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Physical activity and environmental enrichment: Behavioural effects of exposure to different housing conditions in mice

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Enriched environments and exercise provide complex environmental stimulation that can induce emotional and cognitive changes; however, few studies have evaluated the effects of these two components on other behaviours, such as novelty seeking or pain sensitivity. The aim of the present study was to investigate the influence of voluntary physical activity provided through different housing conditions on anxiety, locomotor activity, pain sensitivity, and exploration. Male mice at postnatal day (PND) 21 and were randomly assigned to one of four different conditions on PND 28: Marlau cages (MC), a standardized cage designed to provide a complex environment; physical exercise in large groups (PE-8); physical exercise in small groups (PE-4); or a standard environment (SE). After seven weeks, animals were evaluated in the hole-board task, the elevated zero maze, actimeter, and hot plate test. In the hole-board task, MC animals displayed more exploration than animals in the PE-8 and PE-4 groups, but no significant differences were observed between groups in the actimeter. In the elevated zero maze, MC and PE-8 animals exhibited an anxiogenic-like profile as compared to the SE group. When pain sensitivity was evaluated, the PE-8 group displayed a higher sensitivity to noxious thermal stimuli than the SE group. These data suggest that the complexity of the environment in which physical activity and environmental stimulation are provided can influence animal behaviours such as novelty seeking, emotional response, and pain sensitivity. These animal models could be useful for designing more personalized interventions that include physical, social, and cognitive stimulation to promote a more active lifestyle in humans. Such interventions could be useful in the prevention and treatment of aging-related decline or neurodegenerative diseases.

Key words: environmental enrichment, physical activity, mice, anxiety, exploratory behaviour, motor activity, Marlau cages

INTRODUCTION

Basic research shows that preclinical models may be useful for identifying the factors that can promote more successful cognitive ageing (Daffner, 2010) and for identifying the main mechanisms that can explain the reported beneficial effects of complex environments (Redolat and Mesa-Gresa, 2012; Fischer, 2016). Animal models of environmental enrichment (EE) seem to be an adequate experimental approach to recreate an active lifestyle in humans (Nithianantharajah and Hannan, 2006; Rogers et al., 2019; Aujnarain et al., 2018) and could be applied to evaluate experience-related changes induced by modifications in the quality and quantity of environmental stimulation (Sampedro-Piquero and Begega, 2017; Leon and Woo, 2018; Sale et al., 2018).

In an enriched environment, rodents can be stimulated at physical, social, cognitive, and somatosensory levels, and problem-solving opportunities are offered (Nithianantharajah and Hannan, 2009; Rogers et al., 2019; Gelfo et al., 2018). According to Pang and Hannan (2013), novelty and complexity are likely the main factors underlying the beneficial effects of enriched environments. One of the main limitations of studies employing enriched environments is the lack of consensus regarding a standardized EE model. To

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date, little research has been published concerning the standardization of enriched environments for rodents with the purpose of increasing the reproducibility of results across laboratories (Sztainberg and Chen, 2010; Mesa-Gresa et al., 2014). Marlau™ cages (MCs) were designed to standardize the stimulation of cognitive functions by exposing rodents to problem-solving opportunities during enrichment procedures that involve mazes and running wheels (Fares et al., 2012; Kentropp et al., 2018). In a prior study, it was reported that male Sprague-Dawley rats allocated to MCs displayed decreased anxiety-like behaviour in the elevated plus maze (EPM), enhanced performance in the Morris water maze, and stronger emotional memories (Fares et al., 2013). However, a prior experiment with male National Medical Research Institute (NMRI) mice did not confirm the beneficial effects of exposure to MCs on anxiety-like response and habituation to novel environments (Mesa-Gresa et al., 2014). In that study, the authors observed that mice reared in these environments showed an increase in exploratory behaviour in the hole board (HB) test, as well as, an increase in motor activity in the EPM. More recently, Kentrop et al. (2018) confirmed a more rapid habituation to novel situations in both adolescent and adult rats reared in complex environments via MCs.

At the behavioural level, although some contradictory results can be found (Azar et al., 2012), experimental evidence suggests enhanced performance in learning and memory tasks and reduced depressive- and anxiety-like behaviour after exposure to enriched environments in animal models (Mesa-Gresa et al., 2013a; Rogers et al., 2019; Aujnarain et al., 2018). Previous experiments with mice and rats suggest that animals usually show an improvement in the ability to learn both spatial and non-spatial tasks (Leggio et al., 2005; Diniz et al., 2010; Viola et al., 2010), faster adaptation to novel environments (Zimmermann et al., 2001; Hughes and Collins, 2010), and diminished exploratory behaviour (Mesa-Gresa et al., 2013b) following exposure to an enriched environment. Furthermore, exposure to enriched environments may also have beneficial effects on behavioural recuperation after stroke in young male Sprague-Dawley rats (Buchhold et al., 2007). In male Wistar rats, these complex environments have also been shown to attenuate the impairment in cognitive flexibility induced by basal forebrain lesions (De Bartolo et al., 2008), and improve spatial learning in rats with subicular lesion-induced neurodegeneration (Dhanushkodi et al., 2007). Different enriched environments can also have differential effects on pain sensitivity in rats; in particular, more complex enriched environments are associated with a more effective ability to diminish pain sensitivity (Kimura et al., 2019). However, no analogous studies comparing different types of housing environments (e.g. with or without running wheels and with different enrichment items) have been published in mice. Prior research suggests that EE improves neuropathic pain (Almeida et al., 2015; Parent-Vachon and Vachon, 2018). However, the mechanisms involved in the effects of EE on pain sensitivity and related behavioural and emotional changes are not well known. Lima et al. (2017) reported that five days of voluntary exercise on the running wheel induced analgesia in mice. This effect appears to be mediated by mu-opioid receptors and serotoninergic mechanisms. Wang et al. (2019a) observed that EE may improve the pain threshold reduction and the long-term memory impairment induced in nerve-injured mice. In another report, the same authors indicated that hippocampal NPSAS4 (neuronal PAS domain protein 4) may play a role in the positive effects of EE on pain sensitivity, depressive symptoms, and cognitive performance (Wang et al., 2019b). It has been suggested that motor behaviours should be taken into account when evaluating the influence of EE on pain modulation (Parent-Vachon and Vachon, 2018). For that reason, the current study aimed to evaluate the effects of EE on both pain sensitivity in the hot plate and emotional measures obtained in the elevated zero maze.

One of the most common basic components of EE models is physical activity. In animal models, the effects of physical exercise can be examined in isolation through environmental interventions that provide only running wheels (Pang and Hannan, 2013). Two different experimental paradigms have predominantly been employed to investigate the effects of physical activity on rodents' brain and behaviour; these paradigms involve exposing animals to daily or weekly sessions of forced running on a treadmill or allowing free access to a running wheel (Pietropaolo et al., 2008). However, there is still little consensus regarding the extent of the influence of EE compared to physical activity. While some studies performed in mice point to physical exercise per se as the main neurogenic and neurotrophic stimulus in EE (Kobilo et al., 2011; Clemenson et al., 2015; Shevtsova et al., 2017), others (Fabel et al., 2009) suggest that enriched and complex environments are more effective in increasing adult hippocampal neurogenesis. Some studies indicate that exercise can improve attention, enhance learning and memory, and reduce emotional response in a similar way to changes induced by EE exposure (Rogers et al., 2019). Exercise interventions may also reduce stress and anxiety, depression, and addiction patterns in animals, as well as, protect against the development of neurodegenerative disorders (Falls et al., 2010; Basso and Morrell, 2017; Shevtsova et al., 2017). Mason and Würbel (2016) recently established a basis for evaluating the effects of the running wheel, to distinguish adaptive from pathological effects of voluntary physical activity. This model could be extrapolated to human exercise by using enriched and non-stressful environments.

Taking the aforementioned literature into account, the main aim of the present research was to evaluate the influence of exposure to different housing environments characterized by a varying degree of environmental complexity and/or physical activity on the behavioural performance of NMRI male mice at an early age. Specifically, the current study aimed to analyze the effects induced by physical activity – which is considered to be a key component of enriched environments – on anxiety-like response, exploration, locomotor activity, and pain sensitivity in mice. The novelty of this investigation lies in the observation that little research has been carried out to assess the impact of physical activity on behaviour performed in environments that differ in the complexity of the enrichment provided. Moreover, no previous research has compared the effects of MCs with those associated with voluntary physical activity.

METHODS

Subjects

Sixty-four male NMRI mice (Charles River, Barcelona, Spain) arrived at our laboratory on postnatal day (PND) 21, and weighed between 12 g and 14 g. After a one-week adaptation period (temperature 20 – 24°C, humidity 55 ± 10%, 12-h light-dark cycle, lights on at 8: 00 h, and water and food access *ad libitum*), animals were randomly assigned to one of four different housing conditions on PND 28. Until their arrival to our laboratory at PND 21, the mice had been maintained in cages with Enviro-dri® (Serlab) (recycled paper composed of 50% virgin fibre and 50% Kraft paper). No other nesting or enrichment items were added until the EE protocol began in our laboratory. When the experimental procedure started, mice were maintained in the different housing conditions for a period of seven weeks, followed by behavioural testing. Adequate measures were taken to minimize pain or discomfort of animals. All procedures were approved by the local ethical committee (the Committee of Ethics in Experimental Research of University of Valencia) and complied with national (Real Decreto 1201/2005; Decreto 13/2007) and international guidelines (European Community's Council Directive of November 24, 1986–86/609/EEC; 2007/526/CE; 2010/63/EU) for the care and treatment of animals, as well as, with the "Guidelines for the use of animals in research" (Animal Behaviour, 1991; 41: 183-186).

Housing conditions

Animals arrived at the laboratory at PND 21. After a one-week adaptation period, animals were randomly assigned to one of four different housing conditions on PND 28: Marlau™ cages (MC): n=16, in which mice were housed in groups of eight; physical activity with social interaction in large groups (8 mice per cage) (PE-8): n=16, in which mice were housed in groups of eight in larger cages containing running wheels; physical exercise with social interaction in small groups (4 mice per cage) (PE-4): n=16, in which mice were housed in groups of four in a spontaneous activity wheel cage; and a standard environment (SE): n= 16, in which mice were housed in groups of four in standard cages (i.e., 42 cm × 26 cm × 19 cm). All cages contained sawdust (Enviro-dri®) and free access to food (Tekald Global Rodent Diet, supplied by Harlan) and water. Sawdust was changed and the cages were cleaned once a week for all of the housing conditions.

Marlau™ cages (MCs)

MCs were employed to provide animals with a complex environment in which the physical, social, and cognitive components of an enriched environment were present. This type of cage, which was designed with the aim of standardizing cognitive stimulation through the use of mazes, has been proposed as a new model of enriched environment for rodents (Fares et al., 2012; 2013; Kentrop et al., 2018). The cage (length: 580 mm × width: 400 mm × height: 320 mm; weight: 13 kg) consists of two floors: a ground floor, comprising the compartments G1 and G2, and an upper floor containing a maze with 12 possible configurations. G1 contains only food pellets and a one-way access to G2, which contains three water bottles, three running wheels (wheel diameter: 12 cm), and a ladder to the second floor. For a more detailed description, see Fares et al. (2012) and Mesa-Gresa et al. (2014). The model offers the following aspects: complexity, as water and food pellets are placed in two separated compartments connected only through the maze; activity, encouraged by the large exploration area and free access to running wheels; novelty, as the maze configuration is changed three times per week; and social interaction, promoted by the housing of a large number of animals in the same cage.

Physical exercise in large groups (PE-8)

Under this rearing condition, animals were provided with both physical and social components of an enriched environment. Two large cages (55 cm × 36 cm × 19 cm) were individually equipped with two activity wheels for voluntary exercise, sawdust, and *ad libitum* access to food and water. To allow for greater social interaction opportunities, eight mice were housed in each cage.

Physical exercise in small groups (PE-4)

Four different cages (37 cm × 26 cm × 36 cm) were individually equipped with a stainless-steel running wheel (25 cm diameter) (Ugo Basile, Italy) that occupied almost half of the cage to provide an enriched environment that is based purely on physical activity. Four mice were assigned to each cage and were allowed free access to the wheel. Animals in the PE-4 condition were allocated in groups of four animals per cage to avoid isolation housing. It has been suggested that isolation housing should be considered an impoverished and stressful environment that has been associated with a pathological running wheel response (Mason and Würbel, 2016). In all housing conditions employed in the current study, running activity was voluntary. Food and water were also provided *ad libitum*. The electronic system embedded in the wheels allowed for the quantification of rodents' spontaneous activity in their home cage environment (i.e., number of wheel turns performed). Recording took place throughout the entire 7-week period that animals spent in the cage, to analyse the differences in activity and exercising patterns induced by the environmental manipulation.

Experimental procedure

Animals arrived at the laboratory on PND 21 and on PND 28, were randomly assigned to one of four housing conditions from PND 28 to PND 77. After exposure to the experimental conditions, a battery of behavioural tests was performed in the following order: hole board (HB, PND 77), elevated zero maze (EZM, PND 77), actimeter (PND 78), and the hot plate test (PND 81). Animals were taken to the experimental room one hour before behavioural testing began. All tests were carried out under light conditions and all cages were cleaned before and between animals testing with a water and ethanol solution (2%).

Behavioural testing

Hole board (HB)

The hole board (HB) test was employed to evaluate novelty seeking and exploratory activity through head-dipping behaviour displayed by the animals (Boissier et al., 1964). A lower number of head-dips (HD) (i.e., the animal introduces its snout or whiskers in the hole in order to explore it) is thought to reflect lower levels of novelty seeking and exploratory behaviour (Zhu et al., 2009; Mesa-Gresa et al., 2014). The session consisted of a unique trial that each mouse underwent individually. At the beginning of the test, the mouse was placed in the centre of the board and allowed to freely explore the cage during a five-minute period. An experimenter was present during the testing but remained out of the scope of the animals to avoid interference. The following measures were recorded: latency to the first HD ('LATENCY 1 HD'); the total number of HDs during the first minute ('HD 1 MIN'); and total number of HDs during a five-minute period ('HD 5 MIN'). The apparatus employed (Cibertec, Barcelona, Spain) was an acrylic black board (31.5 cm × 31.5 cm × 20.5 cm) with 16 holes (hole diameter: 2 cm; distance between holes: 5 cm). The hole sensors were situated at a depth of 1 cm and automatically recorded the number of HDs performed by animals.

Elevated zero maze (EZM)

The EZM (Ugo Basile, Italy) was employed to evaluate the animals' anxiety-like responses. The EZM is a modification of the EPM model of anxiety for rodents. The EZM incorporates both traditional and novel ethological measures to analyse pharmacological or environmental effects (Shepherd et al., 1994). The EZM used for this study was specific for mice. It consists of a 55 cm-diameter circular (ring shaped) runway made of metal alloy and raised 60 cm from the floor. The corridor width is 5 cm and is divided into two open quadrants (open sections) facing two enclosed quadrants (close sections) with a 15 cm-high wall. The fact that the EZM lacks a central space like the one found in the EPM allows for easier assessment and interpretation of the exploration performed by the animals. Mice underwent the test individually. The test began by placing the animal in an open section, facing a closed one, and allowing it to freely explore the maze for five minutes. The following measurements were taken: percentage of time (%TO) and total time (TO) spent in the open sections; percentage of time (%TC) and total time (TC) spent in the enclosed sections; percentage of entries (%FO) and frequency of entries into the open sections (FO); percentage of entries (%FC) and frequency of entries into the closed sections (FC); total entries (TE); and latency of the first entry into the open sections (LO) and into the closed sections (LC). Animals were scored as being in the open or closed section when all four paws were placed inside the corresponding quadrant. A researcher remained in the room during the testing and sessions were recorded with a video camera for further analysis. Video recordings of the EZM were analysed by a blind researcher using "Raton-Time" software, which allows for the analysis of the behavioural parameters (Mesa-Gresa et al., 2013a; 2013b).

Actimeter

An actimeter was employed to assess the locomotor activity displayed by the animals in a novel cage over a period of 30 min (divided into six 5-min periods). The measure obtained was the number of counts. Mice underwent the test individually. The apparatus employed (Actimeter, Cibertec, Madrid, Spain) consists of an individual novel cage (30 cm × 14 cm × 12 cm) attached to a continuous recording system that detects the horizontal movements of the animals using an infrared photocell system.

Hot plate test

The hot plate test (UGO Basile, Italy) was used to evaluate pain sensitivity by providing mice with a noxious thermal stimulus. To do this, mice were individually placed on a plate surrounded by a plexiglass cylinder. The hot plate was gradually heated to a maximum of 52°C, with a cutoff of 120 s to prevent injury to the animals. Latency of the first reaction was measured as the time span (s) between placing the animal in the hot plate and the moment at which it either jumped or licked its hind paws.

Statistical analysis

Statistical analysis was carried out with IBM® SPSS® Statistics 24.0 (IBM Corp, NY, USA) for Windows. For the behavioural data obtained in the HB, EZM, actimeter, and hot plate tests, a one-way analysis of variance (ANOVA) with "Housing condition" as the between-subjects factor was performed, followed by a Tukey HSD. *Post hoc* tests were carried out when appropriate. Significance levels were set at $p<0.05$ and parametric assumptions for ANOVA were considered. All data are presented as mean ± standard error of the mean (SEM).

RESULTS

The hole board test

ANOVA revealed a significant effect of Housing conditions on the following variables: latency to the first head-dip (LATENCY 1 HD) [$F_{3,60}=2.96$, $p<0.05$], HDs in one minute (HD 1 MIN) [$F_{3,60}=5.65$, $p<0.01$], and HDs in five minutes (HD 5 MIN) [$F_{3,60}=4.91$, $p<0.01$] (see Table I). *Post hoc* Tuckey test showed that animals housed in the PE-4 group displayed lower LATENCY 1 HD than mice housed in SE cages ($p<0.05$) (see Fig. 1A). Regarding the variable HD 1 MIN, MC-housed animals performed more HDs than PE-8 ($p<0.05$) and SE animals ($p<0.05$). Mice allocated to the MC group also performed more HDs in 5MIN than mice in the PE-8 ($p<0.01$) and PE-4 groups ($p<0.05$) (see Fig. 1B).

Elevated zero maze

One-way ANOVA showed statistically significant differences among housing conditions with respect to

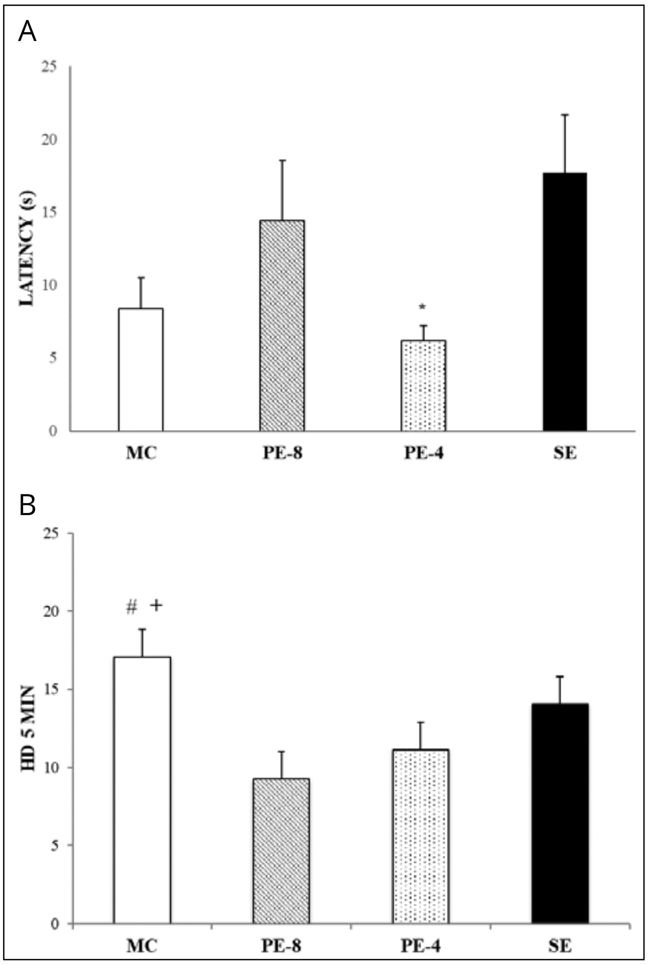

Fig. 1. (A) Latency to the first reaction ('LATENCY (in sec)) and (B) total number of head-dips in 5 minutes (HD 5 MIN) displayed in the hole-board test across the four experimental groups: Marlau cages (MC), physical activity in large groups (PE-8), physical activity in small groups (PE-4), and the standard environment (SE). Data are presented as mean ± SEM. (*) $p<0.05$, PE-4 *vs.* SE. (#) $p<0.01$, MC *vs.* PE-8. (+) $p<0.05$, MC *vs.* PE-4.

Table I. Effects of housing in the four experimental groups: Marlau cages (MC), physical activity in large groups (PE-8), physical activity in small groups (PE-4) and standard environment (SE) on the behavioral parameters displayed in the hole board test by male NMRI mice.

Behavioural categories	MC	PE-8	PE-4	SE
Latency 1 HD*	8.37 ± 2.13	14.38 ± 4.13	6.15 ± 1.06	17.68 ± 3.94
HD in 1MIN**	7.44 ± 0.83	3.88 ± 0.68	5.12 ± 0.70	3.81 ± 0.63
HD in 5 MIN##, #	17.06 ± 1.77	9.25 ± 0.77	11.12 ± 1.67	14.06 ± 1.74

Data are presented as mean values ± SEM; *Abbreviations:* (HD) Head-Dips; (*) p<0.05 PE-4 *vs.* SE; (**) p<0.01 MC *vs.* PE-8, MC *vs.* SE; (##) p<0.01 MC *vs.* PE-8; (#) p<0.05 MC *vs.* PE-4.

the following variables: frequency of entries into the FO [$F_{3,60}$=2.907, $p<0.05$] (see Fig. 2A), frequency of entries into the FC [$F_{3,60}$=2.923, $p<0.05$] (see Fig. 2B), TE [$F_{3,60}$=2.918, $p<0.05$], percentage of time spent in the open sections (%TO) [$F_{3,60}$=5.291, $p<0.01$] (see Fig. 2C), percentage of TC [$F_{3,60}$=5.341, $p<0.01$] (see Fig. 2D), and latency to enter into the LO [$F_{3,60}$=3.034, $p<0.05$].

Post hoc Tukey's HSD test revealed that the PE-8 group showed less FO and TE than the SE group ($p<0.05$) and more FC than the SE group ($p<0.05$). PE-4 and SE animals displayed higher %TO compared to MC animals ($p<0.05$), and SE animals showed higher %TO than PE-8 animals ($p<0.05$). In contrast, the MC mice displayed higher %TC compared to PE-4 and SE mice ($p<0.05$), and PE-8 animals showed higher %TC than SE group ($p<0.05$). Regarding latency to enter into closed areas, the MC group showed a shorter latency than SE mice ($p<0.05$) (see Table II).

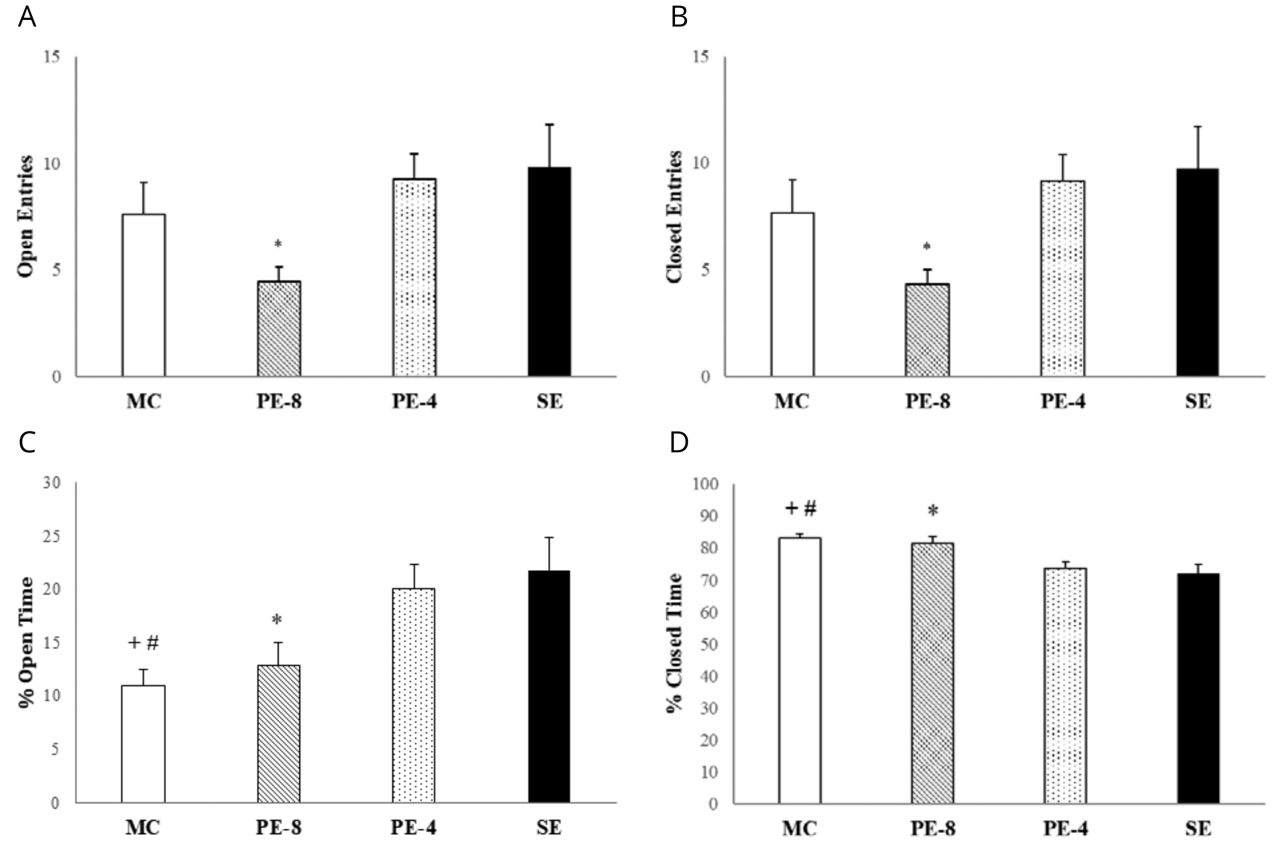

Fig. 2. (A) Frequency of entries into open sections (FO); (B) Frequency of entries into closed sections (FC); (C) Percentage of time spent into open sections (%TO); and, (D) Percentage of time spent into closed sections (%TC) displayed in the elevated zero maze test across the four experimental groups: Marlau cages (MC), physical activity in large groups (PE-8), physical activity in small groups (PE-4), and the standard environment (SE). Data are presented as mean ± SEM. (*) $p<0.05$, PE-8 *vs.* SE. (+) $p<0.05$, MC *vs.* PE-4. (#) $p<0.01$, MC *vs.* SE.

Table II. Effects of housing in the four experimental groups: Marlau cages (MC), physical activity in large groups (PE-8), physical activity in small groups (PE-4) and standard environment (SE) on the behavioral parameters displayed in the elevated zero maze by male NMRI mice.

Behavioural categories	MC	PE-8	PE-4	SE
Total entries*	15.31 ± 3.01	8.75 ± 1.40	18.43 ± 2.37	19.56 ± 3.96
Open entries*	7.62 ± 1.49	4.44 ± 0.70	9.25 ± 1.18	9.81 ± 1.98
Closed entries*	7.69 ± 1.52	4.31 ± 0.71	9.18 ± 1.19	9.75 ± 1.98
Open Time**,+	32.93±4.45	38.54±6.65	60.37±6.78	65.44±9.12
Closed Time**,+	248.93±4.30	244.09±6.92	220.91±6.75	216.61±9.24
% Open entries	49.80 ± 0.33	51.46 ± 1.16	50.24 ± 0.24	50.19 ± 0.48
% Closed entries	50.21±0.33	48.54±1.16	49.75±0.24	49.81±0.48
% Open time**,+	10.98 ± 1.48	12.85 ± 2.22	20.12 ± 2.26	21.81 ± 3.04
% Closed time**,+	82.98±1.43	81.36±2.31	73.64±2.25	72.02±3.08
Latency open entry	1.36±0.85	1.19±0.26	0.44±0.05	0.83±0.29
Latency closed entry#	4.02±0.86	17.99±6.21	16.04±3.38	24.93±7.00

Data are presented as mean values ± SEM; (*) p<0.05 PE-8 *vs.* SE; (**) p<0.05 MC *vs.* PE-4 and SE; (+) p<0.05 PE-8 *vs.* SE; (#) p<0.05 MC *vs.* SE.

Actimeter

ANOVA did not reveal any significant effects of housing conditions on the level of locomotor activity in a novel cage in any of groups during any of the time periods evaluated with the actimeter. Interestingly, mice allocated to the MC, PE-4, and SE conditions generally displayed high levels of locomotor activity. Descriptive data indicated a higher number of counts in the SE group than in the other groups during the following time periods: 0-5 min, 5-10 min, 15-20 min, and 25-30 min (see Fig. 3), although this effect did not reach significance.

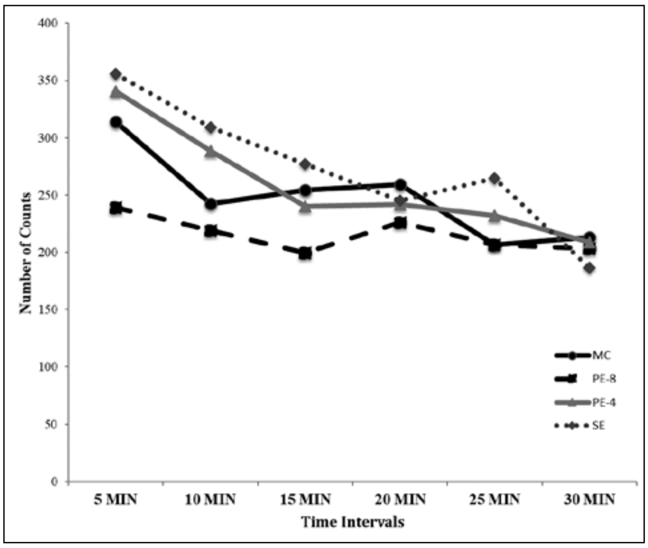

Fig. 3. Motor activity counts for each 5-min time period evaluated over 30 min in the actimeter across the four experimental groups: Marlau cages (MC), physical activity in large groups (PE-8), physical activity in small groups (PE-4), and the standard environment (SE). Data are presented as mean ± SEM.

Hot plate test

One-way ANOVA revealed a significant effect of housing conditions on mean hot plate reaction time among NMRI mice [$F_{3,60}$=4.40, *p*<0.05]. Multiple comparisons by *post hoc* Tukey's HSD test (α=0.05) showed a faster reaction time in PE-8 mice as compared to SE and PE-4 mice (see Fig. 4). The difference between PE-8 and PE-4 mice tended towards statistical significance (*p*=0.064).

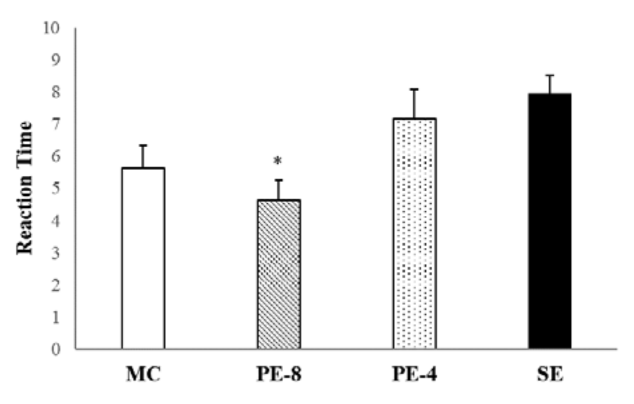

Fig. 4. Hot plate reaction time across the four experimental groups: Marlau cages (MC), physical activity in large groups (PE-8), physical activity in small groups (PE-4), and the standard environment (SE). Data are presented as mean ± SEM. (*) *p*<0.05, PE-8 *vs.* PE-4; PE-8 *vs.* SE.

DISCUSSION

The main aim of this study was to assess the behavioural effects of exposure to different housing conditions that include a key component of enriched environments – physical activity. To this end, we evaluated various behavioural parameters that index exploratory and motor activity, emotional response, and pain sensitivity in NMRI male mice. The different housing conditions employed in the present study differed in the degree of environmental complexity, opportunities for social interaction, type of presentation of voluntary physical activity, and availability of running wheels.

Some authors have suggested that the mechanisms underlying the mental health benefits of physical exercise are related to a reduction in anxiety levels and an increased ability to cope with stress (Smits et al., 2008). These conclusions stem from evidence that, among male and female BALB/C and C57BL/6 mice, enriched environments promote resilience to stress (Chapillon et al., 1999), and that voluntary wheel running has antidepressant and anxiolytic effects in adult male C57BL/6 mice (Schoenfeld et al., 2013) and male Sprague-Dawley rats (Moraska and Fleshner, 2001). The objective of the current study was to evaluate more in depth the effects of physical activity on motor, exploratory, and emotional behaviours using different animal models to compare the effects of physical activity alone, or as a component of more complex environments.

As far as we know, no previous studies on the issue have been published with the aforementioned experimental design and purpose. The results obtained suggest that mice allocated to different housing conditions display significant differences in their sensitivity to a noxious thermal stimulus, exploratory behaviour, novelty seeking, and anxiety-like response. In light of the existing literature, animals allocated to more stimulating environments (such as the MC, PE-8, and PE-4 conditions in the current study) were expected to show a rapid adaptation to novel environments, as well as, diminished stereotyped behaviours, locomotor, and exploratory activity when evaluated in the HB test and in the actimeter (Zimmermann et al., 2001; Viola et al., 2010). Groups provided with physical activity (i.e., MC, PE-8, and PE-4) were also expected to exhibit an anxiolytic-like profile as compared to the SE group, when evaluated in the EZM. However, these hypotheses were only partially confirmed by our data.

In the HB test, HD behaviour exhibited by the animals is interpreted as an exploratory behaviour that differs from spontaneous locomotor activity. It is worth mentioning that some authors use the HB test to assess anxiety-like effects induced by different manipulations, although it is not a test of anxiety *per se* (Mesa-Gresa et al., 2013b). A lower number of HDs is thought to reflect a lower level of anxiety (Simpson and Kelly, 2011), novelty seeking, and exploratory behaviour (Zhu et al., 2009; Mesa-Gresa et al., 2013b; 2014). In the current study, PE-8 animals exhibited a shorter latency to the first HD in comparison to mice allocated to the SE, which was accompanied by a lower total number of HDs (at both one and five minutes). These results suggest more pronounced initial exploratory behaviour in this group of mice, followed by a faster habituation to the novel environment. Furthermore, the group of mice reared in the MC condition performed a higher number of total HDs during the first minute as well as, during the total five-minute period, compared to PE-4, PE-8, and SE animals. These results contrast with those reported by van der Veen et al. (2015) showing a rapid habituation to novel contexts among female rats housed under complex environments such as MCs. The present findings, however, are consistent with those reported by Mesa-Gresa et al. (2014) and suggest that the complex and challenging environment provided by MCs induces greater exploratory activity and novelty seeking behaviours that may be accompanied by greater motor activity. These results also contrast with those typically obtained in non-standardized enriched environments, as well as with those obtained in the PE-8 group of the present study, in which a significant decrease in motor activity and exploratory behaviour has been reported (Simpson and Kelly, 2011; Mesa-Gresa et al., 2013b; 2014). Although previous findings suggest that animals exposed to enriched environments typically display diminished spontaneous locomotor activity when evaluated with the actimeter (Mesa-Gresa et al., 2013b), no significant differences between groups were observed in the current study. This finding does not agree with findings obtained in previous studies that indicate a more rapid habituation to new environments in mice exposed to a completely enriched environment (Mesa-Gresa et al., 2016) or to a rearing environment that includes voluntary access to running wheels. This discrepancy could be related to the longer rearing period in the current study (i.e., 7 weeks) than in previous experiments (Mesa-Gresa et al., 2013b), and to the age of the mice at the exposure to the behavioural tasks.

To understand the results obtained in the current experiment regarding anxiety-like behaviour, we must also take into account the fact that the cage sizes differed for each group, and in some cases, this resulted in less space provided for each animal. It is therefore possible that, in these groups, the housing conditions induced higher crowding which could influence the obtained results. We must also take into account the fact that the MCs included three running wheels in each cage. Thus, it is difficult to separate the effects induced by the complex environment present in these cages (e.g. doors, labyrinths, tunnels, ladders) from the physical activity induced by the presence of the three running wheels. The algorithm used to assign different cages to each group was based on our prior research in EE cages (Mesa-Gresa et al., 2013a; 2013b; 2016) and in MCs (Mesa-Gresa et al., 2014). In future studies, it would be of interest to design different enriched environments that included an equal number of animals per square unit in each cage. Such a design would allow for better standardization of the different housing conditions.

Regarding emotional response, data obtained in the EZM revealed a statistically significant effect of rearing conditions on the anxiety-like behaviour of mice. Animals allocated to the PE-8 condition performed fewer entries into the open areas, and more entries into the closed areas than mice allocated to the SE. Mice reared in the MCs performed a similar number of entries into both the open and closed sections as compared to mice reared in the PE-4 and SE conditions. Nevertheless, the MC and PE-8 groups displayed a higher percentage of time in the closed areas and a lower percentage of time in the open sections of the maze, which are both suggestive of increased anxiety-like behaviours. In line with these results, the MC group also displayed less latency to enter into closed areas compared with the SE group. Some studies have observed that exposure to exercise can increase anxiety-like behaviour in mice (Fuss et al., 2010; Rogers et al., 2019; Uysal et al., 2018), but do not obtain the same results when exercise is combined with EE conditions (Sampedro-Piquero et al., 2013; Rogers et al., 2019; Aujnarain et al., 2018). These data are in line with a prior study from our laboratory in which an increase in anxiety-like response was reported in mice allocated to MCs conditions. On the other hand, there is no clear consensus on the influence of the complex environment provided by MCs on anxiety response. The results of the current study regarding the anxiogenic-like behavioural profile of animals housed in MCs are inconsistent with results of previous studies carried out with rats (Fares, 2013). However, the present results are consistent with findings obtained in our laboratory with the same strain of mice (i.e., NMRI) when evaluated in the EPM task (Mesa-Gresa et al., 2014). In our previous study, we compared the behaviour of mice allocated to the MC, EE, and standard conditions. In the EPM, the MC group spent a lower percentage of time in the open arms, while this group did not show significant differences with respect to categories related to the time spent in each part of the maze as compared with standard mice (Mesa-Gresa et al., 2014).

One possible reason underlying the obtained contradictory results concerning anxiety response in the enriched groups is the high level of activity displayed by the animals in their own cages (Fares et al., 2012). In the study by Fares et al. (2012), the hyperactivity displayed by animals allocated to the MC condition may have accounted for the lack of correlation between the number of entries into open arms and the percentage of time spent in that area of the maze. The behavioural profile displayed by the animals in our current study might be due to the same reason, and the effects observed could be more closely related to a high level of activity than to an emotional response. In this context, the joint use of both the EPM and EZM would allow for a more integrated perspective regarding the effects of different environmental conditions on anxiety response (Sampedro-Piquero et al., 2018).

The nociceptive response is an interesting measure that is related to thermal pain sensitivity, but few studies have analysed the effects of exposure to EE conditions on this response. Data obtained from the hot plate test suggest that animals housed either in MCs or in an environment provided with voluntary wheel-running and social interaction displayed greater pain sensitivity to a noxious thermal stimulus than animals housed in an environment enriched purely by physical exercise or in a standard environment. Prior studies have suggested that both physical and social aspects of enrichment have a significant influence on pain sensitivity and duration, as evaluated in rats (Gabriel et al., 2010). In a study carried out by Vachon et al. (2013), exposure to EE conditions reduced hypersensitivity to mechanical and cold stimuli as compared to mice allocated to impoverished conditions, but there were not significant differences in pain sensitivity as assessed by the hot plate test. Moreover, physical exercise and EE have recently been proposed as a multimodal non-pharmacological strategy with neuroprotective effects, or as an environmental treatment to promote rehabilitation after stroke injury and different types of brain lesions (Sale et al., 2014; Livingston-Thomas et al., 2016; Gelfo et al., 2017). Such interventions have also been proposed for the treatment of chronic pain (Vachon et al., 2013) or neuropathic pain.

The effects of exposure of laboratory animals to EE have been extensively studied, including the differential impact of the main components of these housing conditions on relevant behavioural and neurobiological changes. Physical activity has been considered the most important component of enriched housing conditions and has been clearly related to the positive consequences induced by EE (Mustroph et al., 2012; Rogers et al., 2019). Following this question, the present study was designed to evaluate if differential behavioural responses can be observed in animals exposed to diverse housing conditions that include physical activity. We also aimed to support prior findings obtained in our laboratory in animals exposed to EE in comparison with standard-housed mice. Findings obtained in the present study are not conclusive with respect to this question, since they do not confirm the previously reported significant effects of housing conditions on animals exposed only to physical activity. These results could be related to those obtained by Mármol et al. (2017) showing that, in contrast to data reported in previous research, exposure to EE conditions without physical activity can induce beneficial effects in mice. The lack of concordant outcomes could be related to multiple factors including the heterogeneity of enriched environments employed in different studies (Mesa-Gresa et al., 2016; Aujnarain et al., 2018). We must take into account the sex of the animals employed, which can also influence results obtained in the present study. According to previous studies carried out in our laboratory, male NMRI mice were used in the present study to compare our data with our previous results. It is possible that an increase in aggressive behaviour and/or greater competition for the resources included in the enriched cages could be related to the lack of effects observed in some group comparisons. The use of male mice could also explain some discrepancies with data reported in prior research carried out with female rodents. Moreover, the number of available running wheels varied between the groups and housing conditions; thus, competition for access to running activity could vary and mask the effects of physical activity. Future studies should explore the comparison between more types of housing conditions that allow for the evaluation of both additive and interactive effects of the running wheel with cognitive, social, and somatosensory components of EE.

CONCLUSION

In conclusion, data provided by the current study suggest that the complexity of the rearing environment – which includes physical activity as a component – modulates the behavioural responses displayed by mice. Our results suggest that housing mice in a complex environment – such as the one provided by MCs – can induce anxiogenic-like responses and greater motor activity in the EZM, accompanied by an increase in exploratory behaviour in the HB. In contrast, housing mice in environments that provide voluntary physical activity plus social enrichment leads to lower levels of exploratory behaviour in the HB, and lower locomotor activity in the EZM. On the other hand, allocating mice to environments that provide physical activity only in the form of voluntary wheel running seems to induce a decrease in anxiety-like behaviours. Nevertheless, mice allocated to these housing conditions (i.e., PE-4) did not differ significantly from animals allocated to standard conditions in the behavioural parameters evaluated in the EZM, or in the HB test. Further research should be carried out to better understand the effects of complex environments and physical activity on behaviour and its neurobiological correlates, in both animals and humans. It would be of interest to not only evaluate exploration and anxiety-like behaviour, but also to evaluate the effects of these environments on cognitive functioning. In addition, new studies should address the issue of optimization of physical activity, as well as, cognitive and social stimulation to obtain the maximum benefits. The findings of basic research encourage new investigations to focus on the interaction between different drug treatments and physical activity provided by complex environments, the resulting effects on behavioural, cognitive, and emotional functioning. Such research could facilitate new approaches that are based on prevention and intervention strategies that are more personalized and effective. These studies might also be of interest in the design of non-pharmacological interventions aimed at improving cognition and other behavioural and psychological symptoms of Alzheimer's disease and other dementias (Du et al., 2018; Wang and Holsinger, 2018; Redolat and Mesa-Gresa, 2018).

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