Environmental Enrichment Duration Differentially Affects Behavior and Neuroplasticity in Adult Mice

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Environmental enrichment is a powerful way to stimulate brain and behavioral plasticity. However the required exposure duration to reach such changes has not been substantially analyzed. We aimed to assess the time-course of appearance of the beneficial effects of enriched environment. Thus, different behavioral tests and neurobiological parameters (such as neurogenesis, brain monoamines levels, and stress-related hormones) were concomitantly realized after different durations of enriched environment (24 h, 1, 3, or 5 weeks). While short enrichment exposure (24 h) was sufficient to improve object recognition memory performances, a 3-week exposure was required to improve aversive stimulus-memory performances and to reduce anxiety-like behavior; effects that were not observed with longer duration. The onset of behavioral changes after a 3-week exposure might be supported by higher serotonin levels in the frontal cortex, but seems independent of neurogenesis phenomenon. Additionally, the benefit of 3-week exposure on memory was not observed 3 weeks after cessation of enrichment. Thus, the 3-week exposure appears as an optimal duration in order to induce the most significant behavioral effects and to assess the underlying mechanisms. Altogether, these results suggest that the duration of exposure is a keystone of the beneficial behavioral and neurobiological effects of environmental enrichment.

Keywords: Anxiety and depressive like-behavior, enriched environment, long-term memory, neurogenesis, serotonin

Introduction

Environmental enrichment consists of complex housing conditions that enhance sensory-motor activities, social interactions, cognitive performances, and stimulate brain plasticity (Rosenzweig 1966). The enriched condition (EC) is well known to prevent age-related cognitive decline in rodents (Freret et al. 2012) and to alleviate those occurring in models of neurodegenerative disorders (for review, see Nithianantharajah and Hannan 2006; van Praag et al. 2000). Even when applied in healthy adult rodents, EC globally leads to an improvement of both spatial (Kempermann et al. 1998; Huang et al. 2007) and non-spatial (Rampon et al. 2000; Tang et al. 2001; Bruel-Jungerman et al. 2005) memory performances. EC also exerts anxiolytic-like (Benaroya-Milshtein et al. 2004; Galani et al. 2007) and antidepressive-like effects (Hattori et al. 2007; Llorens-Martin et al. 2007; Xu et al. 2009), despite controversial results (Hattori et al. 2007; Silva et al. 2011). Indeed, recently reviewed by Redolat and Mesa-Gresa (2012), numerous varieties of EC paradigms are available in the literature, possibly leading to a high heterogeneity of the results. Nevertheless, while beneficial effects on cognition have been reported both after short (several hours per day) (Rampon et al. 2000; Tang et al. 2001; Bruel-Jungerman et al. 2005) and long (weeks to months) (Kempermann et al. 1998; Huang et al. 2007; Leger, Bouet et al. 2012; Leger, Quiedeville et al. 2012) EC exposure, the comparison of increasing EC durations remains lacking.

The neurobiological changes that may underlie the behavioral plasticity induced by EC have been extensively studied. Many reports show an increase in cortical weight and thickness (Diamond et al. 1964; Bennett et al. 1969) as well as in regional hippocampal volume (Kempermann et al. 1997) in rodents housed in EC. Some modifications affecting various growth factors such as brain-derived neurotrophic factor (BDNF) (Ickes et al. 2000) and neurotransmitters have also been observed. For example, higher serotonin and noradrenalin concentrations in hippocampus have been reported in EC animals (Galani et al. 2007; Brenes et al. 2009). It is important to underline that these neurotransmitters are known to be crucially involved in anxiety, depressive behavior and memory (see for reviews, Buhot 1997; Pardon 2010). Hippocampal neurogenesis and synaptogenesis enhancement have also been proposed as changes supporting the behavioral effects of EC (Kempermann et al. 1997; Bruel-Jungerman et al. 2005). However, literature data suffer from heterogeneity regarding the EC paradigms leading to such beneficial effects. Thus, the aim of this study was to assess to what extent the duration of EC affects the behavior and whether these behavioral changes are associated with neurobiological modifications. To this end, several neurobiological parameters were assessed, especially hippocampal neurogenesis and brain monoaminergic neurotransmitter concentrations. By these investigations, our work extends our knowledge on the onset and duration of behavioral and brain modifications occurring during EC and thus contributes to the understanding of the neurobiological mechanisms involved in behavioral effects of EC.

Materials and Methods

Animals

Naval Medical Research Institute (NMRI) male mice aged 10 weeks at the beginning of the experiments were used (local breeding facility, F1 Centre d’Élevage Roger Janvier, Le Genest, France). All animals were maintained in a room with reversed 12 h light-dark cycle (20:00–8:00), regulated temperature (21 ± 1°C) and humidity (55 ± 10%). Water and food were available ad libitum. All experiments were carried out in accordance with the European Communities Council Directive (2010/63/UE) regarding the care and use of animals for experimental procedures, and approved by the regional ethical committee.
Environmental Enrichment

For each experiment, mice were subjected to standard condition (SC) or EC. SC mice were maintained in transparent polycarbonate cages (42 × 29 × 15 cm³, 5 mice per cage) containing nesting material and a cardboard tube. As already described (Leger et al. 2012), EC mice were continuously housed in environmental enrichment that consists of large polycarbonate cages (80 × 60 × 60 cm³, 12 mice per cage), provided with various objects of different shapes, sizes, colors, textures, and material (wood, plastic, and metal) and a large running wheel. Most of the objects and their locations were renewed twice a week to ensure novelty. During the cage cleaning, some objects were either changed or nor cleaned. At each cage cleaning and for each housing condition, a part of the nesting material from the previous cage was placed in the clean one to limit inter-individual male agonistic behaviors (Van Loo et al. 2003). Mice were placed continuously in SC (n = 12) or EC (n = 12) for a duration of 24 h, 1 week, 3 weeks, or 5 weeks before the start of the behavioral assessment and maintained in the same conditions during the behavioral experiments. For the object recognition test experiments, mice were also housed for 6 weeks in EC.

Behavioral Experiments

Mice were placed in the experimental room 30 min before the beginning of the behavioral experiments. To minimize the influence of tests on each other, 4 different cohorts were used (Fig. 1A). A cohort was used to assess the spontaneous locomotor activity, a second one used for the anxiety and depressive-like behavioral tests, and the 2 others were used for long-term memory tests (passive avoidance and object recognition). The analyses were conducted by an experimenter blind to the housing condition.

Spontaneous Locomotor Activity Test

Actimetry

The apparatus consisted of a photoelectronic actimeter (APELAB®) that automatically counts the number of interruptions of 2 perpendicular infrared light beams of an individual box (Boissier and Simon 1965). As previously described (Leconte et al. 2012), the number of light beams interruptions was recorded during a single session of 30 min and used as an index of spontaneous locomotor activity.

Anxiety-Like Behavior Tests

Two tests were used to measure anxiety-like behavior. Both are based on the innate preference of mice for dark and confined compared with light and open spaces. The light–dark box test and the elevated-plus-maze test were performed in the morning (9:00–12:00) and in the afternoon (14:00–17:00) of the first day of experiments, respectively.

Light–Dark Box

The apparatus (LETICA LE 810, Biosbeh®, France) consisted of 2 compartments separated by a squared opening (Costall et al. 1989). As already described (Leconte et al. 2012), the mouse was placed in the center of the illuminated white compartment (28 × 27 × 27 cm, 900 lux) and left to freely explore the apparatus for 5 min. The latency to enter the dark compartment (17 × 27 × 27 cm, 100 lux, red light), the number of transitions between the compartments and the time spent in each compartment were collected. The percentage of time spent in the lit compartment was used as an index of anxiety-like behavior.

Elevated-Plus Maze

The apparatus was elevated 40 cm above the floor and consisted of 2 opposite white open arms (30 × 5 × 40 cm, 40 lux) crossing 2 black closed arms (30 × 5 × 15 cm, 10 lux) with a central platform (5 × 5 cm) (Lister 1987). As already described (Leconte et al. 2012), the mouse was placed onto this platform, facing an open arm, and left to explore the apparatus for 5 min. The total number of entries and the time spent in the different arms were collected. The percentages of number of entries and time spent in the open arms were used as an index of anxiety-like behavior.

Depressive-Like Behavior Tests

Tail suspension and forced swimming tests are based on the immobile posture adopted by mice when exposed to an inescapable stressor. Changes in immobility time are considered to reflect changes in mood (Porsolt et al. 1977; Steru et al. 1985). These tests have been widely used to detect potential antidepressant effects of drugs. Both tests were conducted in the morning and in the afternoon of the second day of experiments (i.e., the day following the anxiety-like behavior tests), respectively.

Tail Suspension Test

The apparatus consisted of a support elevated 30 cm above the floor (10 lux). The mouse was suspended by the tail using an adhesive tape (at 1.5–2 cm from the tip). The latency for the first period of immobility and the immobility time during the last 4 min of the whole 6 min testing period were collected as already described (Leconte et al. 2011). Forced Swimming Test

The apparatus consisted of a transparent glass cylinder (30-cm height, 15-cm diameter) containing water (20-cm deep) at 25 ± 1 °C. The mouse was placed in the water and the immobility time, the time of swimming and the time of climbing were collected during the last 4 min of the 6 min testing period as already described (Aguila et al. 2007). The latency for the first immobility was also recorded.

Long-Term Memory Tests

Two memory tests were performed in 2 sessions separated by a 24 h-intersessions interval (24 h-ISI).

Passive Avoidance

Passive avoidance behavior reflects the ability of an animal to inhibit an innate preference for a dark and confined space after a single association with an aversive stimulus (Jarvik and Kopp 1967). As described before (Leger et al. 2012), the apparatus consisted of 2 compartments with an electric grid floor, separated by an automated sliding door (LETICA LE 812, Biosbeh®, France). For the acquisition session, the mouse was placed in the large illuminated white compartment (20 × 21 × 20 cm, 1000 lux), with the door closed. After 30 s, the door opened and latency to enter into the small dark compartment (7.3 × 7.5 × 14 cm, 10 lux) was measured (maximum latency: 50 s). Immediately after entering, the door was closed and the mouse received an inescapable electric foot shock (0.4 mA; 2 s) before being placed back in its home cage. For the test session (24 h-ISI), the experimental procedure was the same as for acquisition except no electric shock was used (the maximal length of this test session was 500 s). Because differences in latencies during the acquisition session were found between groups, data were normalized as previously described (Leger et al. 2012). The ratio of acquisition versus test session latencies to enter the dark compartment was used as an index of memory performance.

Object Recognition in Y-Maze

Object recognition test was performed in Y-maze as previously described (Leger, Quiiedeville et al. 2012). The apparatus consisted of a gray plastic Y-maze with 3 arms (33 × 8 × 16 cm each). During the presentation session, 2 identical objects were placed in the distal third areas of 2 arms. The mouse was placed in the starting arm containing no object, facing the experimenter, for free exploration (maximum: 10 min). When a criterion of 20 s of total exploration of both objects was achieved, the mouse was returned to the home cage. During the test session (24 h-ISI), a copy of the familiar objects and a novel object were placed in 2 arms of the maze. As previously, the mouse was allowed to freely explore the 3 arms until the criterion of 20 s total exploration was achieved.
object exploration time was reached. The nature of the objects (Lego® vs. Falcon®) and the position of the novel object (left vs. right) were randomized among animals. As previously described (Leger et al. 2013), object exploration was considered when the mouse sniffed the object with the nose directed towards the object at a distance <2 cm. The time of exploration of each object was manually collected and used as an index of memory performances by comparison with the chance level (10 s). Mice failing to reach the criterion within 10 min for the presentation or the test session were excluded from the analysis.

A 3-week exposure seemed to be a crucial duration to achieve beneficial effect in most behavioral tests made. Besides, among those tests, the object recognition test was the more sensitive to EC exposure (revealing performances modifications even after a short duration). Thus, we aimed to assess whether this 3-week exposure beneficial effect could persist after a similar time of EC interruption (i.e., 3 weeks). For this specific study, 3 additional groups were thus included (n = 12 per group; Fig. 1A). The first group was exposed to 3 weeks in EC and replaced in SC for 3 additional weeks. As control groups for this study, 2

**Figure 1.** Experimental design. (A) After 24 h, 1 week, 3 weeks, or 5 weeks in either SC (n = 12 per duration) or EC (n = 12 per duration), behavioral experiments were conducted during 2 consecutive days (D1 and D2). The first cohort was used to assess the spontaneous locomotor activity while the second cohort was used to assess anxiety and depressive-like behaviors (light–dark box [LD], elevated plus maze [EPM], tail suspension test [TST] and forced swimming test [FST]). The third and fourth cohort were used to assess the long-term memory through the passive avoidance (PA) test and the object recognition test performed in a Y-maze (OR). (B) Immunohistochemical experiments were conducted after 1, 3, or 5 weeks in either SC or EC. For the DG cell proliferation study, mice of each condition (n = 6 SC and n = 6 EC) were injected with one singly BrdU dose (75 mg/kg) 2 h before killing. To assess the newborn cell survival, mice of each condition (n = 6 SC and n = 6 EC) were injected with 2 daily BrdU doses (50 mg/kg) the first 4 day of housing and killed after 3 or 5 weeks of housing exposure. (C) Neurochemical changes were assessed after 1, 3, or 5 weeks in either SC or EC. Brain regions of interest were collected at the end of the housing period (BLA, baso-lateral amygdala; FrC, frontal cortex; Hpp, hippocampus). (D) Stress-related hormones were measured after 1, 3, or 5 weeks in either SC or EC. Blood samples were collected at the end of the housing period, (1) either in resting condition, (2) or in stressed-induced condition (FST).
other groups were placed either in EC or in SC for 6 weeks before object recognition memory assessment.

**Neurobiological Experiments**

To avoid the potential influence of behavioral testing on neurobiologic-
al parameters, 3 other cohorts of mice were used for immunohisto-
chemistry (Fig. 1B), measures of monoamine concentrations (Fig. 1C) and endocrine response (Fig. 1D).

**Immunohistochemistry**

**Cell Proliferation**

To assess the influence of EC duration (i.e., 1 week, 3 weeks, and 5 weeks) on hippocampal cell proliferation, mice (n = 6 SC and 6 EC for each housing duration) were injected intraperitoneally with a single dose of 5-bromodeoxyuridine (BrdU, 75 mg/kg) on the last day of housing, 2 h before killing (Paizanis et al. 2010) (Fig. 1B).

Mice were deeply anesthetized with an intraperitoneal injection of pentobarbital (120 mg/kg) and transcardially perfused with phosphate buffered saline (PBS 0.1 M) supplemented with sodium nitrite (0.1%) at 2 °C in cryoprotective solution (30% glycerol, 30% ethylene glycol, 40% PBS) until immunohistochemistry was processed (Vibratome VT 1000S, LEICA). Free-floating sections were then stored at −20 °C in cryoprotective solution (30% glycerol, 30% ethylene glycol, 40% PBS) until immunohistochemistry was processed (Paizanis et al. 2010).

Briefly, series of 1 in 4 free-floating sections were rinsed in PBS 0.1 M before being incubated in 3% H2O2/10% methanol/0.1 M to quench endogenous peroxidases. After 5 min, they were rinsed and incubated in 2 M HCl/0.1% Triton X-100/PBS 0.1 M (30 min; 37 °C) to de-
nature the DNA; pH was adjusted by incubation in 0.1 M sodium tetraborate buffer (30 min; pH 8.6). After several washes, sections were incubated for 1 h in 5% normal rabbit serum/0.1% Triton X-100/PBS 0.1 M before overnight incubation at 4 °C in the same solution supplemented with the primary antibody (monoclonal rat IgG anti-BrdU, 1:100, AbCys, France). After several washes, sections were incubated for 2 h at room temperature with the secondary antibody (biotinylated rabbit anti-rat IgG, 1:200, Vector Laboratories, France) and stained using ABC staining system (Vectastain ABC, Elite kit, Vector Laboratories, France). Peroxidase activity was detected by incubation of sections in 0.05% diaminobenzidine/0.01% H2O2/PBS 0.1 M for 5 min. Finally, free-floating sections were rinsed, mounted on gelatinized slides, dehydrated and coveredslipped with R.A. Mounting Medium™ (Richard-Allan Scientific, Kalamazoo, MI, USA).

Quantification of hippocampal BrdU-immunopositive cells of the granular cell layer (GCL) and adjacent subgranular zone of the dentate gyrus (DG) (2-cell body-wide zone along the border between the GCL and the hilus) was performed in a blind manner on an Olympus micro-
scope (×20–×40 objectives). GCL surface areas were measured with ImageJ 1.60© software. Results were expressed by the total number of hippocampal BrdU-immunopositive cells (total number of BrdU-stained cells × 4). GCL volume was calculated by multiplying the sum of surface areas by 160 µm.

**Newborn Cells Survival**

To assess the influence of EC duration on newborn hippocampal cell survival, mice (n = 6 SC and 6 EC for each housing duration) were treated with BrdU at the beginning of housing. Two daily injections of BrdU (50 mg/kg) every 12 h were administered during the first 4 days of EC or SC (Tashiro et al. 2007). After 3 and 5 weeks of housing, mice were killed as described above and brains were removed and sectioned as previously described (Paizanis et al. 2010) (Fig. 1B).

A second series of one in 4 free-floating sections was immuno-stained and analyzed as described before.

A second series of 1 in 4 free-floating sections was double-stained for BrdU and neurospecific nuclear protein (NeuN) using rat anti-BrdU (monoclonal rat IgG, 1:100, AbCys, France) and mouse anti-NeuN (1:500, Chemicon International, Millipore, France) antibodies. Immunofluorescence was revealed with the secondary antibody (biotinylated rabbit anti-rat IgG, 1:200, Vector Laboratories, France) coupled with streptavidin-Alexa Fluor 488 (1:1600, Molecular Probes, USA) and an Alexa Fluor 568 goat anti-mouse (1:1600, Molecular Probes, USA). Free-floating sections were finally mounted on gelatinized slides, dehydrated and coveredslipped with Fluoromount-G solution (Clinisciences, France). The neuronal phenotype was determined by analyzing 100 BrdU-positive cells per mouse labeled both by BrdU and NeuN staining with an Olympus Fluoview FV 1000 microscope in the entire z-axis (reso-
lution of 1 µm/pixel), using a step interval of 0.03 µm (×60 water-
immersion objective; Olympus Fluoview Ver. 3.1,© software).

**Neurochemistry**

At the end of each housing condition exposure for which the main be-
havioral effects of EC were observed (1, 3, and 5 weeks), mice were killed by cervical dislocation (n = 10 per housing condition) (Fig. 1C). The brains were rapidly removed and dissected on a cold plate. The frontal cortex and the hippocampus were rapidly isolated, weighed and stored at −80 °C for further analysis. Considering the neuronal activity changes induced by a 3-week EC exposure in such brain structures (Leger, Bouet et al. 2012; Leger, Quiedeville et al. 2012), the baso-lateral amygdala was additionally isolated for the 3-week exposed animals. Using high-performance liquid chromatography (HPLC), the concentrations (pg/mg of wet tissue) of serotonin (5-HT), dopamine (DA), and their metabolites, that is, 5-hydroxyindolacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were quantified.

The tissue samples were homogenized for 30 min in 100 µl of an extraction solution (pH = 3) constituted the mobile phase supplemented with perchloric acid 0.1 M. The samples were then centrifuged (10,000 rpm, 10 min), the supernatant was isolated and centrifuged again (10,000 rpm, 5 min). A sample volume of 20 µl of the super-
natant was injected. The apparatus consisted of a Waters Series system (Waters Corporation®, Milford, MA, USA) composed of an online de-
gasser and a binary pump (Waters 1525) coupled with an electrochem-
ic detector (Waters 2465: ISSAC reference, VT-03 Glassy carbon WE). A reverse phase column (Perkin Elmer, USA: C18 Brownlee® RP5, 5 µm pore size, 2.1 mm in diameter and 220 mm long) was used at 33 °C and a mobile phase (adapted from Dzahini et al. 2010) was delivered at 0.5 µl/min flow rate. The mobile phase constituted of 2 mM KCl, 50 mM KH2PO4, 0.1 mM ethylenediaminetetraacetic acid (EDTA)—Na2, 0.28 mM sodium octyl sulfate and 6% methanol (pH = 4.5). The analy-
cal system’s best sensitivity average value was 3 pg/10 µl with a signal/noise ratio of 3:1. Specific limits of detection were 490 fmol for DOPAC, 530 fmol for DA, 446 fmol for 5-HIAA, 560 fmol for HVA and 496 fmol for 5-HT. Using a standard of 5 and 50 pg and performing the analysis 5 subsequent times, repeatability was found to be better than 4% relative standard deviation for peak area and the method shows good linearity for all analytes (correlation coefficients > 0.991). Chromatogram analysis was obtained with Waters eSATIN and Breeze2® data acquisition software. The neurochemistry experiments were performed in a double blind design.

**Stress-Related Hormones Measurement**

At the end of 1, 3, and 5 week housing durations, half of the mice were exposed to the forced swimming test (same protocol as described before). Immediately after this stress procedure (n = 6 per housing condition) or after resting condition (n = 6 per housing condition), mice were decapitated and trunk blood samples were collected (between 10:30 and 12:00) in EDTA-coated tubes (adapted from Keeney et al. 2000) (Fig. 1D). Samples were rapidly centrifuged (5 min at 4 °C) and plasma supernatants were isolated and stored at −20 °C until radioimmunossay procedure. Corticosterone plasma concentration was determined with a radio-
immunoassay kit (ImuChem® Double antibody, Corticosterone-125I kit, MP Biomedicals, LLC, Orangeburg, NY, USA).

The average assay sensibility was 7.7 ng/mL and corticosterone levels were determined in duplicates with an intra-assay variation coef-
ficient of 4.4%. All samples were run together in a single assay to avoid inter-assay variability.
Statistical Analyses
Statistical analyses were processed with Statview® 5.0 software. For all experiments, we checked with an analysis of variance that SC mice presented similar behavioral performances, whatever the duration of exposure (data not presented). To compare the behavioral performances between the housing conditions, analysis of variance (ANOVA) and, when appropriate, post hoc Bonferroni/Dunn test were used to reduce Type I errors. Because 4 comparisons were made (comparison between respective same duration of SC and EC housing, i.e., 24 h, 1 week, 3 weeks, and 5 weeks), the significance level was adjusted to *P* = 0.0125 for the behavioral experiments. In the object recognition test, a univariate t-test was used to compare the exploration time of novel object with the chance level of exploration (10 s). The comparison between the 5 housing durations (24 h, 1, 3, and 5 weeks) was done with a post hoc Bonferroni/Dunn test and the significance level was adjusted to *P* ≤ 0.01. For the neurochemistry analysis, because 3 comparisons were made (comparison between respective same duration of SC and EC housing, i.e., 1, 3, and 5 weeks), the significance level was adjusted to *P* = 0.0167. Values are expressed as means ± SEM. For data that were not normally distributed (ratio of latency between the acquisition and the test sessions in the passive avoidance test) or obtained from small sample sizes (total number of BrdU-stained cells and GCL volumes, corticosterone levels), results are expressed as median ± quartile and analysis was performed with the nonparametric Kruskal–Wallis test followed when appropriate with a Mann–Whitney *U* test. A significant difference was considered when the *P* value was <0.05.

Results

**EC Decreases Spontaneous Locomotor Activity From the 1-Week Exposure**
Analysis of 30-min locomotor activity showed a significant reduction in the number of light beams interruptions in EC compared with SC mice at all exposure times from the 1-week housing duration upwards (ANOVA: *F*~3,88~ = 5.73, *P* < 0.001, Bonferroni/Dunn test *P* < 0.0025 for the 1- and 5-week exposure; *P* < 0.0125 for the 3-week exposure) but not with the shortest exposure of 24 h (Fig. 2).

**Exposure to EC Does Not Modify the Anxiety-Like Behavior in the Light–Dark Box Test**
The number of transitions between the 2 compartments did not differ between groups (data not shown, ANOVA: *F*~3,88~ = 1.03, *P* > 0.05), suggesting no modulation of exploratory and/or spontaneous activity. There was no difference between groups for the percentage of time spent in the aversive lit compartment (ANOVA: *F*~3,88~ = 1.21, *P* > 0.05). However, EC mice entered significantly more rapidly in the dark compartment than SC ones (ANOVA: *F*~3,88~ = 4.30, *P* < 0.001, Bonferroni/Dunn test: *P* < 0.0125 for the 3- and 5-week exposure) (Fig. 3A).

**The 3-Week EC Exposure Exerts Anxiolytic-Like Effects in the Elevated-Plus-Maze Test**
The total number of entries in the closed arms did not differ between groups (data not shown, ANOVA: *F*~3,88~ = 2.13, *P* > 0.05; followed by a Bonferroni/Dunn test that did not reveal difference between groups), suggesting no modulation of exploratory and/or spontaneous activity by EC in the elevated-plus maze. A significantly higher percentage of time spent and entries in the open arms was observed in mice exposed to the 3-week EC duration compared with the corresponding SC group (ANOVA: *F*~3,88~ = 4.57, *P* < 0.001 and *F*~3,88~ = 2.62, *P* < 0.05, respectively, *P* < 0.0025 and *P* < 0.00025, respectively) (Fig. 3B).

**EC Does Not Influence Depressive-Like Behavior**
There was no effect of EC on the immobility time or on the latency for the first immobility, in the tail suspension test (ANOVA: *F*~3,87~ = 1.77, *P* > 0.05 and *F*~3,87~ = 1.85, *P* > 0.05, respectively), or in the forced swimming test (ANOVA: *F*~3,87~ = 1.44, *P* > 0.05 and *F*~3,87~ = 1.13, *P* > 0.05, respectively). This is complemented by the absence of difference in time spent to swim and to climb between groups in the forced swimming test (ANOVA: *F*~3,87~ = 1.47, *P* > 0.05 and *F*~3,87~ = 0.63, *P* > 0.05, respectively) (data not shown).

**The 3-Week EC Exposure Improves the Memory Based on Aversive Stimulus**
Because mice exposed to 3 and 5 weeks of EC entered the dark compartment significantly more rapidly compared with SC mice in the light–dark box test, we checked whether this behavior occurred in the passive avoidance, since at the acquisition session, the mouse also has to enter a dark compartment. A significant difference in the latency to enter in the dark compartment was found between the 3-week SC (23.7 ± 4.4) and EC (8.9 ± 2.8) groups (data not shown, ANOVA: *F*~3,85~ = 2.43, *P* < 0.05, Bonferroni/Dunn test: *P* < 0.0025). The ratio of latencies test/acquisition was significantly higher in mice exposed to the 3-week EC duration indicating better memory performance compared with the SC group (Kruskal–Wallis test: *H* = 17.12, *P* < 0.05, Mann–Whitney *U* test: *Z* = -2.21, *P* < 0.05, for the 3-week EC exposure) (Fig. 4A).

**Whatever the Housing Duration, EC Improves Object Recognition Memory but This Effect Does Not Persist After Return to SC**
During the presentation session, no difference in the time to explore the 2 objects was observed, whatever the group considered (data not shown, univariate *t*-test: *t*~116~ = 0.90, *P* > 0.05). During the test session, the time spent to explore the novel object was overall significantly higher than chance (10 s) (univariate *t*-test: *t*~116~ = 5.63, *P* < 0.001; Fig. 4B). While SC mice globally did not discriminate the novel object compared with the chance level (univariate *t*-test: *t*~55~ = 0.47, *P* > 0.05), EC mice significantly discriminated the novel object, at all housing durations (univariate *t*-test: *t*~11~ = 2.41, *P* < 0.05 and *t*~11~ = 2.62, *P* < 0.01, Bonferroni/Dunn test: *P* < 0.00025 for the 3- and 5-week exposure).
< 0.05 for the 5- and 6-week exposure, respectively, 
\( t_9 = 5.06, \ P < 0.01 \) for the 24 h exposure, 
\( t_6 = 4.82 \) and \( t_9 = 6.19, \ P < 0.001 \) for the 1- and 3-week exposure, respectively). By contrast, the 3-week exposed group that had been transferred for 3 weeks to SC did not discriminate the novel object (univariate \( t \)-test: \( t_{11} = 1.42, \ P > 0.05 \)). Moreover, a significantly higher exploration time of the novel object was observed in mice exposed to EC from 1 week up to 5 weeks compared with the respective SC groups (ANOVA: \( F_{10,106} = 5.18, \ P < 0.001 \), Bonferroni/Dunn test: \( P < 0.002 \) for the 1- and 3-week exposure and \( P < 0.01 \) for the 5- and 6-week exposure).

**EC Does not Modify Hippocampal Cell Proliferation**

Whatever the housing duration, EC did not modify the GCL volume (Kruskal–Wallis test: \( H = 5.61, \ P > 0.05 \)). Besides, there was no difference in the total number of BrdU-immunopositive cells between EC and SC groups (Kruskal–Wallis test: \( H = 11.10, \ P < 0.05 \), Mann–Whitney \( U \) tests: \( P > 0.05 \); Fig. 5).

**Exposure to 3 and 5 Weeks of EC Promotes the Neuronal Phenotype of Surviving Cell**

There was no difference in GCL volume between groups (data not shown, Kruskal–Wallis test: \( H = 2.90, \ P > 0.05 \)). Concerning...
the newborn cell survival, only the 5-week EC exposure significantly enhanced the cell survival compared with the respective SC group (Kruskal–Wallis test: $H = 9.22, P < 0.05$, Mann–Whitney $U$ tests: $Z = -1.12, P > 0.05$, and $Z = -2.36, P < 0.05$, for the 3- and 5-week exposure, respectively) (Figs 5B and 6A). Confocal immunofluorescence analysis revealed a significantly higher number of positive cells coexpressing BrdU and NeuN, after both 3 and 5 weeks of EC, compared with SC groups (Kruskal–Wallis test: $H = 16.13, P < 0.01$, Mann–Whitney $U$ tests: $Z = -2.25, P < 0.05$, and $Z = -2.88, P < 0.01$, for the 3- and 5-week exposure, respectively) (Figs 5C and 6B).

The 3-Week EC Exposure Enhances Serotonin Concentration in the Frontal Cortex

Brain monoamines analysis revealed a global significant effect of EC duration (1, 3 and 5 weeks) compared with SC on serotonin concentration in the frontal cortex (Table 1) but not in the hippocampus or the baso-lateral amygdala (data not shown). A significantly lower 5-HIAA concentration (~30%) was found in mice exposed to 1 week of EC, without modification of the 5HT concentration (ANOVA: $F_{3,54} = 8.54, P < 0.001$ and $F_{5,54} = 5.89, P < 0.001$, Bonferroni/Dunn test: $P < 0.0001$ and $P > 0.0167$, respectively). This was associated with a significant decrease in the serotonergic turnover (5-HIAA/5HT; -31%) (ANOVA: $F_{3,54} = 11.93, P < 0.001$, Bonferroni/Dunn test: $P < 0.0002$). After a 3-week exposure to EC, a significantly higher 5HT concentration was observed (+29%), as well as a trend to higher 5-HIAA concentration (ANOVA: $F_{3,54} = 5.89$, $P < 0.001$ and $F_{5,54} = 8.54, P < 0.001$, Bonferroni/Dunn test: $P < 0.0167$ and $P = 0.0338$, respectively) without modification of the serotonergic turnover. There was no effect of EC on the other monoamine and metabolite concentrations in the frontal cortex (data not shown, ANOVA: $F_{5,54} = 1.25, P > 0.05$ for the DA, $F_{5,54} = 1.09, P > 0.05$ for the DOPAC, $F_{5,54} = 1.12, P > 0.05$ for the HVA).

EC Neither Modifies Basal nor Stress-Induced Plasma Corticosterone Levels

Radioimmunoassay revealed in both groups a significant enhancement of the corticosterone levels under stress-induced condition (forced swimming test exposure) compared with the basal condition (Kruskal–Wallis test: $H = 33.70, P < 0.001$; Mann–Whitney $U$ tests: $Z = -3.44, P < 0.001$ in SC groups, and $Z = -4.05, P < 0.001$ in EC groups). However, there was no effect of EC on these corticosterone levels neither in basal condition (Kruskal–Wallis test, $P > 0.05$; Fig. 7A), nor in stress-induced condition (Kruskal–Wallis test, $P > 0.05$; Fig. 7B), compared with SC group.

Discussion

The aim of this study was to assess how the duration of EC affects the behavioral performances and whether these behavioral changes are related to neurobiological modifications. As reported by others (Ramon et al. 2000; Tang et al. 2001; Huang et al. 2007; Leger, Bouet et al. 2012), among the main

![Figure 5. Effect of EC duration on DG cell proliferation (A), cell survival (B), and neuronal phenotype (C) compared with SC. Data are expressed as boxplots. (A) No difference between the number of BrdU-stained proliferative cells (left) and of GCL volume (right) was observed between groups, whatever the housing duration (1 week ($n = 5$ per group), 3 weeks ($n = 6$ SC; $n = 4$ EC), and 5 weeks ($n = 6$ per group)). (B) A significantly higher number of BrdU-stained survival cells was detected in mice exposed to 5 weeks of EC compared with SC corresponding group (Mann–Whitney $U$ test: $^* P < 0.05; n = 6$ per group). (C) A significantly higher number of BrdU–NeuN-stained cells was observed in EC both after the 3- and 5-week exposure compared with respective SC groups (Mann–Whitney $U$ test: $^* P < 0.05; ^{**} P < 0.01; n = 6$ per group).]
behavioral effects, we observed a promnesiant effect of EC on 2 kinds of memory, either based on an aversive stimulus (passive avoidance) or on recognition memory (object recognition). We also found a reduced spontaneous locomotor activity, a behavioral change probably related to a faster habituation to novelty that we identified to occur after the 1-week exposure, and an anxiolytic-like effect in the elevated-plus maze. These cognitive effects may be related to neurobiological changes induced by EC. Indeed, serotonergic changes in the frontal cortex and hippocampal cell survival associated with a neuronal phenotype increase were detected. Finally, we might suppose that NMRI mice are less sensitive to the antidepressive-like effect of environmental modulation observed in despair-like paradigms (Hattori et al. 2007; Llorens-Martin et al. 2007) as no effect of EC on the depressive-like behavior was detected in both tests used.

In accordance with recent investigations in rats (Birch et al. 2013), our findings highlight the importance of the duration of exposure in EC-related effects. While short EC exposure (24 h) was sufficient to improve object recognition memory, the enhancement of hippocampal cell survival required longer EC exposure (5 weeks). Moreover, the anxiolytic-like effect and the memory improvement in the passive avoidance test induced by
EC were detected after the 3-week exposure, a duration after which an increase in serotonin concentration was observed in the frontal cortex. The potential relationship between the timing of appearance of the neurobiological and the behavioral effects are now discussed.

**EC Does not Modulate the Plasma Corticosterone Levels**

Whatever the duration of exposure, EC did not modify the plasma corticosterone concentration compared with SC mice, neither in resting, nor in stress-induced condition. Because both agonistic behavior and stressful situation are known to induce higher corticosterone levels (Haemisch and Gartner 1994; Haemisch et al. 1994; Von Holst 1998), the absence of corticosterone levels changes could reflect a less stressful effect of the EC in NMRI mice, as since reported in a docile inbred strain (Marashi et al. 2004). Besides, it has been demonstrated that the antidepressive-like effects of EC required glucocorticoids elevation in mice (Xu et al. 2009). The unchanged depressive-like behavior observed between our groups may thus be underlain by the absence of hyperactivity of the hypothalamic–pituitary–adrenocortical axis.

**EC Affects Hippocampal Neuronal Survival From the 3-Week Exposure Duration**

Hippocampus-dependent memory training has been involved in modifications of survival and incorporation of immature newborn cells in neuronal networks (for review, see Zhao et al. 2008). To isolate the effects of EC on neurogenesis from those potentially related to the memory test, we explored the effects of EC on neurogenesis in animals that did not experience any task training. In opposition to the higher cell proliferation reported after short EC exposures as used herein, that is, 1 week (Steiner et al. 2008; Llorens-Martín et al. 2010), we did not observe a modification of DG cells proliferation, whatever the EC duration. In these studies, however, BrdU was injected at the beginning of the EC, which could be more stimulating than the end of the exposure, and explain the discrepancy with our results. By contrast, we report a higher number of BrdU-stained surviving cells, which reached the significance level after the 5-week EC duration, as similarly found after longer exposure periods ranging from 6 weeks to 6 months (Kempermann et al. 1998; Brown et al. 2003; Rossi et al. 2006; Llorens-Martín et al. 2010). In agreement with other studies (Kempermann et al. 1998; Tashiro et al. 2007), a significantly higher percentage of surviving cells displaying the neuronal phenotype was observed in EC mice compared with SC, an effect that we identified from the 3-week EC exposure. Thus, we show for the first time that the minimal duration of EC exposure of 3 weeks is necessary to induce neurogenesis changes in mice. While the 3-week EC exposure resulted in a higher proportion of new neurons, the neuronal survival is prolonged specifically after 5 weeks of EC. This indicates a duration-dependent effect of EC on neurogenesis, an observation similar to the results described by Birch et al. (2013) in rats. Because of the nature of the EC that we chose (social interactions, object interactions, and exercise), it is difficult to determine here the role of each aspect of the EC on the neurogenesis changes observed. Considering the recent works published, the running activity definitely plays a major contributing role on hippocampal neurogenesis increase (Marlatt et al. 2013) as well as neurotrophine levels enhancement such as BDNF (Kobilo et al. 2011; Vivar et al. 2013). Despite no recording of the running activity in our study, the large running wheel used in our experiment allowed access to several mice at the same time. The physical activity may thus largely be involved in the neurogenesis stimulation. Interestingly, as stress-induced release of glucocorticoid hormones (i.e., corticosterone) is known to impair the neurogenesis (Lucassen et al. 2010; Schoenfeld and Gould 2013), the beneficial effects of EC reported here on neurogenesis occurred without any corticosterone changes. Our results tend to confirm those from Xu et al. (2009) demonstrating that the enhanced rate of adult neurogenesis was maintained in mice exposed to EC independently of the prevention of elevated corticosterone

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Table 1

<table>
<thead>
<tr>
<th>Monoamines</th>
<th>Group</th>
<th>1 Week</th>
<th>3 Weeks</th>
<th>5 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HT</td>
<td>SC</td>
<td>72.8 ± 7.1</td>
<td>78.2 ± 4.5</td>
<td>63.1 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>74.0 ± 5.7</td>
<td>101.3 ± 8.4*</td>
<td>65.6 ± 4.2</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>SC</td>
<td>399.5 ± 15.2</td>
<td>300.8 ± 18.3</td>
<td>371.9 ± 17.0</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>281.0 ± 13.7***</td>
<td>349.3 ± 17.1</td>
<td>373.9 ± 17.8</td>
</tr>
<tr>
<td>5-HIAA/5HT turnover</td>
<td>SC</td>
<td>5.8 ± 0.5</td>
<td>3.9 ± 0.2</td>
<td>6.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>4.0 ± 0.4***</td>
<td>3.6 ± 0.2</td>
<td>5.8 ± 0.2</td>
</tr>
</tbody>
</table>

Note: Values are means ± SEM, ANOVA followed by a post hoc Bonferroni/Dunn test, *P < 0.0167, ***P < 0.0002 compared with the corresponding SC group (n = 10 per group).
release. In rats exposed to EC, elevated hippocampal BDNF levels negatively correlated with corticosterone levels (Bakos et al. 2009). Considering the importance of BDNF for the newborn cell survival and differentiation in the DG (Sairanen et al. 2005; Taliaz et al. 2010), it would be relevant to further investigate how much the BDNF may be involved in the behavioral, neurochemical and neurogenesis changes induced by EC.

**EC Exposure Transiently Affects Serotonin Concentration in the Frontal Cortex**

The monoamine levels were measured in 3 brain regions known to take part in the beneficial effects of EC on memory, namely the hippocampus, the frontal cortex and the basolateral amygdala (Leger, Bouet et al. 2012; Leger, Quiedeville et al. 2012). To our knowledge, the brain monoamine levels following different EC durations have never been investigated. Here, we report a transient serotonergic modulation in the frontal cortex after a 3-week EC exposure, suggesting that compensatory mechanisms leading to normalization of brain serotonin levels appear after longer exposure. This could explain the absence of serotonergic changes observed in mice exposed to a longer duration of EC, that is, 5 weeks in our experiments or 40 days in a previous study (Naka et al. 2002). Thus, EC as well as novelty exposure protocols (Miura et al. 2002; Antoniou et al. 2008; Ford et al. 2008), modulate the serotonergic system with a regional selectivity as there was no change in the hippocampus, or the baso-lateral amygdala.

**EC Exposure Transiently Affects Anxiety-Like Behavior in a Task-Dependent Manner**

Our main findings concern the transient anxiolytic-like effect in the elevated-plus maze induced by 3 weeks of EC but not by longer exposure (5 and 8 weeks, data not presented). If this anxiolytic-like effect has already been described in the literature after longer EC duration (5–6 weeks) (Benaroya-Milshtein et al. 2004; Galani et al. 2007), it is the first time that it is shown to be transient. This emotional response does not seem to be related to a hypothalamic–pituitary–adrenocortical axis response. Indeed, EC had no effect on plasma corticosterone concentration under resting or stress conditions. Interestingly, the control of emotions, and especially anxiety, has been shown to be supported by the prefrontal cortex (Griebel 1995; Buhot 1997; Gross et al. 2002), a brain region receiving a dense serotonin innervation (Azmitha and Segal 1978). Considering their similar kinetics of onset, the anxiolytic-like effects induced by 3 weeks of EC could thus be supported by the serotonergic adaptation observed in the frontal cortex. This hypothesis could be further studied by focusing on the serotonergic changes induced by EC, not only on basal condition, but also during the elevated-plus-maze test.

By contrast, the anxiety-like behavior was not modified by EC in the light–dark box test, an effect already observed by others (Augustsson et al. 2003; Chourbaji et al. 2005), that we found to be independent of the exposure duration. The shorter latency to enter the dark compartment found after the 3 and 5-week exposure to EC may be related to a faster habituation to novelty, as also reported by Chourbaji et al. (2005) after similar exposure duration. Because of its openness and elevation, the elevated-plus-maze is probably more aversive than the light–dark box and could facilitate the apparent anxiolytic-like effect induced by EC.

**Aversive Stimulus-Based Memory and Object Recognition Memory are Differentially Affected by the Duration of EC Exposure**

The major effects of the 3-week EC exposure on anxiety are associated with an improvement in memory performances assessed in the passive avoidance test. Again, such a beneficial effect was transient and disappeared after 5 weeks of exposure. Because the test requires the use of an aversive stimulus, one could suppose that the reduced anxiety-like behavior elicited by the 3-week EC exposure was related to the memory improvement. However, such a hypothesis does not seem to be supported by the pharmacological studies reporting that anxiolytic drugs produce memory impairment in the passive avoidance test (Nabeshima et al. 1990; Lelong-Boulouard et al. 2006). Moreover, no changes in anxiety-like behavior were observed when assessed in the light–dark box which is a testing condition very close to the passive avoidance test.

Despite the involvement of adult hippocampal neurons in the expression of contextual fear memory (Saxe et al. 2006; Drew et al. 2010; Denny et al. 2012), it seems that the increased number of cells expressing the neuronal phenotype observed from the 3-week EC exposure may not fully contribute to the transient effects of EC on memory based on aversive stimulus. Indeed, the neurogenesis changes were induced from the 3-week EC exposure and persisted after the 5-week duration. By contrast, the onset of memory improvement was only observed for the 3-week duration and not after longer duration of exposure in the passive avoidance test. Thus, other neurobiological mechanisms may also be involved in the transient beneficial effects of EC on memory based on aversive stimulus. Interestingly, we found a transient increase in serotonin levels specifically after 3 weeks of EC in the frontal cortex, a brain region previously reported to be recruited during the passive avoidance test in EC mice (Leger, Bouet et al. 2012). These results suggest a possible involvement of the serotonergic system in the transient beneficial effects of EC on this memory.

Further investigations will nevertheless be required to assess the mechanisms underlying the higher serotonergic release observed in EC, for example, by regarding the evolution of the expression of serotonergic receptors known to play a role in anxiety and memory (King et al. 2008; Meneses and Liy-Salmeron 2012). Because the prefrontal cortex is particularly rich in 5-HT1a receptors (Puig and Gullledge 2011), these serotonergic receptors may represent a first interesting target. Considering emerging evidence on the role play by other receptor subtypes on memory processes, such as 5-HT6 (Quiedeville et al. 2014) and 5-HT7 (Gasbarri and Pompili 2014) receptors, it could also be particularly relevant to assess the influence of EC duration on the expression of these receptors, that as so far never been investigated.

Finally, the transient nature of the behavioral and neurochemical effects observed after the 3-week EC exposure has never been reported before and may be surprising. However, these results complement some other transient effects of EC that have recently emerged from the literature. For example, a decrease of dendritic arborization was reported as a transient phenomenon in the prefrontal cortex of rats housed for only a few days in EC (Comeau et al. 2010). More recently, a transient
increase in hippocampal cell activity and plasticity in both CA1 area and DG was found after a 10-day EC exposure in rats (Eckert and Abraham 2013). Thus, our work provides new support to the view of duration-dependent effects of EC exposure on both behavioral and neurobiological changes.

When investigating object recognition memory performance, we found a rapid memory enhancement following short-term exposure to EC (24 h). Such early beneficial effects of EC have never been reported before and could be related to the nature of the environmental enrichment used, that is, the exposure to various objects. Suggested by others (Birch et al. 2013) and supported by our previous findings (Leger, Quiedeville et al. 2012), this early improvement in object recognition memory could be related to a strengthening of the neuronal networks involved in novelty processing following renewed objects exposure. Interestingly, the memory enhancement induced by EC did not persist after 3 weeks of EC cessation. These results indicate that continuous EC exposure seems to be required to enhance the object recognition memory. In opposition, the behavioral changes induced in rodents exposed to EC, such as reduced locomotor activity (Amaral et al. 2008), enhanced exploratory activity (Fernandez-Teruel et al. 2002), or anxiety-like effects (Friske and Gammie 2005), were found to persist under SC. Among the neurobiological changes proposed to support the EC-induced object recognition memory, one may take into consideration neurogenesis modulation. Indeed, some studies support a role for increased proliferation and enhanced survival of DG cells in the beneficial effect of EC on memory, be it in adult (Kempermann et al. 1997; van Praag et al. 1999; Bruel-Jungerman et al. 2005) or aged (Kempermann et al. 1998; van Praag et al. 2005) rodents and especially for hippocampal-dependant spatial memory (Dupret et al. 2008). Interestingly, a higher cell survival in association with better object recognition memory performances was reported in rats exposed to EC (Bruel-Jungerman et al. 2005), a view also supported by the recent works of Birch et al. (2013). After similar EC durations to those we used, Birch and collaborators found an object recognition memory improvement from the 3-week EC exposure in rats and a delayed effect on neurogenesis (i.e., after 6 weeks of EC). We report here earlier beneficial effects of EC (beginning from 24 h of exposure) on the recognition performances. Taken together with the works of Birch et al. (2013), our study suggests that the duration of EC differentially affects the memory trace prolongation (when SC rodent presents a memory loss) and the memory improvement (when SC rodent discriminates the novel object). Moreover, the onset of neurogenesis changes observed from the 3-week exposure did not match with the early memory improvement observed from the 24-h exposure, suggesting the involvement of other neurobiological mechanisms in the beneficial effects of EC on memory. However, the cell-labeling that we used only allowed us to determine the effects of EC on the proliferation and the survival of neurons. Further investigation would thus be necessary to assess the effects of EC on the morphology and the earlier and intermediate phases of neuronal maturation. Indeed, it has been shown that the morphological alteration of young differentiating doublecortin (DCX)-positive neurons may play a role in the impairment of spatial memory (Oomen et al. 2010). Because some plasticity-inducing experience such as voluntary exercise accelerated the maturation of newborn cell (Zhao et al. 2006), the beneficial effects of EC on memory may be supported by earlier neurogenesis changes. The precise phase of maturation affected by EC could thus be determined with the use of other cell markers as well as investigating the morphology of the cell (dendritic branching of DCX-positive cells for example). Finally, the analysis of different subregions of the hippocampus would also be of great importance as a delayed acceleration of neuronal maturation through a septo-temporal axis of the DG was recently observed in mice exposed to running activity (Piatti et al. 2011).

To conclude, we describe here for the first time, the time course of several behavioral and neurobiological changes occurring after environmental enrichment in mice. While some behavioral components, such as memory based on aversive stimulus and anxiety-like behavior, seem to be sensitive to the duration of EC exposure, enhanced object recognition memory is not or less dependent on EC duration. In addition, the onset of the behavioral effects of EC does not seem to depend on increased survival of newly generated hippocampal neurons. At the molecular level, we hypothesize that the onset of behavioral effects of EC on aversive stimulus-based memory could be related to specific neurochemical changes, with a particular role for serotonin in the frontal cortex. Of note, brain activity in this region had already been reported to undergo modulations during memory tests in EC mice (Leger, Bouet et al. 2012; Leger, Quiedeville et al. 2012). Taken together, these results show that different behavioral components are not equally sensitive to the duration of EC exposure, suggesting that they could be supported by different neurobiological mechanisms on which EC does or does not act with varying time-courses. These peculiarities could partly explain the heterogeneity of the results found in the literature as to the effects of EC in rodents.

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References


