Pharmacology, Biochemistry and Behavior 102 (2012) 434–441

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Early-life social experiences in mice affect emotional behaviour and hypothalamic-pituitary-adrenal axis function

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ARTICLE INFO

Article history:
Received 5 March 2012
Received in revised form 8 May 2012
Accepted 2 June 2012
Available online 9 June 2012

Keywords:
Environmental enrichment
Social isolation
Hypothalamo-pituitary-adrenal axis
Corticosterone
Behaviour

ABSTRACT

Rationale: Early-life stressful experiences are associated to alterations in behavioural responses and development of psychiatric and neurodegenerative diseases. In rodents, individual housing is considered as a stressful condition whilst enriched environment can protect against stress and its negative consequences. Neuroendocrine responses to stress can also be altered by early-life experiences and seem to contribute to behavioural alterations induced by changes in housing conditions.
Objective: To develop an improved procedure of social isolation throughout development (from pre-adolescence to adulthood) in CD1 mice and to elucidate its effects on behavioural parameters related to stress and neuroendocrine responses compared to enriched or social conditions.
Materials and methods: CD1 male mice (PND 21) were housed in social/standard conditions, enriched conditions or isolated conditions during seven weeks. After that, different relevant behaviours were evaluated, including locomotor activity, anxiety-like and despair behaviour. Levels of plasma corticosterone were also analysed before and after a stressful event.
Results: CD1 mice exposed to an isolated environment exhibited higher locomotion and anxiety-like responses than animals exposed to social or enriched conditions. In addition, isolated animals showed lower basal plasma corticosterone than social or enriched ones but after a stressful event the elevation of plasma corticosterone was higher, suggesting an enhanced response of the HPA axis to a novel and stressful situation.
Conclusions: Social interaction is an important feature to display an appropriate behavioural and neuronal development. Habituation to novel stimuli is impaired in subjects exposed to social isolation and induces increased excitability response to stressful events. Social deprivation increases the possibility of altered neuronal function and could facilitate the development of neuropsychiatric disorders in adulthood.

1. Introduction

Early-life stressful experiences are associated to alterations in behavioural responses, such as cognition, motivation and emotional behaviours (Levine, 1985; De Kloet et al., 2005; Lai and Huang, 2011; Pechtel and Pizzagalli, 2011). Social positive interaction may lead to a normal development of the mentioned functions and protection against negative events in life.

In rodents and other experimental animals, long-term specific housing conditions can be adequately enriched with environmental stimuli and affect the ulterior development of cognitive and emotional controlling systems in the adulthood (Gutman and Nemeroff, 2002). In this sense, environmental enrichment (EE), which consists on housing the animals in large cages containing a variety of sensory-motor stimulus, such as tunnels, running wheels, toys; which are routinely changed and removed during the experimental period to enhance novelty, exploration, sensory-motor and cognitive stimulation (Nithianantharajah and Hannan, 2006; van Praag et al., 2000), has been shown to benefit behaviour in rodents, such as reduced anxiety in BALB/c and C57BL/6 mice (Roy et al., 2001; Sztainberg et al., 2010), increased exploratory behaviour or improved learning processes in rats (Cummins et al., 1973; Hoffmann et al., 2009; Peña et al., 2009). Additionally, it promotes neuronal protection through the increase of trophic factor levels, enhanced cell survival, increased neurogenesis and enhanced dendritic branching and synaptogenesis (Mattson and Magnus, 2006; van Praag et al., 2000). Furthermore, enrichment in the early-life can protect against stress, which can be a vulnerability factor for the development of neurodegenerative (Laviola et al., 2008) and other central nervous system (CNS) diseases. In contrast, social isolation/individual housing without cognitive and motor stimulation is considered as a stressful

Abbreviations: EE, environmental enrichment; CNS, central nervous system; HPA, hypothalamo-pituitary-adrenal; CRH, corticotrophin releasing hormone; ACTH, adrenocorticotrophin; CORT, corticosterone; EC, enriched condition; SC, social condition; IC, isolated condition; RIA, radio-immunoassays; EPM, elevated plus maze; TST, Tail suspension test.

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doi:10.1016/j.pbb.2012.06.001

condition (Valzelli, 1973). In this sense, it is known that stressful events can activate the hypothalamo-pituitary-adrenal (HPA) axis and increase the release of corticotrophin releasing hormone (CRH) from the hypothalamic paraventricular nucleus, causing, in mice, the stimulation of the secretion of glucocorticoids, such as corticosterone (CORT) from the adrenal cortex (De Kloet et al., 1990; Armario et al., 2004; 2006; Marin et al., 2007; Jankord and Herman, 2008). Although hormonal responses to environmental clues have been studied, there is little consistent data about changes in neuroendocrine status following different home environments (Moncek et al., 2004). Therefore, several studies have found basal elevated CORT secretions in mice (Marashi et al., 2003) as well as in other mammals (De Jong et al., 2000) kept under environmental enrichment. Other studies have reported no differences in CORT levels exposed under different environmental conditions (Schrijver et al., 2002) and some have even found a reduction of CORT in enriched adult rats (Belz et al., 2003). More recently, no differences in basal CORT during the light period between enriched and standard housed post-weaned rats (Peña et al., 2009) and a lower increase in enriched ones after the exposure to a novel stimulus have been reported.

Home environmental conditions during childhood and adolescence are relevant for the development of a spectrum of important issues related to early-life experiences, such as neuronal development, behavioural responses, and potential future psychopathological states in humans (Penza et al., 2003; Carroll, 2003; McGowan and Szyf, 2010).

Many studies have investigated the effects of environmental enrichment or social isolation on behaviour. However, most of them considered only two different conditions, for instance, enrichment vs standard conditions (Konkle et al., 2010; Moncek et al., 2004; Zhu et al., 2009) or isolation vs standard conditions (Bartolomucci et al., 2003; Karim and Arslan, 2000). Additionally, it needs to be taken into account that behavioural effects of environmental conditions on rodents' development depend on the specie and even the strain used (Võikar et al., 2005; Abramov et al., 2008; Silva et al., 2011). Thus, the aim of this study was to develop an improved model of environmental enrichment in CD1 mice in which we have compared the consequences of three different home environmental conditions maintained throughout development (from pre-adolescence to adulthood) in CD1 mice: social conditions vs enriched conditions vs isolated conditions, on behavioural parameters. Indeed, pre-adolescent and adolescent periods compromise a critical period where social and environmental complexity can impact to the brain and behaviour in adulthood (Workman et al., 2011). In particular, we have analysed the capability of this mouse strain housed in different environments to adapt to a novel situation. Furthermore, we have investigated the effect of these different housing conditions on exposure to stress and its hormonal responses (plasma CORT). For that, the effects of these different housing conditions were assessed by the following parameters: i) body weight gain along the whole study (49 days); ii) locomotor activity and habituation; iii) anxiety-like responses; iv) depressive-like behaviour; and v) HPA axis functionality under a stressful situation; that is, levels of plasma CORT before and immediately after the exposure to dark–light box (an anxiety-like environment). A different environmental housing was maintained throughout the behavioural testing. The use of CD1 mice as experimental subjects allows us i) to assess the effects of different housing conditions in this particular mice strain on behaviour and HPA axis function and ii) to use, in future studies, mutant mice to elucidate the particular contribution of specific mutations.

2. Materials and methods

2.1. Subjects

Male CD1 mice (Charles River, France) weighing 11–12 g (PND 21) at the beginning of the experiments were used for this study. Mice were housed in a temperature (22º ± 1 °C), humidity (55% ± 10%), and light cycle controlled room (light was on between 8.00 AM and 8.00 PM). Standard diet (SDSdiets RM1 P) and water were available ad libitum. All animal care and experimental procedures were conducted according to the guidelines of the European Communities Directive 86/609/EEC, regulating animal research and were approved by the local ethical committee (Comitè Ètic d'Experimentació Animal-PRBB). All procedures were conducted by an experimenter blind to the procedure.

2.2. Home environment

Upon arrival to the laboratory, mice were randomly assigned to one of the three different environments: (1) a social condition (SC), containing social partners only; (2) an enriched condition (EC), containing novel objects and social partners; and (3) an isolated condition (IC), without objects or social partners. SC mice were housed (5 per cage) in a Plexiglas box (25 cm wide × 25 cm long × 14 cm high). EC mice were housed (10 per cage) in a large Plexiglas box (24 cm wide × 40 cm long × 18 cm high). This box was connected to another box of the same characteristics through a tunnel made of a PVC pipe. The environment contained plastic wheels and 6–8 novel hard plastic objects which were changed twice per week, during the cleaning of the home cages. IC mice were housed individually in small Plexiglas boxes (12.5 cm wide × 22 cm long × 12.5 cm high). All animals were maintained in the same vivarium in the corresponding home environment for 7 weeks, that is from PND 21 (pre-adolescence) to PND 70 (adulthood); and throughout the behavioural procedures described below.

2.3. Behavioural procedures

2.3.1. Experiment 1: body weight, locomotor and emotional-related behaviour

Body weight was individually evaluated twice a week (every Monday and Thursday) during the 7 weeks that mice were housed under the aforementioned conditions (n = 20 mice per group).

After the 7 weeks of exposure to different environmental conditions, mice were exposed to different behavioural tests (Fig. 1). Thus, on days 1, 2 and 3 (PND 71, 72 and 73) animals were subjected to locomotor activity test, on the 4th day (PND 74) to elevated plus-maze paradigm and, on the 5th day (PND 75) to tail suspension test (Fig. 1). During these behavioural testing days, animals were maintained in the corresponding home cages, with the environmental conditions previously described. Thus, after each behavioural test mice were returned to the corresponding home cage. The experiments were ranked from less to more stressful conditions, leaving at least a pause of 24 h between performances of each assay. Prior to each test, mice were acclimatized to the experimental room for at least 30 min. All behavioural tests were conducted during the first hours of the light cycle (always between 8.00 h and 14.00 h), as fairly reported (Marashi et al., 2003; Marin et al., 2007; Xu et al., 2009; Peña et al., 2009; Silva et al., 2011).

The *locomotor activity* of animals exposed to different environments was evaluated using locomotor activity boxes (9 × 20 × 11 cm) (Imetronic, Lyon, France) in a low luminosity room (5 lx). For each mouse locomotion was measured as the number of beam breaks on horizontal and vertical movements. Locomotor activity was recorded for 3 consecutive days during 20 min each day.

The *elevated plus-maze* (*EPM*) consisted of a black plastic apparatus with four arms (16 × 5 cm) set in a cross from a neutral central square (5 × 5 cm). Two opposite arms were delimited by vertical walls (closed arms); whereas the two other opposite arms had unprotected edges (open arms). The maze was elevated 30 cm above the ground and illuminated from the top (100 lx). Each mouse was placed in the centre of the maze facing to the open arms for a 5 min period. Percentage of entries in the open arms and percentage of time spent in open arms were measured (Simonin et al., 1998). As a measure of general activity, closed arm entries and total entries were also measured. An

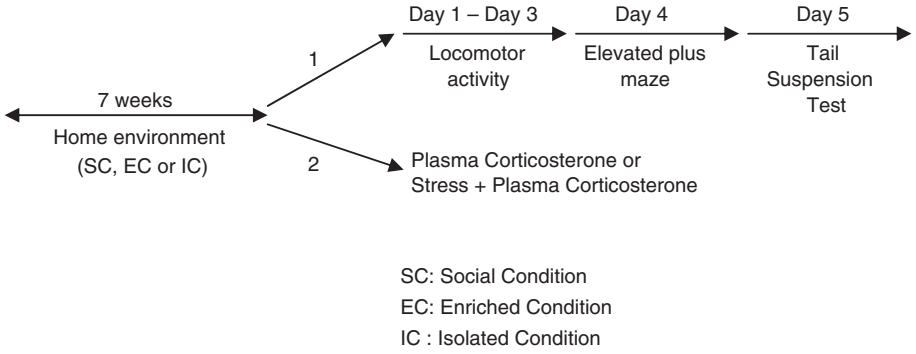

Fig. 1. Experimental procedure (see Materials and methods section).

entry was considered when the animal placed all four paws into the arm. Behaviour was recorded and monitored simultaneously with a video camera interfaced to a computer situated outside the room.

The *tail suspension test* (*TST*) consisted of suspending the animal individually by adhesive tape 1 cm from the tip of the tail 50 cm above a bench top for a 6 min period as described by Steru et al. (1985) and adapted to our experimental conditions (Aso et al., 2008; Ros-Simó et al., 2011). Total time of immobility was recorded for each mouse.

2.3.2. Experiment 2: plasma corticosterone after a stressful situation

In a separate group of mice (n = 20 mice per group), plasma corticosterone was determined (Fig. 1). After housing, half of the animals were decapitated and trunk blood was collected and centrifuged (10 min, 3500 rpm at 4 °C) to determine basal levels of plasma CORT. The rest of the animals were subjected to a stressful situation (a dark–light box test) and immediately after, mice were also decapitated to collect trunk blood and determine levels of plasma CORT after such stressful situation.

The *dark–light box* (DLB) consisted of two plastic chambers connected by a small tunnel. The dark measured chamber (20 × 15 cm) and the adjacent chamber, measuring 30 × 15 cm, were white and illuminated from above with 600 lx. Mice were placed into the dark compartment and percentage of time spent in the lit compartment was recorded for 5 min (Valverde et al., 2009).

Plasma corticosterone was determined by double antibody radioimmunoassays (RIA). The corticosterone RIA (Carrasco et al., 2008) used 125I-carboximethyloxime-tyrosine-methyl ester (ICN-Biolink 2000, Barcelona, Spain), synthetic corticosterone (Sigma) as the standard and an antibody raised in rabbits against corticosterone ± carboximethyloxime-BSA kindly provided by Dr G. Makara (Institute of Experimental Medicine, Budapest, Hungary). Intra-assay and inter-assay coefficients of variation were 6.9% and 9.1%, respectively.

2.4. Statistical analysis

All data are expressed as mean ± SEM. Data was analysed using two-way ANOVA repeated measures (factor 1: condition and factor 2: day, with day as the repeated factor) for results from body weight gain and locomotor activity, and subsequent one-way ANOVA analysis was assessed at each time point between groups. For locomotor activity experiments, one-way ANOVAs (within groups) were calculated to analyse the effects of the days. One-way ANOVA (between subjects) was used for the rest of the behavioural experiments. Two-way ANOVA (between subjects) with environmental conditions and application of stress as factor of variations was calculated. For post-hoc comparisons Tukey contrast test was calculated. Differences were considered significant when $p<0.05$.

3. Results

3.1. Experiment 1: body weight, locomotor and emotional-related behaviour

3.1.1. Body weight

At the beginning of the experiment mice from different groups did not differ in body weight [$F_{(2,56)} = 2.9$, n.s]. During the seven weeks of different home environment, body weight was recorded twice a week (every Monday and Thursday). Two-way ANOVA repeated measures analysis revealed significant differences in the body weight gain depending on the environmental condition [$F_{(2,56)} = 4.3$, $p<0.01$], the day [$F_{(13,728)} = 739.2$, $p<0.001$] and interaction between both factors [$F_{(26,728)} = 4.78$, $p<0.001$]. One-way ANOVA calculated for housing conditions indicated that differences in body weight gain started on day 15, and continued until the end of the experimental procedure (day 49) (Fig. 2a). EC and SC groups showed similar body weight gain along the experimental procedure, reaching an increase of 45.5% and 46.4%, respectively from their initial body weight. In contrast, the IC group exhibited lower weight gain and only reached an increase of 41.5% from its initial weight ($p<0.01$) (Fig. 2b).

3.1.2. Different environmental condition effects on locomotor activity

Horizontal and vertical activities were evaluated during three consecutive days after seven weeks from the beginning of the procedure. Two-way ANOVA with repeated measures revealed significant differences in *horizontal activity* depending on the environmental condition [$F_{(2,49)} = 28.5$, $p<0.001$] and the day [$F_{(2,98)} = 17.7$, $p<0.001$] without interaction between both factors [$F_{(4,98)} = 2.3$, n.s]. One-way ANOVA indicated that significant differences were found between animals housed in EC vs IC ($p<0.001$) and SC vs IC groups ($p<0.001$) every day, and between EC vs SC group ($p<0.01$) on the third day (Fig. 3a). Only mice from enriched housed groups showed differences between days [$F_{(2,47)} = 7.7$, $p<0.001$], indicating a habituation to the locomotor activity boxes that subjects housed in other environmental conditions did not show. Post hoc analysis indicated that these differences were observed between days 1 and 3 ($p<0.001$) (Fig. 3a).

Concerning the explorative activity, measured by the *vertical activity*, two-way ANOVA revealed significant differences in vertical activity depending on the environmental condition [$F_{(2,49)} = 4.02$, $p<0.05$] and the day [$F_{(2,98)} = 30.7$, $p<0.001$] without interaction between both factors [$F_{(4,98)} = 2.03$, n.s]. One-way ANOVA showed that differences were observed on the third day [$F_{(2,51)} = 7.85$, $p<0.01$], specifically between EC vs SC group ($p<0.01$) and EC vs IC group ($p<0.01$) (Fig. 3b). The three groups showed differences within the three days. For enriched housed animals [$F_{(2,47)} = 14{,}3$ $p<0.001$], specifically between days 1 and 2 ($p<0.01$) and 1 and 3 ($p<0.001$). For social housed animals [$F_{(2,53)} = 3.3$, $p<0.05$], on day 1 vs 2 ($p<0.05$). Finally, for isolated ones [$F_{(2,53)} = 5.5$, $p<0.01$], on day 1 vs 3 ($p<0.05$) (Fig. 3b).

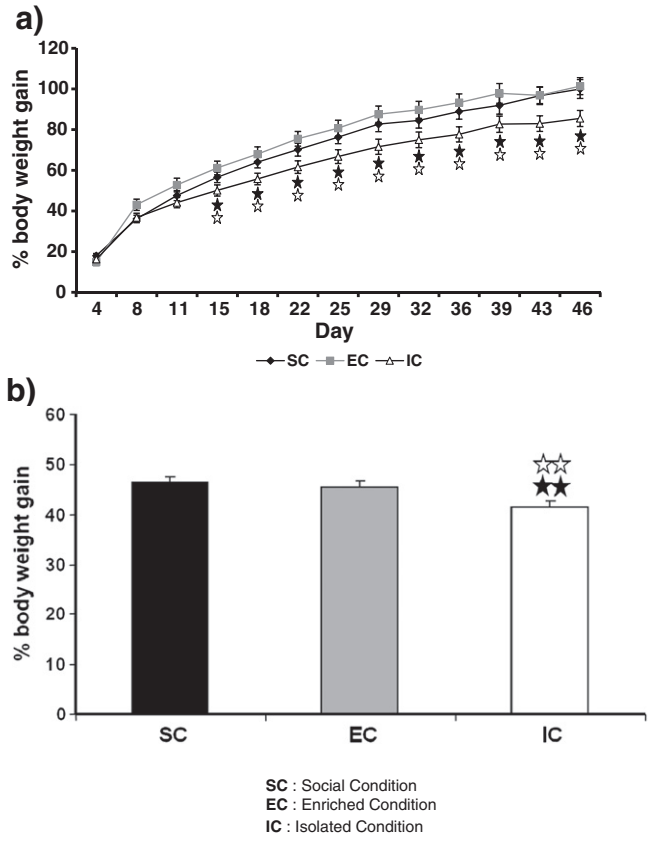

Fig. 2. Percentage of increase in body weight (mean ± SEM) during housing period. a) Progression of the increase or b) total increase at the end of the 7 weeks (n = 19–20 mice per group). ★p<0.05 and ★★p<0.01 vs SC, ☆p<0.05 and ☆☆p<0.01 vs EC.

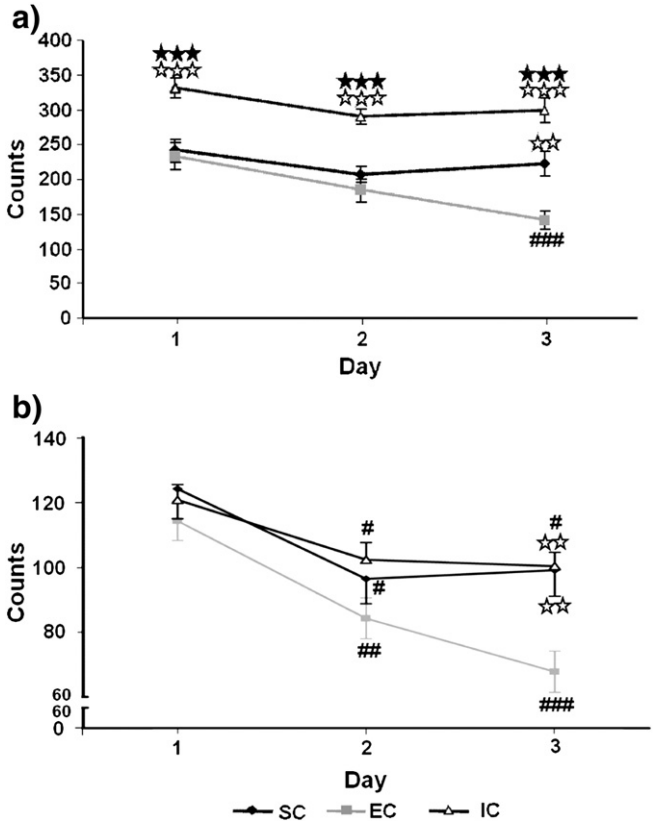

Fig. 3. Horizontal (a) and vertical (b) locomotor activities were recorded during three consecutive days (only the first 20 min is shown) (n = 16–18 mice per group). ★★★p<0.001 vs SC, ☆☆p<0.01 and ☆☆☆p<0.001 vs EC. #p<0.05, ##p<0.01 and ###p<0.001 vs first day for each group.

3.1.3. Isolation induced anxiety-like responses in the EPM

Anxiety-like responses were evaluated using the EPM. One-way ANOVA revealed a significant effect between groups on percentage of entrances in the open arms of the maze [$F_{(2,29)} = 14.9$, $p<0.001$]. Ulterior post-hoc analysis indicated a decrease in the entrances in open arms for the IC group than those registered for SC ($p<0.01$) or EC ($p<0.001$) (Fig. 4a). Moreover, one-way ANOVA revealed a marginally significant effect between groups on percentage of time spent in open arms of the EPM [$F_{(2,29)} = 3.2$, $p = 0.054$]. Post-hoc analysis showed that the difference was observed between IC and SC groups ($p<0.05$) (Fig. 4b), indicating that home environment is critical for the development of anxiety-like responses in adulthood.

As a measure of general activity, closed arm entries and total entries were also evaluated. One-way ANOVA showed marginally significant differences in closed arm entries [$F_{(2,29)} = 3.2$, $p = 0.053$] and no significant effects in total entries [$F_{(2,29)} = 1.2$, p = n.s]. Post-hoc analysis showed the difference in closed arm entries between EC and IC groups ($p<0.05$). These results reveal that the differences in the number of entries and the time spent in open arms are due to anxiety-like behaviour exhibited by mice of the IC group rather than locomotor activity differences.

3.1.4. Environmental housing effects on the TST

Depressive-like behaviour was tested using the TST. One-way ANOVA revealed significant differences in the tail suspension test [$F_{(2,17)} = 5.6$, $p<0.05$]. Post-hoc analysis showed that the IC group spent less time immobile than the other two groups ($p<0.05$) (Fig. 5), suggesting a higher degree of hyperactivity in isolated animals, according with results described above.

3.2. Experiment 2: plasma corticosterone after a stressful situation

After the seven weeks of different housing conditions (PND 71), half of the animals of each group were used to determine basal levels of plasma CORT and the rest of the animals were subjected to a stressful event, which was the exposure to a DLB (an anxiety-like environment). To evaluate functionality of the HPA axis after stress exposure, immediately after the DLB levels of plasma CORT were determined.

One-way ANOVA revealed significant differences between groups in the time spent in the white box [$F_{(2,26)} = 10.35$, $p<0.001$]. Post-hoc analysis revealed that IC spent less time in the white box than the EC group ($p<0.001$) (Fig. 6), consistent with results obtained in the EPM (Fig. 4a).

Two-way ANOVA for plasma CORT indicated significant effect of time (basal or after) [$F_{(2,56)} = 11.3$, $p<0.001$] and marginally significant effect of the house conditions [$F_{(2,53)} = 2.8$, $p = 0.066$] without interaction between both factors [$F_{(2,53)} = 2.3$, n.s]. Post-hoc analysis showed that differences were observed between SC and IC groups under basal conditions ($p<0.05$). In order to dismiss the cohort removal effect, we compare basal levels of SC animal #1 and animal #10 and no differences were found between them. Indeed, a significant increase in plasma levels of CORT after the DLB was observed in the IC group ($p<0.001$) compared to its basal levels (Fig. 7); which was observed in neither SC nor EC animals. The results obtained indicate that IC animals show a higher degree of anxiety-like responses than the other groups and only plasma CORT levels of this condition (isolation) were significantly increased after the DLB, suggesting an abnormal functionality of the HPA axis when animals are exposed to stressful situations.

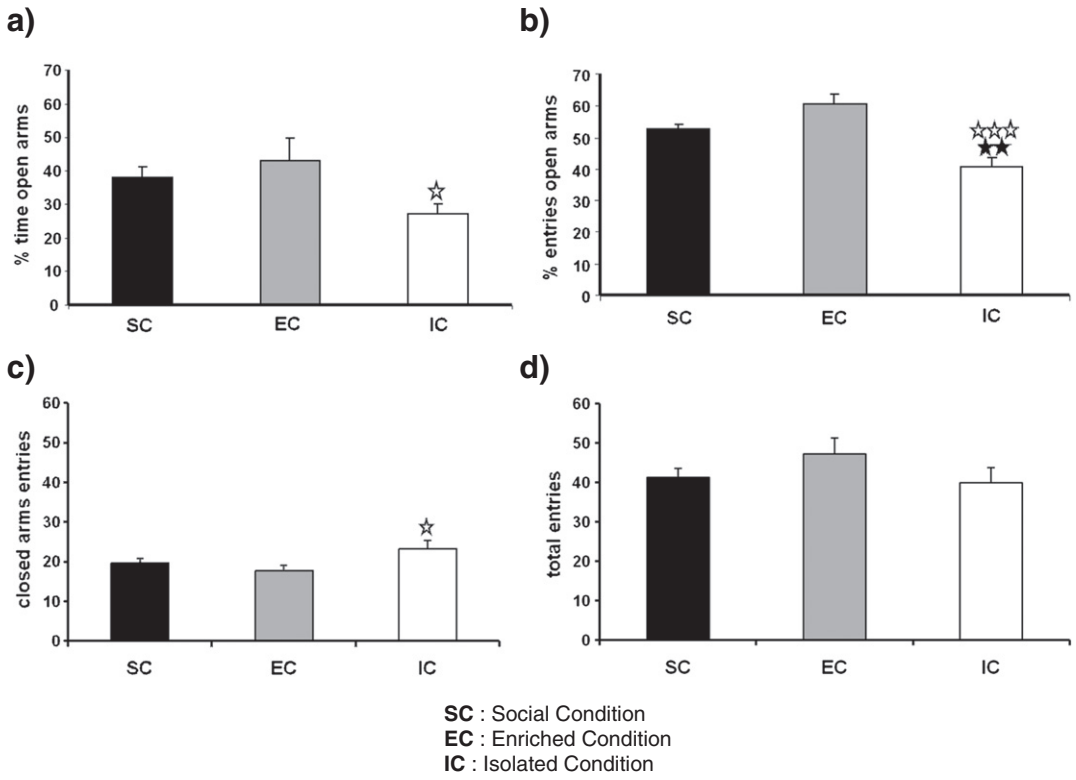

Fig. 4. Anxiety-like effects observed in isolated animals in the elevated plus maze. a) Percentage of time in open arms, b) percentage of entries in open arms, c) closed arm entries, and d) total entries (n = 10 mice per group). ★★p<0.01 vs SC and ☆p<0.05 and ☆☆☆p<0.001 vs EC.

4. Discussion

The present study attempts i) to design an improved experimental model of different home environmental conditions from pre-adolescence to adulthood periods in CD1 mice, and ii) to elucidate the behavioural and neuroendocrine responses induced by the exposure of CD1 mice to different housing environmental conditions. As stated above, previous studies investigating the importance of environmental conditions during adolescence have frequently considered only two different conditions, for instance, rats exposed to enrichment vs standard conditions (Konkle et al., 2010; Moncek et al., 2004; Zhu et al., 2009) or isolation vs standard conditions (Bartolomucci et al., 2003; Karim and Arslan, 2000). In some other studies, including the present one, three environmental possibilities (SC, EC and IC) have been considered, which are methodologically more appropriate. However, most of the previous studies were developed in rats (Varty et al., 2000; Deehan et al., 2007) or in a different mouse strain (Võikar et al., 2005; Workman et al., 2011; Silva et al., 2011). As stated above, differences between strains have been reported and consequently, our study will increase the knowledge about effects of environmental enrichment/isolation in CD1 mice. We are particularly interested in using this type of mice is to standardize the neurobiological substrate involved in behavioural and neuroendocrine responses produced by different environmental conditions, by the use of mutant mice. Taken together, this procedure has allowed us to demonstrate that social isolation during pre-adolescence and adolescence affects behavioural coping to novel situation of stress in adulthood, a fact that correlates with changes in HPA axis functionality. Pre-adolescent and adolescent periods are critical ages where social and environmental conditions can strongly influence

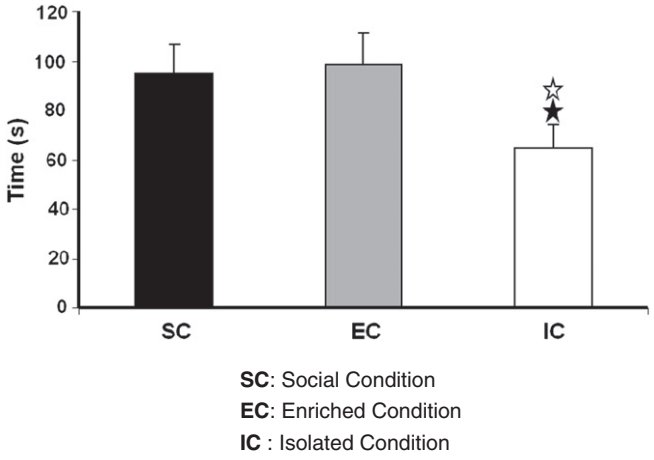

Fig. 5. Less immobility in the tail suspension test was observed in isolated animals (n = 6–9 mice per group). ★p<0.05 vs SC and ☆p<0.05 vs EC.

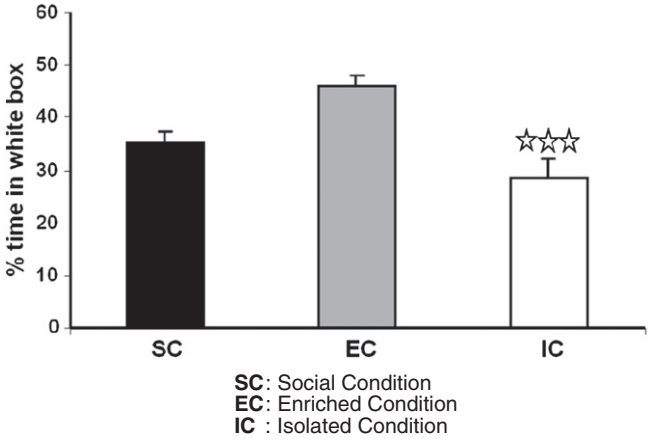

Fig. 6. Isolated animals showed a higher degree of anxiety-like responses in the dark–light box test (n = 9 mice per group). ☆☆☆p<0.001 vs enriched group.

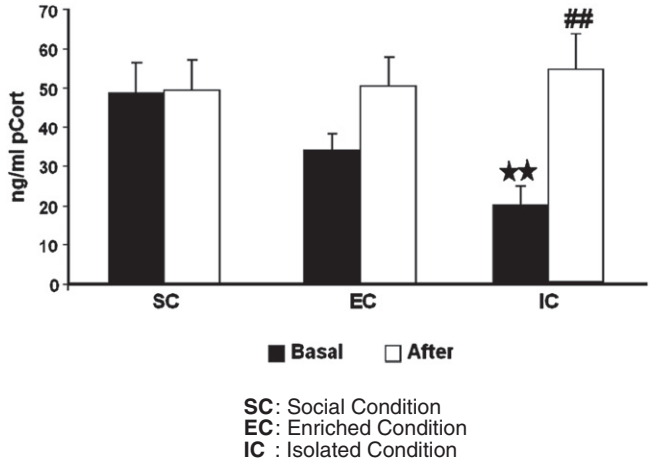

Fig. 7. Basal plasma corticosterone levels were lower in isolated animals (★★$p<0.01$ vs SC) but immediately after a stressful situation the increase was higher in isolated animals (##$p<0.01$ vs basal levels) ($n=9$ mice per group).

adult phenotype (Brenes et al., 2009; Workman et al., 2011). The fact of few works using pre-adolescent CD1 mice and the importance of studying the effects of environmental housing during this period of mice post-natal development, led us to develop this study.

Especially, our results reveal that subjects exposed to isolated conditions reached a lower body weight gain after 7 weeks of exposure to specific environmental conditions, indicating a retardation of growth in this group of animals. This fact may be due to reduced food intake or even to difference in the energy spent to maintain body temperature (Peña et al., 2009). Isolated animals are alone in the cages whilst five and ten mice are housed simultaneously in SC and EC, respectively. This could lead to a lower energy spent to maintain their body temperature than the IC group. In fact, whilst reduced body weight in EC rats is fairly reported (Moncek et al., 2004; Peña et al., 2006, 2009), results using mice are more controversial (Haemisch and Gärtner, 1994; Roy et al., 2001; Tsai et al., 2002). In all experiments the mouse strain used was different so, there is the possibility of differences in metabolism according to the strain evaluated.

In our experimental conditions, different housing conditions also induced significant differences in locomotor activity. Thus, isolation provoked an enhancement of locomotor activity, as observed in the horizontal activity data. This result could be explained by an increase of excitability and nervousness when IC mice are exposed to a novel environment such as the locomotor activity boxes. Results obtained in the anxiety-like tests (EPM and DLB) clearly demonstrate that this group of mice has abnormal response to a novel/stressful situation. Indeed, isolated mice did not show a habituation to the locomotor activity boxes in the three consecutive days evaluated. There are controversial results regarding the effect of home environment on locomotion, however, our results agree well with previous studies showing the development of hyperactivity in rats exposed to 6–18 weeks of isolation (Rilke et al., 1998) or 8 weeks (Varty et al., 2000). In addition, our findings showed that animals maintained under EC exhibited a better habituation to a novel environment. Since the third day of locomotor evaluation this group showed lower activity in the locomotor activity boxes. These results are in accordance with those obtained by Varty and colleagues (2000), who proposed that the lower locomotor activity observed in rats housed in EC for eight weeks was due to the better capability to assimilate stimuli from their environment than other rats reared in isolation (Varty et al., 2000). This data is consistent with our hypothesis of impairment to cope with a novel situation. However, we cannot ignore the possibility that hyperactivity observed would be due to impairment in learning abilities that leads to a decrease in habituation. Further experiments will be needed to explore this possibility. Our data, taken together, agree well with these previous studies since we demonstrate that isolation leads to impairment in behavioural coping as shown by the increased response to stress in the EPM and DLB as well as increased mobility in the TST and elevated plasma CORT levels in mice maintained under IC when exposed to an anxiety-like environment. As stated above, a clear anxiety-like response was observed in the elevated plus maze in animals individually housed compared to social and enriched conditioned ones, in agreement with previous studies conducted in rats and mice (Hellemans et al., 2004; Wei et al., 2007). In the anxiety experiment, using the dark–light box test, animals housed in IC spent less time in the light box than enriched ones. Thus, in both anxiety experiments carried out, animals housed in IC exhibited an enhanced anxiety-like response observed as less time in open arms and in lit box, respectively. Additionally, data obtained using a model of despair behaviour, revealed that isolated mice spent less time immobile. The interpretation of these data could suggest a higher degree of hyperactivity behaviour of isolated animals in front of novel stimuli rather than smaller despair behaviour; as previously reported in rats (Karim and Arslan, 2000) and in accordance with results found in locomotor activity. Other authors have reported no differences in the TST in Swiss mice (Silva et al., 2011) or reduced immobility in C57BL/6J under EC (Xu et al., 2009). According to Silva et al. (2011), these differences reported are probably due to variations related to the strain of mice used. All together, our behavioural data indicate that behaviour adaptations are impaired in animals exposed to isolation and this impairment could be attributed to an abnormal functionality of the HPA axis (Herman and Cullinan, 1997; Ma and Morilak, 2005). Activation of HPA axis leads to an increase in the release of different neuroendocrine hormones, such as CORT (De Kloet et al., 1990; Armario et al., 2004; Marin et al., 2007), which can be easily detected in plasma mediating a RIA assay (Carrasco et al., 2008). In order to get more fully acquainted with this hypothesis, plasma CORT was evaluated at basal conditions (immediately after the seven weeks of different housing conditions), and after a stressful event which was 5 min exposure to the dark–light box test. Our results showed lower levels of basal plasma CORT during the light period in isolated animals in comparison to enriched and social housed groups when analysed before exposure to stress. Although there are no consistent findings regarding HPA axis functioning after different housing, our results agree well with those found by Miachon et al. (1993) who reported decreased CORT in rats after 13 weeks of isolated housing (Miachon et al., 1993). In addition, Sánchez et al. (1995) found decreased levels of plasma CORT in rats isolated from all social contacts (Sánchez et al., 1995), suggesting a reduced HPA axis activity following prolonged social isolation. Plasma CORT evaluated immediately after the dark–light box test showed a significant increase compared to basal levels only in isolated animals, suggesting a major response of the HPA axis to a stressful situation in this group of mice. These results are in agreement with our behavioural data, and support our hypothesis regarding an abnormal functionality of the HPA axis and an enhanced stress response. No differences were found between animals exposed to EC or SC, respectively. These results suggest a low activation of the HPA axis in individually housed at basal conditions but an increased reaction when faced to stressful events. Similar results were found by Bartolomucci et al. (2003), who proposed that individual housing seems, therefore, not to be stressful per se, but induces an increased reaction to subsequent stressful events indicating that social isolation impairs habituation to novel stimuli (Bartolomucci et al., 2003).

Overall, it seems surprising that no differences were found in the EC group when compared to SC animals. As previously reported, it is well established that environmental enrichment has a strain specific effect on the behaviour of mice (van de Weerd et al., 1994; Marashi et al., 2003; Silva et al., 2011). There is the possibility that CD1 mice kept under enriched environment do not have any effect on its behaviour and, in contrast, social isolation has significant behavioural and neuroendocrine alterations on them.

In summary, this improved procedure of different home environments in CD1 mice has enabled us to show that different housing from pre-adolescence to adulthood affects physiological, behavioural and biochemical parameters in this mouse strain. Differences from normal behaviour were greater in individually housed mice rather than enriched compared to social ones. Even though there are many controversial results in relation to social environment, it seems clear that individual housing leads to altered behaviour in adulthood, and this could be due to abnormal HPA axis function. Habituation to novel stimuli is impaired in subjects in social isolation as well as increased excitability response to stressful events. In conclusion, social interaction is an important feature to achieve an appropriate neuronal development whilst social deprivation increases the possibility of altered neuronal function and possible neuropsychiatric affectations in adulthood.

Acknowledgements

The authors wish to thank Dr. J. Ruiz-Medina for critical reading of the manuscript. This study was granted by the Spanish Ministry of Science and Innovation (SAF2010-15793), the Spanish Ministry of Health (PNSD 2010 and RTA 001/06/1001-FEDER), and the Generalitat de Catalunya (2009SGR684). CR-S was funded by a FPI fellowship (BES-2008-007915).

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