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Age-dependent effects of environmental enrichment on spatial memory and neurochemistry

Andrea Mora-Gallegos [a,*], Mijail Rojas-Carvajal [a], Sofía Salas [a], Adriana Saborío-Arce [a], Jaime Fornaguera-Trías [a,b], Juan C. Brenes [a,c]

[a] Neuroscience Research Center, University of Costa Rica, Costa Rica
[b] Biochemistry Department, Medicine Faculty, University of Costa Rica, Costa Rica
[c] Institute for Pyschological Research, University of Costa Rica, Costa Rica

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ABSTRACT

Although aging and environmental stimulation are well-known to affect cognitive abilities, the question of whether aging effects can be distinguished in already-mature adult rats has not been fully addressed. In the present study, therefore, young and mature adult rats were housed in either enriched or standard conditions (EE or SC) for three months. Open-field (OFT) and radial-maze (RM) behavior, and ex-vivo contents of GABA and glutamate in hippocampus, and of dopamine and DOPAC in ventral striatum (VS) were analyzed and compared between the four groups. In OFT, young rats were more active than mature adults irrespective of the housing condition. Surprisingly, in the RM test, mature adults outperformed young counterparts except for the young-enriched rats, which showed a progressive improvement in RM performance. At the neurochemical level, young EE rats showed higher hippocampal glutamate and GABA concentrations, and DA turnover in VS, which correlated with RM performance. Altogether, the behavioral and cognitive strategies underlying habituation learning and spatial memory seem to be qualitatively different between the two ages analyzed. These results challenge the assumption that mature adult animals are always worse in learning and memory tasks. However, young rats benefited more from the social and physical stimulation provided by the enrichment than mature adult counterparts. The latter effect was evident not just on behavior, but also on brain neurochemistry.

1. Introduction

Environmental enrichment in rodents has been widely used as a model of experience-dependent plasticity in which mice or rats are housed in large cages where social interaction, object exploration and/or physical exercise are promoted (Van Praag, Kempermann, & Gage, 2000; Simpson & Kelly, 2011; Sampedro-Piquero, Begega, Zancada-Menendez, Cuesta, & Arias, 2013; Solinas, Thiriet, Chauvet, & Jaber, 2010). As a result of sensorimotor and cognitive stimulation, subjects in an enriched condition show enhanced spatial processing capabilities compared with animals housed in standard conditions (Harati et al., 2009). From a cognitive perspective, the latter effect may be attributed to the acquisition of spatial abilities promoted by the complexity of the housing environment, which may enhance procedural strategies, working memory, and reference memory (Leggio et al., 2005). Environmental enrichment has been shown to affect not only memory processes but also emotional states (Brenes-Sáenz, Rodríguez, & Fornaguera, 2006; Schrijver, Bahr, Weiss, & Würbel, 2002). Thus, improvement in cognitive performance may also derive from a decreased emotional reactivity conferred by coping with the positive, mild stress of being housed in an enriched environment. Thus, reducing the deleterious consequences of impoverished rearing (e.g., in standard laboratory conditions) may facilitate subsequent learning in unfamiliar situations and contexts (Brenes, Rodríguez, & Fornaguera, 2008; Brenes-Sáenz et al., 2006; Schrijver et al., 2002).

Analyzing performance in spatial tasks has proven to be a good method to evaluate learning and memory in rodents (Bird & Burgess, 2008). Spatial cognition is generated by processing a variety of environmental cues, together with ambulation through that environment, allowing the individual to represent its location and movements in space (Bird & Burgess, 2008; Simpson & Kelly, 2011). The eight-arm radial maze (RM) test has been reported to be an appropriate tool to evaluate spatial working and reference memory, based on analyses of different types of errors that the subject commits (Carrillo-Mora, Giordano, & Santamaría, 2009;

* Corresponding author at: Neuroscience Research Center, University of Costa Rica, Rodrigo Facio Campus, 2060 San Pedro, Costa Rica.
E-mail address: andreamorag@gmail.com (A. Mora-Gallegos).

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Leggio et al., 2005). Spatial working memory combines the storage of spatial information with central executive function during the time that this information is required to complete a task (Buchsbaum & D'Esposito, 2008). The rat has to remember the location of food hidden in specific arms of the RM, avoiding previously visited arms that contain no food. Performance on this task indicates that rats have a spatial short-term memory for multiple places that improves it foraging strategy (Dudchenko, Talpos, Young, & Baxter, 2013; Olton, 1979). Spatial reference memory, on the other hand, involves long term memory acquired through a repetition of experiences. It has longer persistence, and greater resistance to interference than working memory (Buchsbaum & D'Esposito, 2008). In the RM test, working memory allows efficient collection of reinforcers within each session, whereas reference memory is important for performance across multiple trials (Dudchenko et al., 2013).

Spatial working and reference memory have been associated with different brain regions. It is well known that the hippocampus (HPC) has a prominent role in spatial tasks, e.g., creating cognitive maps (Awh & Jonides, 2001; ÓKeefe, 1976, 1979). Moreover, there is evidence for a role of ventral striatum (VS) in learning and memory, specifically associated with motivation and reward (Bowman, Beck, & Luine, 2003; Lucas et al., 2004). A motivated learning process is characterized by the repetition of a rewarding behavior, and it has been linked with an increase in the activity of dopaminergic neurons in the VS (Eagle, Humby, Dunnett, & Robbins, 1999).

Aging has an influence on the decline of spatial functions because of the physiological changes that occur with aging in different brain regions (Carrillo-Mora et al., 2009; Harati et al., 2009). It is known, however, that increasing sensory stimulation that animals receive may counteract the effects of aging on cognitive performance (Simpson & Kelly, 2011). For instance, physical and social environmental enrichment increases neural plasticity (Van Praag et al., 2000), which in turn seems to prevent or delay the negative consequences of aging on learning and memory paradigms (Bennett, McRae, Levy, & Frick, 2006). The duration of environmental enrichment, and the age at which the animal is exposed for the first time to these environmental conditions, vary among studies, and these differences may critically affect the experimental outcome (Bennett et al., 2006; Harburger, Lambert, & Frick, 2007; Leggio et al., 2005; Soffié, Hahn, Terao, & Eclancher, 1999). Although many studies have explored the implications of aging and housing on cognitive abilities (Bennett et al., 2006; Bizon et al., 2009; Harburger et al., 2007), the question of whether aging effects can be distinguished in already-mature adult rats has not been fully addressed. The present study, therefore, investigated the effects of environmental enrichment in both young and mature adult rats on cognitive and neurochemical parameters relevant to spatial memory. In addition to studying memory using a reward-dependent paradigm, we included a non-associative task, the open field test (OFT), in which habituation learning can be easily assessed (Brenes et al., 2008; Simpson & Kelly, 2011). Typically, changes in exploratory activity (i.e., locomotion and rearing) between and within sessions in the OFT have been taken as indicators of such habituation processes (Brenes, Padilla, & Fornaguera, 2009; Brenes et al., 2008). Furthermore, the *ex vivo* contents of glutamate (Glu) and gamma aminobutyric acid (GABA) in the HPC were measured. Glutamatergic and GABAergic transmission have been strongly associated with behavioral and brain plasticity (Simpson & Kelly, 2011), especially in HPC-dependent memory tasks, such as the RM. Considering the prominent role of dopaminergic activity in the VS in instrumental learning and motivation, we also analyzed the contents of dopamine (DA) and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) in this brain region.

2. Materials and methods

2.1. Animals and housing conditions

Seventy two male Wistar rats obtained from LEBi Laboratories (University of Costa Rica, San José) were randomly assigned to two groups of thirty six animals each, namely the young and mature adult groups (n = 3–5 per cage), which were transported to our colony room at post-natal day 21 (PND 21) and at PND 210 (7 months of age), respectively. After a week of acclimatization, animals in both age groups were then randomly distributed into two different housing conditions (n = 18 each): environmental enrichment (EE) and standard control (SC) conditions. All of the rats in the EE group were housed together in a specially designed box (120 cm length × 70 cm width × 100 cm height) containing non chewable plastic objects, PVC tubes, food dispensers and water bottles, which were rearranged at least twice a week as previously described by our group (Brenes & Fornaguera, 2008; Brenes et al., 2008; Brenes-Sáenz et al., 2006). SC rats, in contrast, were housed in small groups (3–5 rats per cage) in standard polycarbonate cages (55 cm length × 33 cm width × 19.5 cm height). All groups were maintained in their respective housing conditions for three months, with two bedding changes per week, food and water *ad libitum*, under a 12:12 h light–dark schedule (lights on at 6:00 h) in a climate-controlled room with 10 air cycles per hour, temperature at 25.5 °C ± 1.20 °C, and 78–87% relative humidity. One hour before behavioral testing, animals were placed in an adjacent dimmed room with red illumination (for OFT and RM test). Animals were tested between 8:00 h and 12:00 h in a pre-determined sequence (one rat of each group randomly assigned during all tests). One week after the last behavioral test, all animals were decapitated and their brains processed for further neurochemical analysis. All experimental procedures were done in accordance with 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 to minimize the number of animals used and to reduce their suffering. One animal from the mature adult enriched group had to be discarded because of disease before the behavioral tests started.

2.2. Open field test (OFT)

The open field arena consisted of a black, square wooden chamber (55 cm × 55 cm × 40 cm). Single animals were placed in the center of the arena and behavior was scored during a 10-min session. Distance traveled (m) was automatically registered using the video tracking system ANY-maze (version 4.72, Stoelting Co., USA). Frequency and time of rearing behavior (posture sustained with only the hind paws on the floor) was manually scored off-line from video recordings using Etholog 2.25 software (Ottoni, 2000). Between subjects, the arena was cleaned with 70% alcohol solution. The OFT was carried out at three different time points for all animals: (1) one day before starting the housing conditions, as a baseline (OFT-1); (2) three months after housing, before the RM test (OFT-2); and (3) three weeks after OFT-2, one week before sacrifice (OFT-3). Animals were kept in their housing conditions during testing (Fig. 1).

2.3. Radial maze test (RM)

The radial maze procedure was conducted as previously described by Görisch and Schwarting (2006), with few modifications. Our radial maze, made of transparent Plexiglas, consisted of a central platform (46 cm diameter) with eight arms (60 cm long × 15 cm wide and 30 cm high) radiating outwards. The appa-

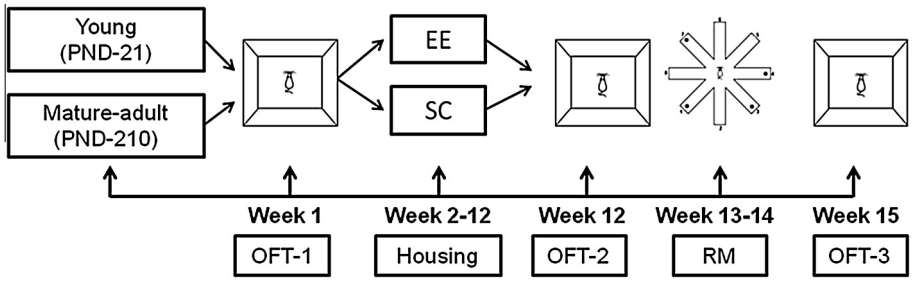


Fig. 1. Experimental design: behavioral tests and housing conditions (SC and EE) over weeks. (PND: Post-Natal Day, EE: Environmental Enrichment, SC: Standard Control, OFT: Open Field Test, RM: radial maze test).

ratus was placed on the floor to avoid elevation-induced anxiety. Prior to RM training, a habituation trial was conducted to minimize the influence of novelty on RM performance. From the habituation day onwards, animals were food deprived to promote food foraging. After each daily RM test (i.e., habituation and 5 days of training), animals received 1 h free access to food in a single cage and, afterwards, they were returned to their home cages. Body weight was monitored daily. If animals lost more than 20% of their body weight, they got longer access to food (i.e., 2 h) after training. The training procedure was as follows: during habituation, each arm contained 4 sweet reinforcers positioned along it, and one at the entry of the arm in the central area (i.e., 40 in total). The habituation trial finished after 30 min or when all reinforcers were eaten. Animals were always positioned in the maze facing arm 6. During the 5 days of training, arms 1, 3, 4 and, 7 were always baited with one sweet reinforcer placed in a food dish that was embedded at the end of the arm. Food dishes and reinforcers were not visible from the central platform or the arm entries. Each training day consisted of five trials spaced by a 3-min inter-trial interval. Each trial finished after 5 min or when all reinforcers were eaten. All other details were exactly as in the habituation.

Between trials and subjects, the maze was cleaned with a 70% ethanol solution. To facilitate spatial orientation, the testing room was decorated with spatial cues located always in the same places. The following variables were scored: frequency of entries into reinforced arms (RA, when the animal entered an arm with its four paws), total frequency of entries into any arm (TA), reference memory errors (RME: frequency of entries into non-reinforced arms), and working memory errors (WME: the number of times that the rat visited an arm more than once) (Görisch & Schwarting, 2006). Additionally, we calculated an indicator of test finalization (IFT) as follows: ((300 s/time of completion of the test) – 1). This was calculated only for animals that consumed all reinforcers.

2.4. Ex-vivo monoamine and amino acid detection

Rats were decapitated one week after OFT-3. Brains were quickly removed and dissected on ice. For neurochemical analysis, 18 animals were used from each age group (i.e., 36 in total); the other 18 subjects were kept for further analyses (not neurochemical), not included in this paper. HPC and VS were dissected and weighted. Samples of VS were analyzed for DA and DOPAC content using reverse phase high liquid performance chromatography with electrochemical detection (HPLC-ED). The mobile phase was delivered by a 515 HPLC pump (Waters Corporation, MA, USA) into an Eclipse XDB-C18 column (150 × 4.6 mm, 5 μm, Agilent Technologies, USA). The column eluate was monitored by a pulsed electrochemical detector (464 Waters Corporation, MA, USA) equipped with a glassy carbon electrode combined with an Ag/AgCl reference electrode, set at a potential of 700 mV. Flow rate was 1.3 mL/min and injection volume was 50 μL. Data were acquired and integrated using Data Apex software (CSW32-Chromatography Station for Windows, Hungary). The sample concentration was determined using the peak area and the internal standard method. Amino acid (GABA and Glu) concentrations in samples from the HPC were analyzed by reverse phase HPLC with fluorescence detection (HPLC-F) (Agilent Technologies, USA). Excitation was at 230 nm and emission was recorded at 394 nm. Amino acids were separated in an Eclipse Plus C-18 column (250 × 4.6 mm, 5 μm, Agilent Technologies, USA) using a guard column (4.6 × 12.5 mm, 5 μm; Agilent). Chromatographic data were processed with ChemStation for LC 3D Systems (Agilent Technologies, USA). Flow rate was 0.5 mL/min and injection volume was 20 μL. The amino acid concentration was determined using the peak area and the external standard method. Concentrations were expressed as nanograms per milligram of wet tissue.

2.5. Data analysis

For behavioral analyses, we used 65 subjects from the initial sample of 72 animals, and we used SPSS (v17). One rat was discarded because of disease. Two rats did not eat the reinforcers in the radial maze and the other four were outliers (exceeding ±1.5 in asymmetry and kurtosis) in more than two behavioral variables (there were no neurochemical outliers, but we also excluded the outliers from these analyses). After exclusion, animal distribution was as follows: 34 young (18 young SC, 16 young EE) and 31 mature adult animals, (16 mature-adult SC and 15 mature-adult EE). For the neurochemical analyses, we used 34 animals from the initial sample of 36 subjects (the other 2 were the behavioral outliers), 17 mature adult and 17 young rats (8 EE, and 9 SC in each group of age).

A mixed factorial ANOVA with age (young vs. mature adult) and housing conditions (SC vs. EE) as between-subject factors, and days (weeks 1, 12, and 15) or trials (1–5) as within-subjects factors was conducted. A MANOVA was performed to evaluate the effects of age, housing, and their interaction on all of the neurochemical variables. In addition, a Pearson correlation analysis was computed between the behavioral parameters (i.e. RA, TA, WME, RME, and IFT) and the neurochemical variables. In this analysis, only the behavioral parameters corresponding to training day 5 were considered, firstly because learning would have already occurred by this day, and secondly because it was the closest time point to the brain extraction and neurochemical analysis. Statistical significance was defined as $p < 0.05$. Values between 0.05 and 0.07 were considered as marginally significant. The source(s) of significant differences were determined *post hoc* using confidence intervals.

3. Results

3.1. Open field test

As shown in Fig. 2A, prior to the start of the differential housing (OFT-1), young rats displayed higher locomotor activity than mature adult rats, which showed a quite stable activity pattern

over testing days (main effect of age: $F(1,60) = 64.24$, $p < .000$, $\eta^2 = .51$). In young rats, re-exposure to the open field environment strongly reduced distance traveled in the second test (i.e., OFT-2) irrespective of the housing conditions; whereas in mature adult rats, neither environmental enrichment nor re-exposure to the OFT affected locomotor activity (interaction of age and days: $F(2,120) = 28.17$, $p < .000$, $\eta^2 = .32$). For housing condition, EE rats (i.e., both young and mature adult rats) showed lower locomotor activity across testing days than their SC counterparts (main effect of housing: $F(1,60) = 7.63$, $p < .008$, $\eta^2 = .11$). No other significant interactions were found.

Regarding rearing behavior (Fig. 2B), a quite different activity pattern was found. First, before the start of the differential housing (OFT-1), no differences in rearing behavior were observed (Fig. 2B). Second, in the OFT-2 and 3 tests, young rats displayed higher levels of rearing (both frequency and time) than mature adult rats (main effects of age: frequency, $F(1,60) = 37.31$, $p < .000$, $\eta^2 = .38$; time, $F(1,60) = 17.38$, $p < .000$, $\eta^2 = .22$). In young rats, rearing behavior increased from OFT-1 to OFT-2, whereas mature adult rats showed the opposite trend. The shift in rearing behavior across OFT sessions was unaffected either by housing condition (OFT-2) or the RM experience (OFT-3) (interaction of age and days: frequency, $F(2,120) = 22.05$, $p < .000$, $\eta^2 = .27$; time, $F(2,120) = 31.28$, $p < .000$, $\eta^2 = .34$). For housing condition, EE rats spent more time rearing than SC rats, especially on OFT-2 and 3 (interaction of days and housing: frequency, $F(2,120) = 6.83$, $p < .002$, $\eta^2 = .27$; time, $F(2,120) = 1.3$, $p < .26$, $\eta^2 = .02$).

Since age had a prominent effect on OFT behavior, we performed a minute-by-minute analysis of distance traveled and rearing time to determine whether there were also differences in the habituation pattern between young and mature adult rats within the 10-min session on each OFT. As expected, both groups showed a decrease in distance traveled and rearing time over minutes and across OFT (main effect of min: distance, $F(9,405) = 172.47$, $p < .000$, $\eta^2 = .79$; rearing time, $F(9,405) = 3.17$, $p < .001$, $\eta^2 = .06$). In mature adult rats, especially for distance traveled, activity decreased faster over time (interaction of min and age: distance, $F(9,405) = 7.88$, $p < .000$, $\eta^2 = .15$; rearing time, $F(9,405) = .40$, $p < .93$, $\eta^2 = .009$). For instance, from minute 1 to the following minutes, mature adult rats displayed less activity than young counterparts (Fig. 3A and B). To better illustrate this effect, we described in percentages how much activity was reduced from minutes 1 to 4, 1 to 7, and 1 to 10. This percentage was calculated as follows: [(Minute 1–Minute 4, 7 or 10)/Minute 1 × 100]) for each behavioral parameter. As shown in Fig. 3A and B (see tables below the graphs), mature adult rats showed a greater reduction in distance traveled and time spent rearing for almost all intervals and OFT.

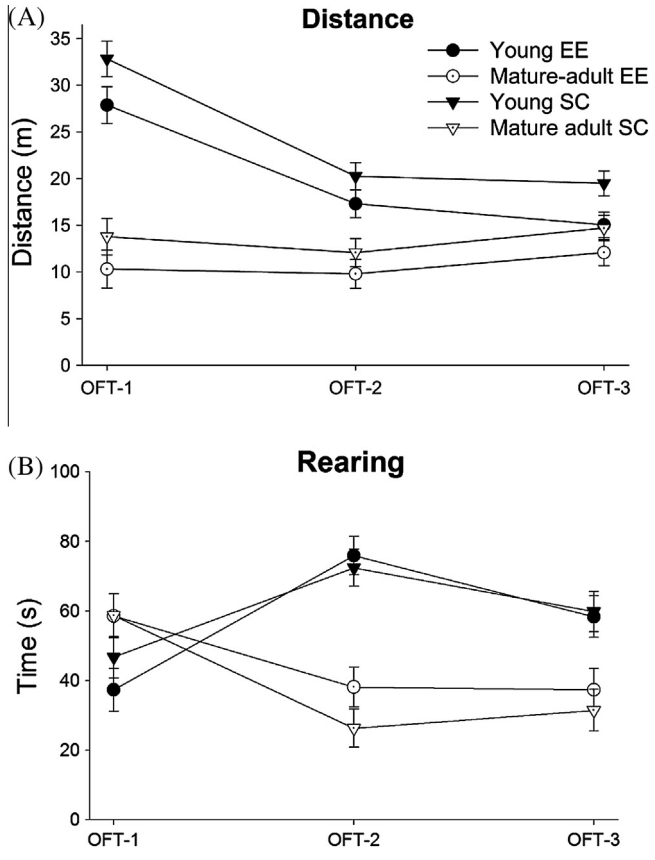


Fig. 2. (A) Total distance traveled and (B) total time spent on rearing in the three OFT.

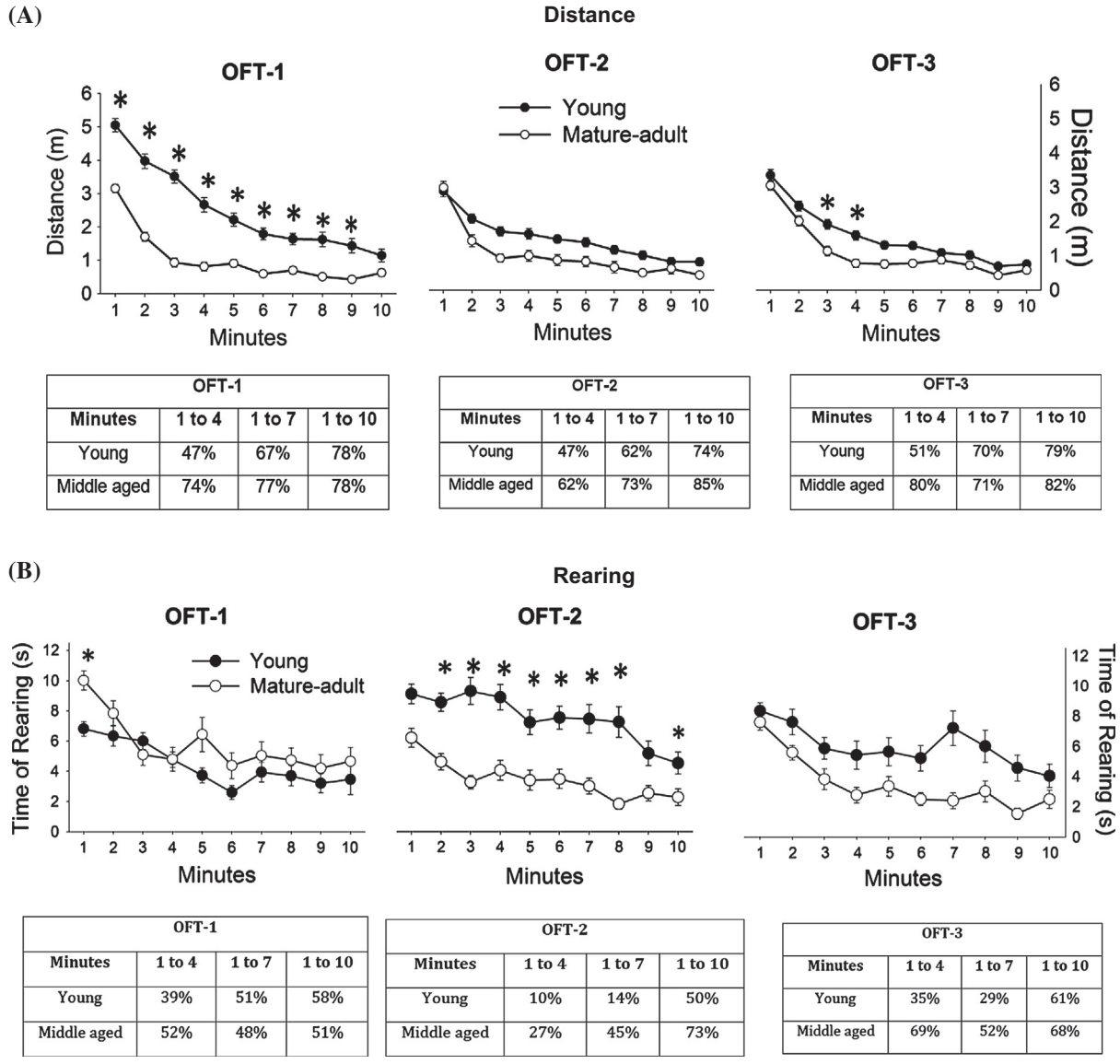
3.2. Radial maze (RM)

The general activity in the RM across days, expressed as the number of total arms visited (TA) (Fig. 4A), was higher in young rats than in mature adult animals (main effect of age: $F(1,61) = 109.70$, $p < .000$, $\eta^2 = .64$). Over time, TA decreased in the young EE rats especially on days 4 and 5; whereas in mature adult animals (i.e., both EE and SC rats) TA entries gradually increased irrespective of housing condition (interaction of days, age, and housing: $F(4,244) = 5.35$, $p < .004$, $\eta^2 = .08$. Entries into the reinforced arms (RA) showed a very similar pattern to TA visits (Fig. 4B). That is, mature adult rats had fewer entries into the RA than young animals (main effect of age: $F(1,61) = 104.39$, $p < .000$, $\eta^2 = .63$), but over days, RA entries increased only in mature adult rats (interaction of age and days: $F(4,244) = 15.25$, $p < .000$, $\eta^2 = .20$). In young rats, specifically in the EE group, the RA entries were reduced on days 4 and 5 (interaction of age, days, and housing: $F(4,244) = 4.63$, $p < .001$, $\eta^2 = .07$). Regarding working (WME) and reference (RME) memory errors (Fig. 4C and D), mature adult rats performed better than the young counterparts, even on the first training day (main effect of age: WME, $F(1,61) = 53.13$, $p < .000$, $\eta^2 = .46$; RME, $F(1,61) = 75.27$, $p < .000$, $\eta^2 = .55$).

Interestingly, in mature adult rats, repeated training had no effect on RM performance, as WME and RME were unchanged over days (Fig. 4C and D). Although environmental enrichment improved RM performance overall, based on WME and RME (main effect of housing: WME, $F(1,61) = 38.18$, $p < .000$, $\eta^2 = .38$; RME, $F(1,61) = 8.30$, $p < .005$, $\eta^2 = .12$), this effect was attributable entirely to the effect of EE in young rats. In the young EE rats, RME and especially WME decreased gradually from testing day 2 onwards (interaction of age, days, and housing: WME, $F(4,244) = 5.58$, $p < .000$, $\eta^2 = .08$; RME, $F(4,244) = 3.86$, $p < .005$, $\eta^2 = .06$); whereas in mature adult EE rats, no change was observed, compared to either the mature SC controls or to their own errors across days (Fig. 4C and D). Regarding the IFT (Fig. 4E), young rats outperformed mature adult animals (main effects of age: $F(1,61) = 115.00$, $p < .000$, $\eta^2 = .65$). Even young SC rats had higher scores than mature adult rats (i.e., both SC and EE). Further, environmental enrichment exerted a robust effect on IFT, but only in young rats (interaction of age and housing: $F(1,61) = 35.59$, $p < .000$, $\eta^2 = .37$). Young EE rats showed the highest IFT values compared to all other conditions, and the greatest increase in IFT across training days (interaction of age, days and housing: $F(4,244) = 7.99$, $p < .000$, $\eta^2 = .11$).

3.3. Neurochemical analysis

In the HPC, young EE rats showed the highest levels of Glu and GABA as compared with all other groups (interaction of age and



OFT-1			
Minutes	1 to 4	1 to 7	1 to 10
Young	47%	67%	78%
Middle aged	74%	77%	78%

OFT-2			
Minutes	1 to 4	1 to 7	1 to 10
Young	47%	62%	74%
Middle aged	62%	73%	85%

OFT-3			
Minutes	1 to 4	1 to 7	1 to 10
Young	51%	70%	79%
Middle aged	80%	71%	82%

OFT-1			
Minutes	1 to 4	1 to 7	1 to 10
Young	39%	51%	58%
Middle aged	52%	48%	51%

OFT-2			
Minutes	1 to 4	1 to 7	1 to 10
Young	10%	14%	50%
Middle aged	27%	45%	73%

OFT-3			
Minutes	1 to 4	1 to 7	1 to 10
Young	35%	29%	61%
Middle aged	69%	52%	68%

Fig. 3. (A) Kinetics of distance and (B) kinetics of time of rearing minute by minute in each OFT by age. The results in tables are shown from minute 1 to minute 4, from minute 1 to minute 7 and from minute 1 to minute 10 to evaluate the kinetics through each test to evaluate habituation (* $p < 0.05$).

housing: Glu, $F(1,30) = 7.30$, $p < .011$, $\eta^2 = .19$; GABA, $F(1,30) = 5.67$, $p < .02$, $\eta^2 = .16$). In mature adult animals, conversely, the amino acids contents in the HPC were almost identical in both housing conditions. In VS, the young rats showed higher levels of DA, DOPAC and DA turnover compared to the mature adult group (Fig. 5A). The only significant interaction found between age and housing was for DA turnover ($F(1,30) = 9.42$, $p < .005$, $\eta^2 = .24$) (Fig. 5B). No significant differences were found for DA in HPC, nor for Glu or GABA in VS (data not shown). Additionally, no significant differences were observed for the weight of the whole brain, or of the dissected samples of HPC and VS (data not shown).

Additionally, to explore a potential relation between RM performance and neurochemical concentrations, correlation analyses were conducted on these parameters. We found that Glu in HPC and DA turnover in VS were positively correlated with IFT ($r = .37$, $p < .01$ and $r = .43$, $p < .001$ respectively). No significant correlations were observed for any other RM parameter and neurochemical variables.

4. Discussion

The present study was designed to evaluate the putative effects of environmental enrichment on cognitive functions and brain ex-vivo neurochemistry, according to the age at which enriched housing conditions started. Many different results have been reported using enrichment protocols starting at different ages (for review see, Simpson & Kelly, 2011). It is well known that sensory and social stimulation may lead to different outcomes when provided outside of critical developmental periods (Hinde, 1983; Pietropaolo et al., 2004; Pryce et al., 2005). Although this is an important issue in the field, few studies have compared directly the effects of housing conditions at different ages (however see, Mora, Segovia, & Del Arco, 2007; Segovia, Del Arco, & Mora, 2009; Segovia, Porras, Del Arco, & Mora, 2001). Even in studies that have addressed this, the primary focus has been on the protective effect of environmental enrichment against the cognitive decline observed in aged subjects, rather than on the effects of enrichment in animals that are not yet experiencing such consequences

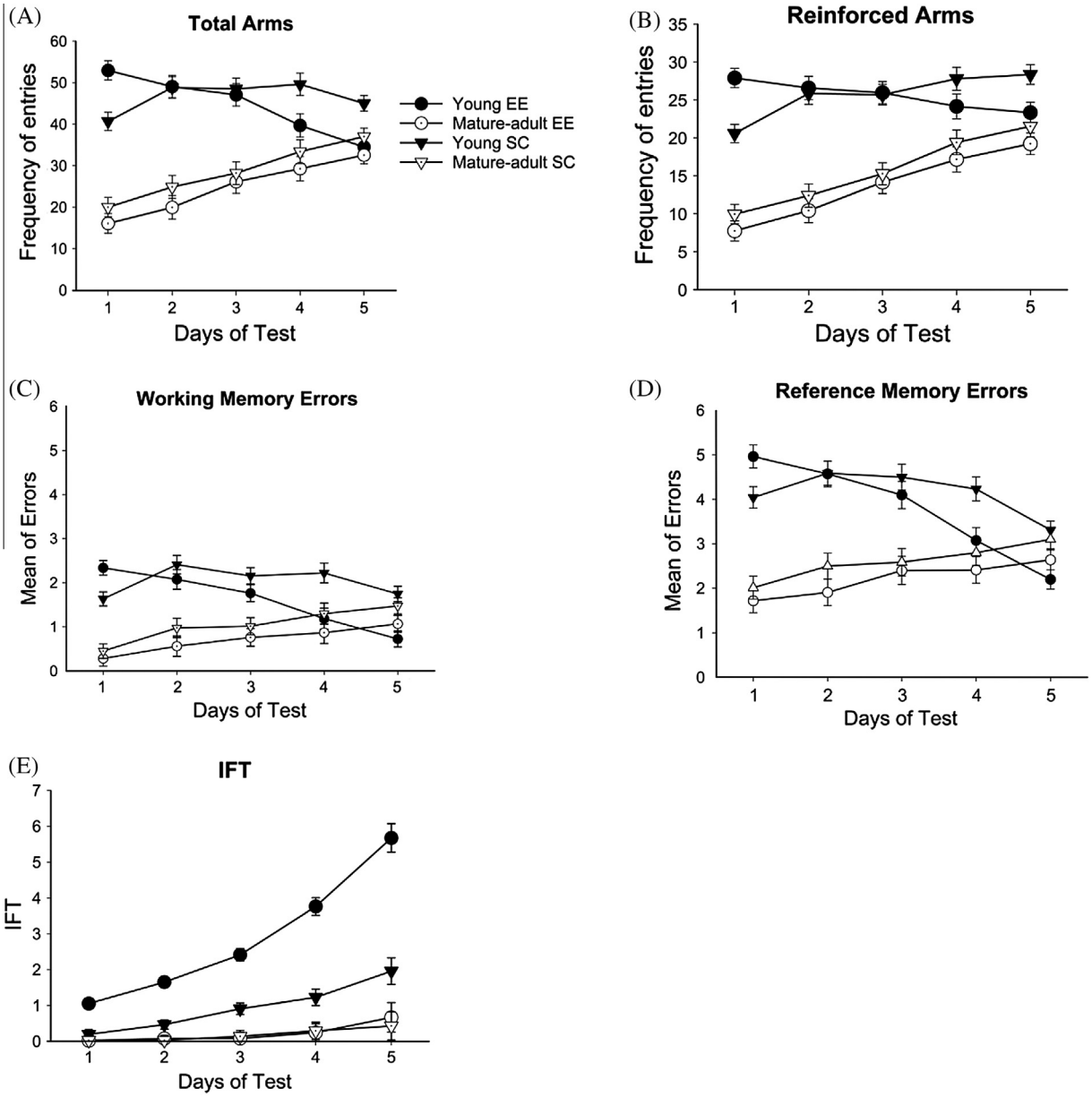


Fig. 4. (A) Total arms entries (TA), (B) visits to reinforced arms (RA), (C) mean of working memory errors (WME), (D) mean of reference memory errors (RME) and (E) indicator of finalization of the test (IFT = (300 s/ time of completion of the test) −1) on trial five of the radial maze test.

(Laviola, Hannan, Macri, Solinas, & Jaber, 2008; Nithianantharajah & Hannan, 2006; Simpson & Kelly, 2011). The present study, therefore, compared young rats with mature adult conspecifics on various aspects of performance on the open field and radial maze tests, and on HPC and VS neurochemistry.

We found, in general, that young and mature adult rats differed in the behavioral and cognitive learning strategies they used. For instance, in the OFT, young rats were more active than mature adult animals irrespective of housing conditions, which is in line with findings showing that young rodents spent more time exploring a novel environment and displayed higher levels of novelty-induced locomotor activity and exploratory behavior than adult conspecifics (Adriani, Chiarotti, & Laviola, 1998; Segovia et al., 2009). Interestingly, locomotor behavior was unaffected by repeated experience in mature adult rats, contrary to young counterparts in which decreased locomotor activity was observed when tested the second and third time. This may at first be interpreted as retarded habituation in mature adult animals. However, the minute-by-minute analysis of distance traveled and time spent rearing showed that mature adult rats habituated faster, both within-session and across testing days compared with young rats. Alternatively, spontaneous open field activity of the mature adult rats may have shown a floor effect that could not go any lower. Then, the decrease in locomotor activity observed in young rats from OFT-1 to OFT-2 may have reflected an effect of age in this group as well, as the young rats were 3 months older when tested at OFT-2. Such an effect of age seems a more likely explanation than long-term retention of the first open field experience (i.e., OFT-1). In agreement with the latter, we have previously found no differences in locomotor activity in young rats when tested again 28 days later (Brenes et al., 2008). Regarding the effects of housing condition, environmental enrichment reduced locomotor activity, especially in young rats. This result is in agreement with findings showing that environmental enrichment effects are more pronounced during early development (Brenes et al., 2009; Hellemans, Benge, & Olmstead, 2004; Larsson, Winblad, & Mohammed, 2002; Segovia et al., 2009). In mature adult rats, envi-

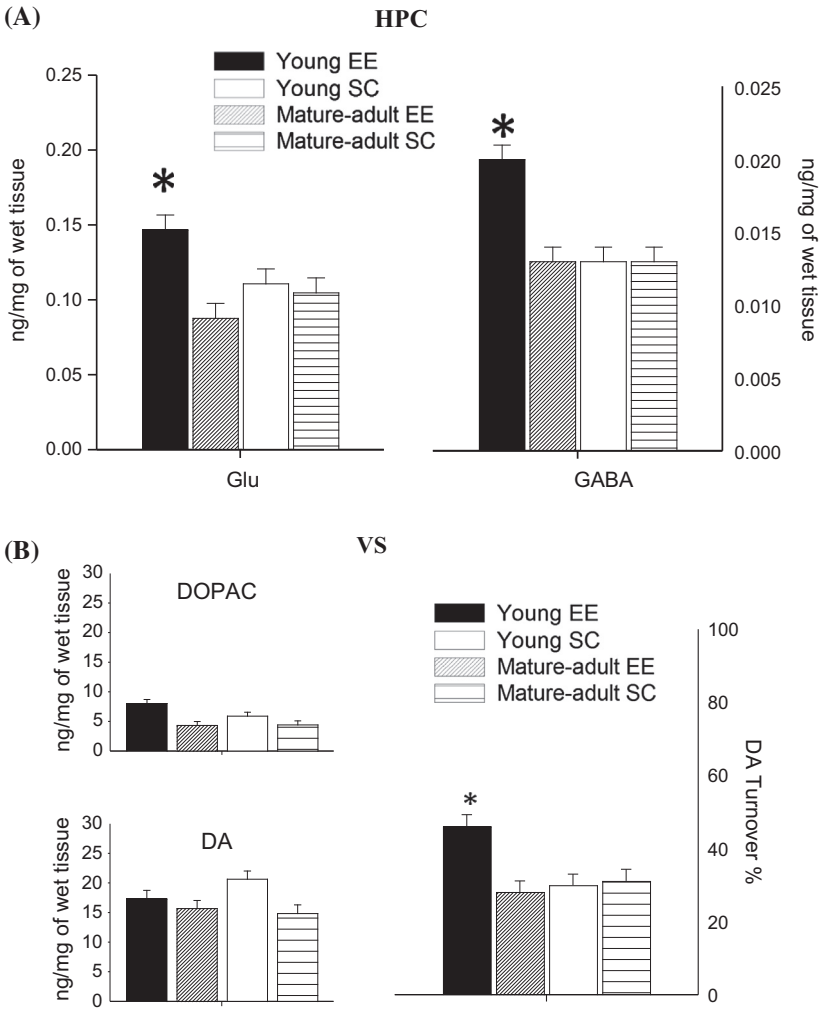


Fig. 5. (A) Contents of Glu and GABA in hippocampus (HPC) by the interaction of age and housing conditions. (B) Contents of DOPAC, DA and DA turnover (percentage DOPAC/DA) in ventral striatum (VS), by the interaction of age and housing conditions (* $p < 0.05$).

ronmental enrichment had no clear effects on locomotor activity or on exploratory behavior. Others have also found that EE-induced decreases in locomotion are less pronounced as the age at which housing conditions are changed increases (Segovia et al., 2009). As with locomotion, young groups also showed significantly higher exploratory behavior than mature adult groups on OFT-2, evident in almost every minute of the test. Also, in accordance with other results (Thiel, Müller, Houston, & Shwarting, 1999), both age groups tended to reduce exploratory behavior within test. The lack of effect of environmental enrichment on rearing is consistent with previous studies indicating that such behavior was either unaffected or even increased when animals were enriched late in development, or when they were switched from one housing condition to another (i.e., from enrichment to standard housing, or vice versa) (Brenes et al., 2009; Hellemans et al., 2004; Larsson et al., 2002). In our study, environmental enrichment and age both produced similar effects on novelty-induced locomotion and exploration, which might suggest that both maturity and environmental enrichment confer an ability to extract information from a novel environment more efficiently (e.g., spending less energy traveling around the OF arena) and to better cope with its anxiogenic features (Brenes et al., 2009; Larsson et al., 2002; Schrijver et al., 2002; Segovia et al., 2009).

In the RM, mature adult rats required fewer arm entries to learn both which arms were food-baited (RME) and which had already been visited (WME). Interestingly, this effect was evident even from the first trials, suggesting that the tendency of mature adult rats to commit fewer memory errors was already established by a maturation-dependent cognitive process and not by enhanced learning after repeated training. Young rats, in contrast, were significantly more active and less accurate in solving the task in the first days, in agreement with spontaneous activity in the OFT. RM performance in young rats improved with training until it reached the level of mature adult rats on test day 5. In addition, environmental enrichment in young animals reduced memory errors, and this effect was more pronounced for WME than for RME. Further, environmental enrichment in young rats reduced WME and RME on test days 4 and 5, at which their performance was no longer different from the mature adult counterparts. Surprisingly, environmental enrichment produced no improvement on RM performance in mature adult animals. However, considering that 5 trials were conducted per day, mature adult EE rats had on average less than one error per trial, a very high level of performance for this type of test (Olton, 1987). Thus, behavior of the mature adult rats may have reflected a ceiling effect, such that environmental effects could not be easily detected. On the IFT measure, young EE rats significantly outperformed all other groups, which mean young EE rats were faster completing the test than rats in all other conditions. Considering this indicator of speed and efficiency in solving the test together with the results of the memory error analyses, our results are in accordance with previous evidence that environmental enrichment during the post-weaning period enhanced brain and

behavioral plasticity, especially as related to memory tasks (Bell, Livesey, & Meyer, 2009; Simpson & Kelly, 2011).

It has been suggested that adult animals may have different learning abilities, derived from utilizing different behavioral traits (Bell et al., 2009; Segovia et al., 2009). It has been found, for instance, that adult rats seem to be more cautious when exploring new environments than younger rats (Bell et al., 2009; Harati et al., 2009; Segovia et al., 2009). This may benefit mature adult animals by allowing them to extract relevant information in a more efficient, but perhaps slower way. We are also aware, however, that our data contrast with findings in which aged rodents remained responsive to environmental enrichment (Bennett et al., 2006; Harburger et al., 2007; Leggio et al., 2005; Soffié et al., 1999). One possible explanation for our data is that during both early development and late aging, organisms are more sensitive to sensory and social stimulation than at other developmental time points (Hinde, 1983; Pietropaolo et al., 2004; Pryce et al., 2005). In the first case, enrichment seems to accelerate the development that is already taking place during early life (Brenes et al., 2009; Hellemans et al., 2004; Larsson et al., 2002). In the second case, on the contrary, enrichment seems to prevent the age-induced cognitive decline (Bennett et al., 2006; Bizon et al., 2009; Harburger et al., 2007). At 7 months of age when the experiment started, our mature rats would not be considered in a late-aging stage. Thus, although it is well-known that EE exerts different effects along the life span, the specificity of age-dependent sensitivity to EE needs further investigation. In regard to the latter, in a multilaboratory study conducted with different strain of rodents, EE during adulthood led to no alterations in open-field behavior and spatial memory tasks, a highly reliable effect observed across different laboratories (Wolfer et al., 2004).

On the other hand, there is evidence that EE alters the chemistry and anatomy of the cerebral cortex, enhances long-term potentiation (LTP), and increases neurogenesis and neurotrophin expression in the HPC. These effects may underlie the enhancement in learning and memory performance observed in enriched subjects (Diamond, 2001; Diamond, Johnson, Protti, Ott, & Kajisa, 1985; Sampedro-Piquero et al., 2013; Speisman et al., 2013; Van Praag et al., 2000). We found significantly higher *ex vivo* concentrations of both inhibitory (GABA) and excitatory (Glu) amino acids in HPC of young EE rats, in agreement with previous reports (for a review see, Solinas et al., 2010). Glu and GABA in the HPC have been linked with learning and memory (Arbuthnott, Ingham, & Wickens, 2000; Brioni, 1993; Lanni, Govoni, Lucchelli, & Boselli, 2009) For instance, high levels of Glu in the HPC may facilitate LTP, a molecular mechanism supporting memory formation (Duffy, Craddock, Abel, & Nguyen, 2001; Lanni et al., 2009; Simpson & Kelly, 2011; Solinas et al., 2010). Also, it has been suggested that sustained exploration of complex environments may be sufficient to modify brain networks functionally, perhaps through induction of LTP and other plasticity processes (Duffy et al., 2001; Sampedro-Piquero et al., 2013). In regard to the latter, we found that animals with higher Glu contents in HPC, especially young EE rats, needed less time to complete the RM task (i.e., high IFT values). In accordance with that, GABA levels, which exert an inhibitory action on HPC activity, correlated negatively with IFT values. The Glu/GABA-glutamine cycle is an astrocytes-dependent metabolic pathway supporting the release of Glu and GABA from neurons (Mora et al., 2007; Soffié et al., 1999). Under normal physiological conditions this cycle remains in balance, and our data are consistent with this. EE increased the level of both amino acids similarly in young rats, whereas in mature adult rats, both neurotransmitters were unaffected by housing condition. It is worth noting, however, that the activity of this neuron-astrocyte unit may be affected by aging, compromising neural plasticity, especially in the HPC (Mora et al., 2007; Soffié et al., 1999). This may explain the lack of effect of EE in mature adult rats on the RM, a well-known HPC-dependent task. However, further studies are warranted to corroborate these assumptions.

In VS, young EE rats had the lowest levels of DA and DOPAC. This is in accordance with previous findings obtained in young enriched rats that described lower DA content in this brain region (Brenes & Fornaguera, 2008). In young EE rats, DA turnover was significantly augmented in VS. High levels of novelty-induced exploration and locomotion, as observed in young rats in the OFT and RM tests, may have resulted from more striatal DA transmission, which translated into increased DA utilization without elevated DA synthesis (i.e., DA *ex vivo* content). Additionally, since DA in VS has been linked with motivation and reward (Bowman, Beck, & Luine, 2003; Lucas et al., 2004), the higher DA turnover in young EE rats may be related with performance in a reward-oriented task, such as the RM. In this regard, we found that young EE rats not only completed the task faster, but also visited more reinforced arms than animals from the other groups. The latter was supported by the correlation analysis in which the highest IFT scores were obtained, particularly, in those rats having the highest DA levels in VS.

5. Conclusions

At the behavioral level, while young rats were more active, mature adult rats were more efficient in coping with a stressful, novel environment in the OFT. Environmental enrichment in young animals reduced locomotor activity, whereas in mature adult counterparts it had no noticeable effects. In this paradigm, rearing behavior was prominently affected by age and not by housing condition. In the RM, mature adult rats are also more efficient in learning which arms were food-baited and which were already visited, especially on the first days of testing compared to young rats. This difference in performance between young and mature adult rats may be attributable to a maturation-dependent cognitive process and not to repeated training. As in the OFT, young rats were also more active displaying reward-oriented behaviors in the RM. Hyperactivity in these animals, however, may have interfered with the cognitive process required to remember which arms were baited and visited, leading to more WME and RME at this age. However, these animals also demonstrated a greater increment in IFT across days. Environmental enrichment improved spatial memory in young animals. Young EE rats were the fastest to complete the task, i.e., visiting all baited arms in the least time. However, the greatest improvement in WME and RME induced by enrichment in young rats brought their performance in those error measures to a level that was comparable to that of mature adult rats. Environmental enrichment did not improve RM performance in mature adult rats, suggesting that mature adult rats were less sensitive to environmental enrichment than young rats. At the neurochemical level, Glu and GABA content were increased by environmental enrichment in young rats, whereas DA and DOPAC contents in VS appeared reduced following enrichment in these animals. According to the correlation analysis, Glu and GABA levels, as well as DA turnover are functionally related with RM performance, especially with the IFT score. Taken together, our data suggest that young and mature adult rats exhibit different behavioral and cognitive learning strategies, specifically in habituation learning and spatial memory tasks, and are differentially affected by the sensory and social stimulation that accompany environmental enrichment.

Disclosure statement

All authors included in this paper have no actual or potential conflicts of interest related with any financial, personal or other relationships with other people or organizations.

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