats have shown naturally more entries into the closed arms and lower entries into the open arm than the Spontaneously Hypertensive and Wistar–Kyoto strains [18]. Thus, illumination and tail-marking could interact synergically with a particular behavioral trait of this strain which ultimately led to high closed arms time, yielding in no relevant group differences on EPM performance. Although we initially supposed that fearfulness may be an important component of coping with novelty (OFT) which could have been well elucidated through the paradigm of EPM, the results did not allow us to clearly elucidate whether differences in open-field behavior were related with changes in EPM behavior.

4.2. Habituation and spontaneous open-field activity

In agreement with previous reports [2,14,17,53,61], the housing effect on open-field behavior showed that enriched animals exhibited faster habituation than did isolated or grouped animals, indicated not only by a lesser and slower locomotor activity, but also by its rapid decrease during the open-field session. In contrast, the isolated rats showed higher, quicker and more sustained locomotor activity than grouped littermates, coinciding with the evidence of hyperlocomotion and delayed habituation in these animals [17,26,56]. This experiment demonstrated that analyzing the open-field activity during only 10 min is enough to detect differences on locomotor habituation and on spatial sensory-motor exploration among rearing conditions. In addition, at the first minute the enriched and isolated rats already differed on several open-field parameters, including the distance traveled in the whole arena. The locomotion at the beginning of the test was a consistent predictor of the following locomotor activity in isolated rats but not in the other groups. Interestingly, the locomotion speed during this period predicted positively the locomotion speed during the whole session in isolated rats. In the other groups the regression coefficients were not only negative but also non-significant. Although locomotor activity differed among all rearing conditions both regression analyses suggest that while in enriched and standard rats locomotion was slowing and reducing along the session, in isolated rats it remained almost invariable, supporting the notion of a retarded novelty processing in these animals [17,26,53]. Moreover, during the first minute the enriched rats stayed longer, traveled and entered more into the central area and did less tigmothatic scanning, contrary to that found in isolated ones, suggesting that enriched rats were not only less fearful but also more efficient to explore the new environment as soon as the test started. During the whole session the distance traveled in the center differed among all conditions, showing the enriched rats the lowest and the isolated ones the highest values. Since the time spent in the center did not differ among groups, the speed of locomotion could explain the significant differences found in the distances traveled therein.

Despite all these data, in our previous study isolated animals tested at 7, 35, 63, 77 days after the onset of isolation never differed from grouped animals on locomotor activity [10]. The differences between the current and our previous study may due to the fact that first, early expositions to the open-field primed animals to react in front of further evaluations damping the heightened locomotor response to novelty, as it has been reported elsewhere [45]; and second that repeated measures led inevitably to more handling periods which is known to reduce hyperlocomotion in isolated rats [28]. However, other studies have not found hyperlocomotor activity in isolated Sprague–Dawley rats when open-field behavior was measured at different time points or following different rearing periods [33,60,61,62], which highlights the importance of the following factors: (i) the rate of decline for locomotion is faster in Sprague–Dawley rats than in other strains [20], which could impede the observation of hyperlocomotion in isolated rats when behavioral recording is performed during long blocks of time; (ii) in Sprague–Dawley rats rather than in other rat strains there is a critical developmental period ranging from 2 to 8 weeks post-weaning required to detect the effect of isolation on locomotor activity (current experiment [4,17,56]); (iii) this particular interaction between strain and rearing protocols cannot be generalized to other environmental manipulations, such as the environmental enrichment. In this condition those experimental factors (i.e., housing duration, age, strain, and the time point when behavior is measured) did not seem to impede its typical effect on locomotor activity [2,14,17,26,32,53].

On the other hand, rearing behavior was gradually reduced in all groups, but this decrease was only significant in enriched rats, in agreement with previous reports [14,26]. These differences in rearing tendency were not mirrored on the total rearing frequency in the entire session. Enriched rats showed the lowest and isolated ones the highest rearing frequencies but they did not differ significantly between each other. In general, full environmental enrichment irrespective of the housing duration, starting

age, and strain, tend to diminish rearing behavior confirming that the fast habituation promoted by environmental enrichment can be extended to other behavioral dimensions of spontaneous open-field activity beyond locomotion [14,26,46,61]. However, rearing has been found either unaffected or even increased when animals were partially enriched (i.e., either socially or physically) or switched from one to another conditions (i.e., from enrichment to isolation, or vice versa) [26,32,46,50], suggesting that continuous structural and social housing are not only required, but also act synergically facilitating rearing habituation.

Although in the current experiment rearing habituation was faster in enriched rats than in standard and isolated ones, our data suggest that the effect for rearing was smaller than that observed for locomotion. Similar has been detected with enriched Sprague–Dawley rats tested either uniquely at 11 week or repeatedly throughout the housing period [10,61]. The difference in the habituation curve between locomotion and rearing is not expected to be equal because locomotion and rearing did not consistently correlated with each other and are not necessarily controlled by the same neurophysiological mechanisms [44,58], thus environmental enrichment could affect them differentially.

The total time spent on grooming was higher in enriched than in grouped and isolated rats. Moreover, enriched rats were progressively more engaged in this behavior than animals from the other groups, showing the shortest latency for the first grooming as well. This behavior was also negatively predicted by the levels of locomotor activity in enriched but not in standard or in isolated animals. A detailed analysis of grooming components showed that from the total grooming time, the enriched rats spent around 70% of the time licking their bodies. When animals start to lick their bodies they usually do not switch to another behavior until they complete this sequence [1,6]. As shown in the current experiment, the body licking episodes are usually longer than elliptical, unilateral and bilateral strokes [1,6,9], and require therefore, that animals redirect their attention from the surroundings to their bodies. Accordingly, we hypothesized that the Phase III of grooming appeared when animals are de-aroused from novelty and disengaged from exploratory and vigilance activities. Since grooming is often displayed as a reaction to unexpected stimuli, injury and conflict [9,30], this behavior may represent a de-arousal mechanism serving homeostasis, which would reflect the output consequence of an activated arousal-inhibition system [11]. Therefore, grooming is not considered here as an anxiety-like behavior itself; in contrast, grooming is thought to appear when a heightened arousal challenge has been overcome [11]. Therefore, grooming, especially body licking, could be taken as important index of habituation, that is, the sooner and the longer the grooming the faster the novelty processing. This coincides with the fact that in the current study enriched rats showed the shortest latency and the highest levels of whole grooming and body licking at the time they were reducing their locomotion and exploratory behaviors as well. In support to the latter, early studies have revealed that animals exposed to more complexes, large and stimulating environments showed higher grooming and lower locomotor activity when they were forced to explore a novel wide open chamber [8,63]. Recently, this effect was also observed in rats and mice housed in an enriched environment [10,46]. This increase in grooming was detected only 7 days after the onset of enrichment [10] and it was prolonged until 63 days later. High levels of grooming have been found to persist several months after the enrichment period finished, irrespective if animals were isolated during several weeks thereafter (unpublished results [46]). Hence, previous and current data indicate that grooming is a consistent and long-lasting behavioral reaction induced by enriched environments which is noticeable when animals are confronted with novelty.

On the other hand, the structural analysis of grooming showed that the isolated rats spent around 80% of their grooming time doing elliptical strokes. This differential distribution of grooming in isolated rats is interesting if one considers that these animals spent only 6.6% grooming from the total open-field time. Since elliptical strokes episodes are very frequent but short [1,6,9] and seem to be associated with the cleaning of vibrissae, we supposed that grooming phase I increases, especially in isolated rats, as a consequence of prolonged locomotion and scanning which demand a high vibrissae cleaning. This assumption is supported by the following data: (1) relative to standard and enriched littermates, the isolated rats showed more but shorter elliptical strokes episodes; (2) isolated animals spent the highest amount of their time moving, scanning and doing elliptical strokes; and (3) scanning and elliptical strokes correlated positively to each other only into the isolated group ($r = 0.80$, $p < 0.001$, data not shown). These data suggest that isolation rearing not only increased unchained elliptical strokes but also disrupted the following stereotyped syntactic chains. Phase I of grooming increased as a consequence of retarded information processing from sensory-motor inputs, which kept the isolated rats scanning longer and traveling higher distances. Thus, it is not surprising that grooming phase I appears here as an inverse marker of locomotor habituation to novelty.

4.3. Depressive-like behavior in the FST

On day 1 enriched rats not only showed the lowest immobility but also the highest swimming, suggesting that environmental enrichment enhanced appropriate coping-stress response in front of an uncontrollable stress situation, as in the FST. Moreover, environmental enrichment prevented the increase of immobility and the decrease of swimming during the retest of the FST, such a phenomenon typically expected in untreated rats during the second session [13]. Already on day 2, this condition reduced immobility due to a large increase in swimming and a small increase in climbing behavior. Therefore, environmental enrichment not only buffered the development of behavioral despair state, but also induced an antidepressant-like effect (day 2) in a similar fashion as antidepressant drugs [13,42,51]. In a previous study we had found that after 76 days of rearing conditions enriched rats differed from standard and isolated ones on all FST behaviors [10]. Two of the most important differences between both experiments were noted on climbing and diving expression in enriched rats. Here, at 35 days of housing the enriched animals looked as if they have not benefited enough from being in an enriched environment as they did at 76 days. It is possible that there was an interaction between the extent of stimulation provided by the environmental enrichment and the own neural maturational processes undergone throughout early rearing which could account for the behavioral differences observed between both experiments. Since climbing and diving are considered the most effective escape-attempt behaviors that rats could ever display in this test [10,11,13], it is possible that the neural mechanisms controlling both stress-coping responses are not sufficiently enhanced by enrichment at the time we carried out the FST in the current experiment. In general, data from both studies indicate that an enrichment period considerably shorter than the previous one used continued being enough to produce antidepressive-like effects, suggesting that lengthened periods are not necessary to observe these outcomes, but the longer the rearing the larger the behavioral effect. In Wistar, Long Evans and Sprague–Dawley rats postweaning enrichment periods ranging from 2 weeks to 2 month have reduced immobility behavior in the FST [10,31,35]. In our knowledge there are not studies with adult rats, but in mice, enrichment during the adult age also produces antidepressive-like effects [25,34]. Taken together, these findings suggest that the duration,

starting age and strain used do not limit the enrichment outcomes.

As we expected, in socially grouped rats immobility behavior increased, and swimming and climbing decreased from one test to another, in agreement with the well-known effect of pretest over the test session in non-pharmacologically treated rats [13,42,51]. Into the isolation group the largest increase in immobility from 1 day to another, and hence, the largest decrease in swimming and climbing was detected. Even though the tendency in both groups followed the same direction, the size of the effect for all behaviors was rather higher in isolated (immobility 74%, swimming 56%, and climbing 49%) than in standard condition (immobility 41%, swimming 30%, and climbing 31%). The latter suggests, following the meaning of this test [13,42], that isolation acted as a predisposition factor in producing either a failure of persistence in escape-directed behavior (called as behavioral despair) or in accelerating the development of a passive behavior that disengages animals from active forms of coping. Nevertheless, a depressive-like effect as a consequence of isolation was not clearly observed in the day 2, although these animals showed the highest immobility time and the lowest levels of active behaviors (swimming and climbing). Therefore, one may think that approximately 1 month under isolation was not enough to produce the expected disrupting effects of this condition on stress-coping response in the test session, as it was clearly noted following 76 days of rearing [10], where isolated rats not only differed from enriched, but also from standard littermates in all FST behaviors, excepting diving. Two mutually related factors could account for these results: the isolation period was too short to affect the neural circuitries involved in regulating coping-stress responses and/or there were physiological mechanisms of compensation which impeded that the effect of social deprivation could be distinguished at this behavioral level and already at this time point. In support to the latter, differences in immobility behavior after longer rearing periods has been found between isolated and enriched, and between isolated and grouped rats [10,27,31], suggesting that depressive-like behavior in the FST may depend on the length of isolation. However, long isolation periods in Wistar and Fawn-Hooded rats did not increased immobility behavior, suggesting that the strain used may become in a critical issue to model depressive-like behavior in isolated rats with this test [23,24].

4.4. 5-HT and NE concentration in hippocampus

Environmental enrichment produced a great increase in the hippocampal concentration of NE and 5-HT. Since control and isolated groups did not differ between one another it is likely that the interaction of social and physical factors, rather than either element by itself, does explain higher neurotransmitter concentrations in the enriched group. Even though neurochemical studies have mainly focused on monoamines alterations induced by social isolation, there is evidence indicating that environmental enrichment slightly increases NE and 5-HT concentration in hippocampus as well as in other brain regions, such as prefrontal cortex and ventral striatum [10,40]. An enhancement in monoamines levels induced by environmental enrichment could be the way by which organisms adapt to cope with a challenging environment. Living in a large colony with hard access to food and water and repeated changes in the objects location may produce a randomized environment which somewhat resemble the natural stress experienced in the wild. Thus, a high environmental demand which lead to optimize the monoamines regulation without overreaching the homeostatic threshold, would explain the highest NE and 5-HT concentration observed in enriched animals. Although current and previous findings suggest that environmental enrichment tend to increase the monoamines content in the brain, it is clear that this effect varies differentially according to the neurotransmitter and the brain region analyzed.

At the behavioral level the concentration of NE and 5-HT was closely related with antidepressant-like effect induced by environmental enrichment in the FST, as we previously reported [10]. That is, the more the tissue levels of NE and 5-HT, the lower the immobility. Our data also demonstrated that an endogenous NE and 5-HT tone correlated positively with climbing and swimming scores, respectively, coinciding with the well-known selective action of noradrenergic and serotonergic antidepressant drugs over these FST behaviors [13,41,42]. Interestingly, NE and 5-HT selective uptake inhibitors and environmental enrichment enhance hippocampal plasticity through increasing the expression of the brain-derived neurotrophic factor (BDNF) and neurogenesis [16,25,34,51,59], factors which have been proposed as two of the most important requirements to achieve an antidepressive effect [16,52,55]. Therefore, the activation of this molecular pathway may be the way by which environmental enrichment led to an antidepressant-like effect via increasing the NE and 5-HT concentration in hippocampus. However, this hypothesis should be fully explored.

On the other hand, we did not find a clear effect of isolation on the tissue levels of NE and 5-HT. However, the 5-HT turnover was much higher in the isolation group. An increased turnover with no differences in 5-HT concentration was also detected in the prefrontal cortex of isolated rats [10]. However, other studies have found alterations in hippocampal 5-HT biosynthesis and increased 5-HT turnover [7,29,36,37,48]. The discrepancies between current and previous analyses may be due to the differences in the length of the rearing period, in the method used for the neurotransmitters detection (in vivo vs. postmortem), and in the variety of challenges applied for eliciting the neurotransmitter release (diverse acute stressors vs. KCl infusions) [7,29,38,39]. In a recent study (unpublished results) social isolation during approximately 8 weeks produced a significant depletion in the 5-HT concentration in hippocampus which could be reversed by the chronic treatment with the antidepressant Fluoxetine. The latter suggests, first, that the lacking effect of isolation rearing on 5-HT contents found here may be due to the housing duration, and second, that alterations in ex vivo 5-HT amount is a consistent neurochemical trait of isolation which could be prevented pharmacologically. Nevertheless, the alteration in 5-HT turnover and its metabolite reported in the present study suggests that isolation rearing could demand a high 5-HT utilization which could not be compensated by an increase in its biosynthesis [3]. As it has mentioned elsewhere, this effect could be addressed as consequence of a dysfunction in 5-HT presynaptic activity and/or of a reduction in 5-HT fibers innervating hippocampus from raphe nuclei [22,39].

On the other hand, within the isolation group there was no association between NE concentration and FST behaviors. Interestingly, in standard group a positive coefficient was found which almost reached the significance level. Moreover, NE and immobility did correlate negatively in this group. Considering the high coefficients detected in enriched group, the association between NE and climbing, and between NE and immobility seems to follow a trend proportional to the extent of social and physical activity allowed by each rearing condition. In contrast, a positive correlation between 5-HT and swimming was detected in the isolation group. Considering that isolated rats showed the lowest 5-HT concentration and swimming scores, this coefficient may be interpreted as the lower the 5-HT the lower the swimming. Interestingly, in the standard group the mid-range values in the 5-HT amount and swimming time were not related to each other, contrary to that found at higher levels, as it did in enriched group. This suggests that social deprivation, as well as physical and social stimulation, affected dif-

ferentially the association between 5-HT and swimming according with the level in which both variables appeared represented in the pairwise distribution. Even though it is out of our current scope, it is likely there was an interaction between the rearing effect and individual differences on the 5-HT concentration and swimming behavior which requires further investigations.

5. Conclusions

In summary, environmental enrichment accelerated locomotor and rearing habituation to novelty during a very short period in which grooming behavior and its structural and sequential components seem to exert a critical role. Grooming, especially body licking, is proposed here as an outcome of a de-arousal inhibition system subserving habituation, specially triggered by the combination of physical and social stimuli. At the first minute of open-field activity rearing conditions could be already differentiated according with their paths of spatial sensory-motor exploration, an index of simple information-processing ability. The performance in the EPM and in the OFT could not be compared to each other due to the ceiling effect observed in the former one. Living in an enriched environment during approximately 1 month produced an antidepressive-like effect in the FST and increased the NE and 5-HT concentration in the hippocampus. Although data were just correlational, those neurochemical changes seemed to account for the behavioral outcomes of environmental enrichment in the FST. On the other hand, isolation rearing exacerbated spontaneous activity, producing hyperlocomotion and delaying open-field habituation. This condition also increased Phase I of grooming which appeared to be related with the elevated locomotion and thigmotactic scanning observed in these animals. On the FST, isolation rearing accelerated the onset of a depressive-like state (i.e., immobile posture) and reduced the active forms of coping (i.e., swimming and climbing) from days 1 to 2, but did not clearly affect behavior the second day. At the neurochemical level, social isolation increased the 5-HT turnover without affecting the tissue levels of 5-HT and NE. Overall, 1 month under environmental enrichment was enough to produce strong behavioral and neurochemical effects. Besides spontaneous open-field activity, the effect of isolation rearing on the FST and on hippocampal monoamines seems to be dependent on the length of housing. The results of the present study not only emphasize the relevance of social and physical factors in determining behavioral plasticity or rigidity in response to experience during a specific developmental window, but also support previous findings regarding the effects of early life events upon shaping emotional behavior and brain neurochemistry in rodents. Finally, the data from FST and hippocampal NE and 5-HT suggest that differential rearing can be a useful procedure for modeling some developmental protective or risk factors underlying depressive disorders.

Acknowledgements

The authors thankfully acknowledge the efforts of the following laboratory members: Diego Quirós and Anthony Ruíz for help with the housing conditions and the behavioral data collection, Elvira Salas for her collaboration on brain dissection and HPLC analysis, and Karen Luedtke for the revision of the final draft of the paper. This work was supported by Universidad de Costa Rica: Project No. 422-A6-609.

References

[1] Aldridge JW. Grooming. In: Whishaw IQ, Kolb B, editors. The behavior of the laboratory rat. A handbook with tests. New York: Oxford University Press; 2005. p. 141–9.
[2] Amaral OB, Vargas DS, Hansel G, Izquierdo I, Souza DO. Duration of environmental enrichment influences the magnitude and persistence of its behavioral effects on mice. Physiol Behav 2008;93:388–94.
[3] Anisman H, Zacharko RM. Depression as a consequence of inadequate neurochemical adaptation in response to stressors. Br J Psychiatry 1992;160:36–43.
[4] Arakawa H. The effect of isolation rearing on open-field behavior in male rats depends on developmental stages. Dev Psychobiol 2003;43:11–9.
[5] Becerra AM, Parra F, Morato S. Effect of different illumination levels on rat behavior in the elevated plus-maze. Physiol Behav 2005;85:265–70.
[6] Berridge KC, Whishaw IQ. Cortex, striatum and cerebellum: control of serial order in a grooming sequence. Exp Brain Res 1992;90:275–90.
[7] Bickerdike MJ, Wright IK, Marsden CA. Social isolation attenuates rat forebrain 5-HT release induced by KCI stimulation and exposure to a novel environment. Behav Pharmacol 1993;4:231–6.
[8] Bindra D, Spinner N. Response to different degrees of novelty: the incidence of various activities. J Exp Anal Behav 1958;1:341–50.
[9] Bolles RJ. Grooming behaviour in the rat. J Comp Physiol Psych 1960;53:306–10.
[10] Brenes JC, Rodríguez O, Fornaguera J. 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. Pharmacol Biochem Behav 2008;89:85–93.
[11] Brenes-Sáenz JC, Rodríguez-Villagra O, Fornaguera-Trías J. Factor analysis of forced swimming test, sucrose preference test and open field test on enriched, social and isolated reared rats. Behav Brain Res 2006;169:57–65.
[12] Burn CC, Deacon RMJ, Mason GJ. Marked for life? Effects of early cage-cleaning frequency, delivery batch, and identification tail-marking on rat anxiety profiles. Dev Psychobiol 2008;50:266–77.
[13] Cryan JF, Valentino RJ, Lucki I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev 2005;29:547–69.
[14] Del Arco A, Segovia G, Garrido P, de Blas M, Mora F. Stress, prefrontal cortex and environmental enrichment: Studies on dopamine and acetylcholine release and working memory performance in rats. Behav Brain Res 2007;176:267–73.
[15] Denenberg VH. Open-field behavior in the rat: what does it mean? Ann NY Acad Sci 1969;159:852–9.
[16] Duman RS, Montaggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry 2006;59:1116–27.
[17] Elliot B, Grunberg N. Effects of social and physical enrichment on open field activity differ in male and female Sprague–Dawley rats. Behav Brain Res 2005;165:187–96.
[18] Ferguson SA, Cada AM. Spatial learning/memory and social and non-social behaviors in the Spontaneously Hypertensive, Wistar–Kyoto and Sprague–Dawley rat strains. Pharmacol Biochem Behav 2004;77:583–94.
[19] Fernández-Teruel A, Giménez-Lort L, Escorihuela RM, Gil L, Aguilar R, Thierry S, et al. Early-life handling stimulation and environmental enrichment. Are some of their effects mediated by similar neural mechanisms? Pharmacol Biochem Behav 2002;73:233–45.
[20] Fone KCF, Porkess MV. Behavioural and neurochemical effects of post-weaning social isolation in rodents—relevance to developmental neuropsychiatric disorders. Neurosci Biobehav Rev 2008;32:1087–102.
[21] Friske JE, Gammie SC. Environmental enrichment alters plus maze, but not maternal defense performance in mice. Physiol Behav 2005;85:187–94.
[22] Gos T, Becker K, Bock J, Malecki U, Bogerts B, Poeggel G, et al. Early neonatal and postweaning social emotional deprivation interferes with the maturation of serotonergic and tyrosine hydroxylase-immunoreactive afferent fiber systems in the rodent nucleus accumbens, hippocampus and amygdala. Neuroscience 2006;140:811–21.
[23] Hall FS, Huang S, Fong CF, Pert A. The effects of social isolation on the forced swimming test in Fawn Hooded and Wistar rats. J Neurosci Methods 1998;78:47–51.
[24] Hall FS, Sundstrom JM, Lerner J, Pert A. Enhanced corticosterone release after a modified swim test in Fawn hooded rats is independent of rearing experience. Pharmacol Biochem Behav 2001;69:629–34.
[25] Hattori S, Hashimoto R, Miyakawa T, Yamanaka H, Maeno H, Wada K, et al. Enriched environments influence depression-related behavior in adult mice and the survival of newborn cells in their hippocampi. Behav Brain Res 2007;180:69–76.
[26] Hellemans KGC, Benge LC, Olmstead MC. Adolescent enrichment partially reverses the social isolation syndrome. Dev Brain Res 2004;150:103–15.
[27] Heritch AJ, Henderson K, Westfall TC. Effects of social isolation on brain catecholamines and forced swimming in rats: prevention by antidepressant treatment. J Psychiat Res 1990;24:251–8.
[28] Holson RR, Scallet AC, Ali SF, Turner BB. Isolation stress revisited-isolation-rearing effects depend on animal care methods. Physiol Behav 1991;49:1107–18.
[29] Jaffe E, De Frias V, Ibarra C. Changes in basal and stimulated release of endogenous serotonin from different nuclei of rats subjected to two models of depression. Neurosci Lett 1993;162:157–60.
[30] Jolles J, Rompa-Barendregt J, Gispen WH. Novelty and grooming behaviour in the rat. Behav Neural Biol 1979;25:563–72.
[31] Koh S, Magid R, Chung H, Stine CD, Wilson DN. Depressive behavior and selective downregulation of serotonin receptor expression after early-life seizures: reversal by environmental enrichment. Epilepsy Behav 2007;10:26–31.

[32] Larsson F, Winblad B, Mohammed AH. Psychological stress and environmental adaptation in enriched vs. impoverished housed rats. Pharmacol Biochem Behav 2002;73:193–207.
[33] Leng A, Feldon J, Ferger B. Long-term isolation and medial prefrontal cortex: dopaminergic and cholinergic neurotransmission. Pharmacol Biochem Behav 2004;77:371–9.
[34] Llorens-Martín MV, Rueda N, Martínez-Cué C, Torres-Alemán I, Flórez J, Trejo JL. Both increases in immature dentate neuron number and decreases of immobility time in the forced swim test occurred in parallel after environmental enrichment of mice. Neuroscience 2007;147:631–8.
[35] Magalhaes A, Summavielle T, Tavares MA, de Sousa L. Effects of postnatal cocaine exposure and environmental enrichment on rat behavior in a forced swim test. Ann N Y Acad Sci 2004;1025:619–29.
[36] Miura H, Qiao H, Kitagami T, Ohta T, Ozaki N. Effects of fluvoxamine on levels of dopamine, serotonin, and their metabolites in the hippocampus elicited by isolation housing and novelty stress in adult rats. Int J Neurosci 2005;115:367–78.
[37] Miura H, Qiao H, Ohta T. Influence of aging and social isolation on changes in brain monoamine turnover and biosynthesis of rats elicited by novelty stress. Synapse 2002;46:116–24.
[38] Miura H, Qiao H, Ohta T. Attenuating effects of isolation rearing condition on increased brain serotonin and dopamine turnover elicited by novelty stress. Brain Res 2002;926:10–7.
[39] Muchimapura S, Fulford AJ, Mason R, Marsden CA. Isolation rearing in the rat disrupts the hippocampal response to stress. Neuroscience 2002;112:697–705.
[40] Naka F, Shiga T, Yaguchi M, Okado N. An enriched environment increases noradrenaline concentration in the mouse brain. Brain Res 2002;924:124–6.
[41] Page ME, Brown K, Lucki I. Simultaneous analyses of the neurochemical and behavioural effects of the norepinephrine reuptake inhibitor reboxetine in a rat model of antidepressant action. Psychopharmacology 2003;165:194–201.
[42] Page ME, Dekte MJ, Dalvi A, Kirby LG, Lucki I. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in rats forced swimming test. Psychopharmacology 1999;147:162–7.
[43] Paulus MP, Bakshi VP, Geyer MA. Isolation rearing affects sequential organization of motor behavior in postpubertal but not prepubertal Lister and Sprague-Dawley rats. Behav Brain Res 1998;94:271–80.
[44] Pawlak CR, Ho YJ, Schwarting RKW. Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. Neurosci Biobehav Rev, 2008, doi:10.1016/j.neubiorev.2008.06.007.
[45] Phillips GD, Howes SR, Whitelaw RB, Wilkinson LS, Robbins TW, Everitt BJ. Isolation rearing enhances the locomotor response to cocaine and a novel environment, but impairs the intravenous self-administration of cocaine. Psychopharmacology 1994;115:407–18.
[46] Pietropaolo S, Branchi I, Cirulli F, Chiarotti F, Aloe L, Alleva E. Long-term effects of the periadolescent environment on exploratory activity and aggressive behaviour in mice: social versus physical enrichment. Physiol Behav 2004;81:443–53.
[47] Pryce CR, Rüedi-Bettschen DC, Dettling AC, Weston A, Russig H, Ferger B, et al. Long-term effects of early-life environmental manipulations in rodents and primates: potential animal models in depression research. Neurosci Biobehav Rev 2005;29:649–74.
[48] Rilke O, Will K, Jähkel M, Oehler J. Behavioral and neurochemical effects of anpirtoline and citalopram in isolated and group housed mice. Prog Neuropsychopharmacol Biol Psychiatr 2001;25:1125–44.
[49] Rosenzweig MR, Bennett EL. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res 1996;78:57–65.
[50] Roy V, Belsung C, Delarue C, Chapillon P. Environmental enrichment in BALB/c mice: Effects in classical tests of anxiety and exposure to a predatory odor. Physiol Behav 2001;74:313–20.
[51] Russo-Neustadt A, Alejandre H, Garcia C, Ivy AS, Chen MJ. Hippocampal brain-derived neurotrophic factor expression following treatment with reboxetine, citalopram, and physical exercise. Neuropsychopharmacology 2004;29:2189–99.
[52] Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 2003;301:805–9.
[53] Schrijver NC, Bahr NI, Weiss IC, Wurbel H. Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. Pharmacol Biochem Behav 2002;73:209–24.
[54] Shepard JD, Myers DA. Strain differences in anxiety-like behavior: association with corticotrophin-releasing factor. Behav Brain Res 2008;186:239–45.
[55] Shirayama Y, Chen AC-H, Nakagawa S, Russell RS, Duman RS. Brain derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 2002;22:3251–61.
[56] Silva-Gomez AB, Rojas D, Juarez I, Flores G. Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. Brain Res 2003;983:128–36.
[57] Stowe JR, Liu Y, Curtis JT, Freeman ME, Wang Z. Species differences in anxiety-related responses in male prairie and meadow voles: the effects of social isolation. Physiol Behav 2005;86:369–78.
[58] Thiel CM, Müller CP, Huston JP, Schwarting RKW. High versus low reactivity to a novel environment: behavioural, pharmacological and neurochemical assessments. Neuroscience 1999;93:243–51.
[59] van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Neurosci 2000;1:191–8.
[60] Varty G, Braff DL, Geyer MA. Is there a critical developmental window for isolation rearing-induced changes in prepulse inhibition of the acoustic startle response? Behav Brain Res 1999;100:177–83.
[61] Varty GB, Paulus MP, Braff DL, Geyer MA. Environmental enrichment and isolation rearing in the rat: effects on locomotor behavior and startle response plasticity. Biol Psychiatry 2000;47:864–73.
[62] Weiss IC, Pryce CR, Jongen-Relo AL, Bahr NI, Feldon J. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res 2004;152:279–95.
[63] Woods P. Behavior in a novel situation as influenced by the immediately preceding environment. J Exp Anal Behav 1962;5:185–90.