Research & Reviews: Neuroscience

Effects of Environmental Enrichment on Anxiety Measurements: A Review

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Review Article

Received date: 31/09/2017

Accepted date: 06/01/2018

Published date: 10/01/2018

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Keywords: Environmental enrichment, Social enrichment, Anxiety, Stress, Animal models, Defence, Animal welfare

ABSTRACT

Environmental enrichment (EE) is an animal management technique, which aims to provide full welfare and better adaptation to the experimental conditions of laboratory animals. Exposing animals to different behavioral tests can assess the effects of EE. Among its several applications, EE has been identified as a protective factor in the treatment of some stress-related emotional disorders, such as depression. The anxiolytic effects induced by EE, on the other hand, are not as clear. In fact, one hypothesis that has been raised is that EE acts as a mild stressor agent. The present study reviews the literature published from 2006 to 2016 on environmental enrichment, stress and anxiety. The terms "environmental enrichment and stress" and "environmental enrichment and anxiety" were searched in MEDLINE, LILACS and Web of Sciences databases. The results obtained demonstrate that there are a great variety of EE protocols adopted. A significant part of these studies shows that EE reduces stress and anxiety measurements and leads to important neuroplastic changes. Nevertheless, since the EE protocols adopted vary greatly among studies, this might contribute to conflicting results. Standardizing EE protocols would help to understand EE effects on anxiety and stress-related measurements.

INTRODUCTION

Environmental enrichment (EE) is an animal management technique that consists of physical, social and sensorial strategies that aim to offer a series of stimuli that can increase the comfort and adaptability, both physiological and psychological, of laboratory animals [1]. EE includes different types of housing, which offer additional physical and social stimuli when compared to the simpler or less stimulating housing accommodations typical for those used in research laboratories. For rats, these include: natural materials to reinforce instinctual nesting behaviors, plastic tunnels and cardboard, wooden gnaw objects, ropes, swings, race wheels, balls, ramps, ladders and other suitable toys [2]. In addition to the use of these objects, social enrichment strategies, that are the grouping of social animals, are sometimes also adopted [3]. Although social enrichment is probably the easiest technique to implement through the introduction of a cage partner, there are situations where animals should be housed individually, and in these cases physical enrichment is particularly useful. A combination of both elements of social and physical enrichment has often been adopted [4-6].

Donald Hebb was the first to formally recognize the importance of EE for laboratory animals in 1940. Hebb observed that when laboratory rats were kept as pets, they showed improved learning when compared to mice housed in standard laboratory conditions. In their natural environment, as in captivity, rats have nocturnal activity, build nests and burrows, and perform coprophagy (consumption of feces), thigmotaxis (preference for the periphery of a new environment), foraging and the gnawing of objects found in their environment. On the other hand, captive rodents exhibit maladaptive and stereotypical behaviors, such as excessive self-cleaning, causing hair and mustache loss and biting of metal bars of cages [7]. One of the reasons for a growing interest in EE is that such maladaptive behavioral repertoires may interfere negatively with studies that are ethologically based. In addition, stereotypies can affect learning and interfere with anxiety and other stress-related measurements [8].

Another reason for the steady growth of EE as a discipline since the 1960s is the observation that rodents raised in enriched

conditions exhibit different neuroplastic changes. Among the neuroplastic changes that have been observed are the following: neurogenesis, increased dendritic branches and increased nerve cell size [9,10]. These changes have implications for the recovery in animal models of neurodegenerative diseases, brain lesions and psychiatric disorders [1,4,6,11,12]. In these studies, EE is introduced as an experimental variable and can contribute to the external validation and robustness of several models, since animals are tested in a variety of environmental conditions.

The effects of EE on stress-related disorders have also been previously investigated [2,3,8,11-20]. There are, however, conflicting results regarding the effects of EE, in particular, in anxiety-related behaviors. Some authors suggest that EE represents a mechanism of stress induction [13]. The repeated introduction of new objects and the opportunity for exploration is comparable to repeated exposures to a mild stressor stimulus [13]. In fact, some studies have shown that with respect to controls, animals exposed to enriched environments expressed slightly elevated levels of corticosterone at the start of the study [14]. Green et al., for instance, observed sucrose neophobia and an increased frequency of fecal cakes, anxiogenic-like effects [15]. EE has also been shown to increase the latency to ejaculate, again suggesting an increase in anxiety [15].

Taking into account the above conflicting results, the purpose of the present review was to better understand the effects of EE on anxiety obtained during the last years.

METHODS

A comprehensive literature search for studies on “environmental enrichment, stress and anxiety” was performed in MEDLINE, LILACS and Web of Sciences databases. The inclusion criteria were: language (English, Portuguese and Spanish), year of publication (articles published between 2006 and 2016) and the use of environmental enrichment in experimental laboratories of behavioral research, more precisely related to anxiety disorders. The results then reproduced 320 articles.

The following articles were excluded: 1) Studies whose objectives were not related to anxiety; 2) Studies that made use of surgical interventions previously and/or during the EE protocol; 3) Studies that have used pharmacological interventions previously and/or during the EE protocol; 4) Studies that have made use of genetically modified rats; 5) Review articles.

After the use of the exclusion criteria, there were 20 articles that are listed in **Table 1**.

Table 1. Environmental enrichment and anxiety-related measurements.

Authors	Protocol of environmental enrichment and duration (in days / weeks)	Population (animals/cage)	Anxiolytic effect?
Abou-Ismail and Mahboub [22]	Cage with 48.5 cm × 33 cm × 21 cm, containing bedding, Nylabone ball, stairs and Nestlets© (24 h /day-6 weeks).	24 males Wistar (1 animal/cage)	Yes
Brenes et al. [23]	Cage with 20 cm × 70 cm × 100 cm cage containing wood shavings, non-chewable plastic objects, two PVC tubes, five food dispensers and two bottles of water (24 h / day-4 weeks).	36 males Sprague Dawley (6 animals/cage)	Yes
Brydges et al. [18]	Cage with 70 cm × 45 cm × 54 cm, lined with a deep layer of shavings, two cardboard tubes, two cardboard boxes, four wooden blocks and a plastic house of 57.2 cm × 18.4 cm and 18.4 cm in height (24 h /day-1 week).	12 males Sprague Dawley (6 animals/cage)	Yes
Connors et al. [8]	Large multi-level cage with shaving, toys, tubes, chewing bone, Nestlets© and ramps (24h/day-7 weeks).	22 females 6 males 10 offsprings Sprague Dawley (4-6 animals/cage)	No
Goes et al. [2]	Cage with 100 cm in diameter and 70 cm in height containing wood, tunnels, sticks and blocks of various sizes, made of wood or plastic and an activity wheel (24 h/ day - 6 weeks).	70 males Sprague Dawley (5 animals/cage)	Partial
Harris et al. [27]	Cage with 56 cm × 38 cm and 20 cm, with shavings, paper materials for nest, a transparent red tunnel (9 × 15 cm), an opaque plastic tube (30 × 10 cm) suspended by chains from the top of the cage, Small paper tubes and boxes, wooden blocks and empty yogurt jars (24 h/day - 15 weeks).	30 females 30 males Wistar (6 animals/cage)	Yes
Li et al. [9]	Cage with 40 cm × 25 cm × 30 cm equipped with shavings, mats, cardboard boxes and plastic toys (24 h / day - 1 or 2 weeks).	12 pregnant females 48 offsprings Sprague-Dawley (4-6 animals/cage)	Yes
Mosaferi et al. [3]	Cage equipped with shaving, two exercise wheels, two food dispensers, two bottles of water, various types of stairs and PVC pipes, balls, rings and a block of plate with non-chewable plastic holes, in addition to such chewable objects Such as ropes and cardboard (24 h/day - 21 weeks).	45 males Wistar (15 animals/cage)	Yes

Peña et al. [24]	Metal cages with 100 cm × 43 cm × 50 cm, containing shavings, stairs, ropes, tunnels, activity wheels (24 h /day - 8 weeks).	24 females 20 males Sprague Dawley (11-12 animals/cage)	Partial
Peña et al. [25]	Metal cage with 100 cm × 43 cm × 50 cm, containing shavings, balls, rings and bells made of metal, plastic or wood (24 h/day - 12 weeks).	28 females 24 males Sprague Dawley (12-14 animals/cage)	Yes
Ravenelle et al. [11]	Metal cage with a smooth metal floor, sized 94 cm × 94 cm × 51 cm, equipped with shavings, nest, plastic and wooden toys such as Lego blocks, buckets, rattles, wheels, skins, and objects to promote movement such as ropes, hanging ladders and hanging chains (24 h/day - 40 days).	60 males Long Evans (10 animals/cage)	Yes
Saenz et al. [20]	Cage with rectangular stainless steel flooring with tree levels (120 cm × 70 cm × 100 cm), walls and ceiling covered with wire mesh, containing shavings, non-chewable plastic objects, two PVC pipes and an activity wheel (24 h/Day - 63 days).	45 males Sprague-Dawley (15 animals/cage)	Yes
Sampedro-Piquero et al. [1]	Cage with 100 cm × 95 cm × 54 cm, containing shavings, toys, exercise wheels, ropes, plastic tubes of different diameters, platforms, wooden houses, odoriferous and sound objects and nest materials (3 hr/day - 8 weeks).	40 males Wistar (10 animals/cage)	Yes
Sampedro-Piquero et al. [4]	Cage of 100 cm × 95 cm × 54 cm, containing shavings, toys, exercise wheels, ropes, plastic tubes of different diameters, platforms, wooden houses, odoriferous and sound objects and nest materials (3 hr/day - 8 weeks).	38 elderly males Wistar (9-10 animals/cage)	Yes
Sampedro-Piquero et al. [5]	Cage of 100 cm × 95 cm × 54 cm, containing shavings, toys, exercise wheels, ropes, plastic tubes of different diameters, platforms, wooden houses, odoriferous and sound objects and nest materials (3 hr/day - 8 weeks).	38 elderly males Wistar (9-10 animals/cage)	Yes
Sampedro-Piquero et al. [6]	Cage of 100 cm × 95 cm × 54 cm, containing shavings, toys, exercise wheels, ropes, plastic tubes of different diameters, platforms, wooden houses, odoriferous and sound objects and nest materials (24 h/day - 69 days e 8 weeks).	40 males Wistar (10 animals/cage)	Yes
Sampedro-Piquero et al. [28]	Cage of 100 cm × 95 cm × 54 cm, containing shavings, toys, exercise wheels, ropes, plastic tubes of different diameters, platforms, wooden houses, odoriferous and sound objects and nest materials (24 h/day - 21 days).	32 males Wistar (8 animals/cage)	Partial
Soares et al. [12]	1. Transparent plastic cage with 40 cm x 25 cm x 20 cm, containing shavings, activity wheels, tunnels, plastic toys, marble, rattles, mirrors and pieces of wood (1 h / day - 21 days). 2. Cage with 40 cm × 60 cm × 90 cm, three floors interconnected by ramps, containing shavings, an activity wheel, plastic toys, rubber balls, wooden objects with different shapes, textures and colors, objects that emit sound and mirrors in the Lateral walls (1 h/day-2 weeks).	20 males and offspring Wistar (10 animals/cage)	Yes
Sparling et al. [26]	Four level cage with 52 cm × 43 cm × 77.5 cm, connected by 6.35 cm diameter tubes, containing wood shavings, included plastic chew toys, plastic links, climbing ropes, bells, nesting materials and plastic balls (24 h/day - 38 days).	10 females Long–Evans (5 animals/cage)	Partial
Turner et al. [21]	Polypropylene cage with 54 cm × 36 cm × 30 cm, high metal cover, containing shavings, nest material, chewing wood, closed shelter 15 cm × 15 cm × 12 cm and activity wheel (24h / day - 6 to 9 weeks).	16 males Sprague Dawley 16 males Long-Evans (8 animals/cage)	Partial

RESULTS AND DISCUSSION

The purpose of this study was to review the literature of the last ten years on the theme environmental enrichment, stress and anxiety. The results obtained demonstrate that the EE protocols used vary greatly among studies. Also, several anxiety measurements were assessed through the use of different animal models. Other factors that varied were: duration and type of the EE protocol, age, species, number of animals included in the study and number of housed animals in the same cage (social enrichment) **(Table 1)**. Results demonstrate that, in general, exposure to EE produces anxiolytic-like effects in different animal models aimed to investigate anxiety-related reactions. There were, however, studies that showed partial effects [2,6,21-26] or negative results [8].

In the open field test (OF) and similar tests involving measures of locomotor activity, studies have shown that EE reduces motor skills and generates faster habituation when enriched animals are compared with control animals [2,11,18-21]. The effects of EE on behavior measured in the OF show that animals submitted to EE are less active in comparison to animals kept alone or in

a social group without enrichment. Also, when using EE from weaning, a lower locomotor activity is observed throughout the test session [8].

Studies conducted with the elevated plus-maze model (EPM), an animal model of anxiety widely used around the world [2,3,8,9,11,12,18,21,22,24-26], also demonstrate that animals submitted to EE present a greater number of entries in the open arm when compared to animals lacking EE housing protocols, an anxiolytic-like effect. There were, however, a few exceptions. For instance, Ravenelle et al. did not observe significant effects of a 20-day exposure to EE in adult animals [11]. However, when the same experiment was performed at the weaning period, the anxiety responses in the EPM were consistently attenuated by the exposure to EE. Goes et al. also implemented EE in adulthood and observed that rats submitted to EE did not explore the maze, tending to choose one of the corners of the closed arms, where they remained immobile for most of the time [2]. No significant differences between control animals and those submitted to EE were found in this study, indicating that EE established in adulthood may be less likely to influence the state of anxiety when evaluated by the EPM.

Abou-Ismail and Mahboub observed important differences between rats raised in variable housing conditions [22]. Rats submitted to EE when compared to control animals presented more frequent sleep, locomotion, eating, self-cleaning, and lower levels of immobility and nesting activity. Still, rats submitted to EE also present increased levels of environmental exploration and locomotor activity compared to rats kept in non-enriched enclosures. The animals also remained for longer periods in the open arms of the EPM [12,22]. Taken together, EPM results indicate that increasing the enrichment of conventional cages for laboratory rats appears to decrease the level of anxiety.

The use of EE during pregnancy and postpartum is a topic that has aroused interest. Connors et al. demonstrated that exposure to EE during the pre- and postnatal periods decreased anxiety responses and altered excitatory and inhibitory neurotransmission systems [8]. Li et al. also observed that environmental enrichment during gestation decreases the observed anxiety behavior in the EPM, and prevents memory-learning deficits observed in rats submitted to stress factors in the prenatal period [9]. This observation supports the notion that EE during gestation can serve as a preventive and effective strategy against damage induced by prenatal stress. The beneficial effects of EE during pregnancy may be due to the preservation of morphology and synaptic function in the hippocampus. However, the underlying mechanisms still require further investigation [9].

The studies also show that the animal strain used influences different behavioral measurements, including locomotion, anxiety, acoustic startling and learning responses. Rats from the Long Evans lineage, when compared to the albino rats of the Sprague-Dawley lineage, are more active, have greater exploration, reduced anxiety, reduced acoustic jitter reflex, and improved cognitive performance [24]. Genetic lineage is not the only influencing factor in the results, but also the animal gender. Peña et al. demonstrated that adult males rats submitted to EE, when compared to control males, presented increased exploration time, representing a good model of social interaction [24]. No significant changes were observed when females submitted to EE were compared to controls. On the other hand, EE increased the percentage of open arms entries, indicating a reduction of anxious behavior in both genders [24,25]. In addition, Peña et al. observed that EE does not differentially affect males and females, but found differences in sex in the 'hole board' and EPM tests, regardless of the use of EE, indicating that females travel long distances, have higher locomotor activity and defecate less than the males [25].

Harris et al. proposed that the addition of new objects in the cage of a rodent could be a stressful experience [27]. However, since no aversive result is experienced when the animal interacts with the new objects, habituation to novelty occurs. Results obtained with the light/dark transition model suggest that rats submitted to EE may even seek novelty (even under aversive conditions), since they are four times faster on entering the light side than the rats not enriched [8,21]. Thus, it is possible that habituation to an enriched environment (social or physical) explains why animals submitted to EE present reduced anxiety when exposed to acute stress situations.

Neurotransmitters, neuro-growth factors and the hypothalamic-pituitary-adrenal axis (HPA) have been investigated for their possible roles in the EE-induced behavior and brain plasticity [28]. It has been shown that one month of EE is sufficient to produce behavioral and neurochemical changes [23]. Pre- and postnatal EE administered is related not only to decreases in anxiety but also to a change in the profile of excitatory and inhibitory neurotransmitters in the offspring. Specifically, an increase in GABA accumulation in the prefrontal cortex of young rats of both sexes has been reported and increases in GluR1 concentrations have been observed only in juvenile males and females [8]. On the other hand, increases in glutamate concentrations were observed only in males [8]. In Long Evans rats, the use of EE from weaning results in increased expression of brain-derived neurotropic factor (BDNF) in the central amygdala and CA2 region of the hippocampus, and is still capable of modulating autonomic and behavioral response to stress [11]. Exposure to EE after weaning, but also during gestation, attenuates the decreased expression of synaptophysin and glucocorticoid (GR) receptors in the hippocampus (CA1 and CA2 regions). In addition, it increases the expression of the gene for the serotonergic type 1A receptor in the hippocampus and frontal cortex [26]. It has also been observed that EE induces a protective affect to cope with anxiety and novelty situations which may be mediated, in part, through increased of the GR expression in the dorsal hippocampus [9].

In studies with elderly rats, a non-continuous EE protocol, initiated during middle age, improves cognitive performance and reduces anxiety [4,5]. In addition, the rats subjected to EE acquire a more precise search strategy, making fewer reference

memory errors. Interestingly, these cognitive enhancements may be correlated, in part, with changes in astrocytes found in the dorsal hippocampus. Quantitative analysis has demonstrated that EE enhances the immune response and the number of cells in the dentate gyrus, which in turn may be correlated with the proliferative characteristic of this hippocampus sub region and with the formation of new synapses. EE also increases the morphological complexity of hippocampal astrocytes. These astrocytes showed longer branches with more crosses and nodes compared to the other experimental conditions. It has been suggested that astrocytic plasticity may represent a component of an EE-induced brain reserve, which in turn could explain the behavioral improvements found in elderly rats [6].

Finally, with respect to the relationship between the HPA axis and EE, Peña et al. concluded that the basal and stress levels of HPA axis hormones have well-described differences between male and female rats, with higher morning and evening ACTH and corticosterone levels, and a higher HPA axis response to stress in females [25]. However, regardless of sex, EE rat models demonstrated normal morning and evening ACTH resting levels, but a greater increase in corticosterone levels from morning to night, indicating a negative result on the effects of EE.

CONCLUSION

The existing literature suggests that EE programs for rats vary widely among laboratories. Most of the studies found suggest that its use exerts anxiolytic-like effects, aside from increasing neuronal proliferation and cognitive improvements in different animal models, which may protect against the development of stereotyped behaviors and the disruption of adaptability that could result in maladaptive behaviors and atypical brain development. Nevertheless, the present results also show that it would be important to standardize EE protocols, in order to better understand the behavioral and physiological data described among different laboratories.

ACKNOWLEDGEMENTS

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Danielle A. Lopes was the recipient of a fellowship grant from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

1. Sampedro-Piquero P, et al. Effects of environmental enrichment on anxiety responses, spatial memory and cytochrome C oxidase activity in adult rats. Brain Res Bull. 2013;98:1-9.
2. Goes TC, et al. Environmental enrichment for adult rats: effects on trait and state anxiety. Neurosci Lett. 2015;584:93-96.
3. Mosaferi B, et al. Enduring effects of post-weaning rearing condition on depressive- and anxiety-like behaviors and motor activity in male rats. Physiol Behav. 2015;142:131–136.
4. Sampedro-Piquero P, et al. Behavioral testing-related changes in the expression of synapsin I and glucocorticoid receptors in standard and enriched aged Wistar rats. Exp Gerontol. 2014;58:292-302.
5. Sampedro-Piquero P, et al. Astrocytic plasticity as a possible mediator of the cognitive improvements after environmental enrichment in aged rats. Neurobiol Learn Mem. 2014;114:16-25.
6. Sampedro-Piquero P, et al. Increase of glucocorticoid receptor expression after environmental enrichment: relations to spatial memory, exploration and anxiety-related behaviors. Physiol Behav. 2014;129:118-129.
7. Hebb DO. The effects of early experience on problem solving at maturity. Amn Psychol. 1947;2:306-7.
8. Connors EJ, et al. Environmental enrichment models a naturalistic form of maternal separation and shapes the anxiety response patterns of offspring. Psychoneuroendocrinology. 2015;52:153-167.
9. Li M, et al. Environmental enrichment during gestation improves behavior consequences and synaptic plasticity in hippocampus of prenatal-stressed offspring rats. Acta Histochemica et Cytochemica. 2012;45:157-166.
10. Petrosini L, et al. On whether the environmental enrichment may provide cognitive and brain reserves. Brain Res Revs. 2009;61:221-39.
11. Ravenelle R, et al. Housing environment modulates physiological and behavioral responses to anxiogenic stimuli in trait anxiety male rats. Neuroscience. 2014;270:76-87.
12. Soares RO, et al. Environmental enrichment reverses reduction in glucocorticoid receptor expression in the hippocampus of and improves behavioral responses of anxiety in early malnourished rats. Brain Res. 2015;1600:32-41.
13. Larsson F, et al. Psychological stress and environmental adaptation in enriched vs. impoverished housed rats. Pharmacol Biochem Behav. 2002;73:193-207.
14. Moncek F, et al. Effect of environmental enrichment on stress related systems in rats. J Neuroendocrinol. 2004;16:423-31.

15. Green TA, et al. Environmental enrichment produces a behavioral phenotype mediated by low cyclic adenosine monophosphate response element binding (CREB) activity in the nucleus accumbens. Biol Psychiatry. 2010;67:28-35.

16. Urakawa S, et al. An enriched rearing environment calms adult male rat sexual activity: implication for distinct serotonergic and hormonal responses to females. Plos One. 2014;9.

17. Crofton EJ, et al. Inoculation stress hypothesis of environmental enrichment. Neurosci Biobehav Rev. 2015;49:19-31.

18. Brenes JC. A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. Behav Brain Res. 2009;197:125-137.

19. Mosaferi B, et al. Enduring effects of post-weaning rearing condition on depressive- and anxiety-like behaviors and motor activity in male rats. Physiol Behav. 2015;142:131-136.

20. Sáenz JCB, et al. Factor analysis of forced swimming test, sucrose preference test and open field test on enriched, social and isolated reared rats. Behav Brain Res. 2006;169:57-65.

21. Turner KM, et al. Comprehensive behavioural analysis of Long Evans and Sprague-Dawley rats reveals differential effects of housing conditions on tests relevant to neuropsychiatric disorders. PLoS One. 2009;9:93411.

22. Abou-Ismail UA and Mahboub HD. The effects of enriching laboratory cages using various physical structures on multiple measures of welfare in singly-housed rats. Laboratory Animals. 2011;45:145-153.

23. Brenes JC, et al. A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. Behav Brain Res. 2009;197:125-137.

24. Peña Y, et al. Environmental enrichment effects in social investigation in rats are gender dependent. Behav Brain Res. 2006;174:181-187.

25. Peña Y, et al. Enduring effects of environmental enrichment from weaning to adulthood on pituitary-adrenal function, pre-pulse inhibition and learning in male and female rats. Psychoneuroendocrinology. 2009;34:1390-1404.

26. Sparling JE, et al. The effects of gestational and postpartum environmental enrichment on the mother rat: a preliminary investigation. Behav Brain Res. 2010;208:213-223.

27. Harris AP, et al. Environmental enrichment enhances spatial cognition in rats by reducing thigmotaxis (wall hugging) during testing. Animal Behaviour. 2009;77:1459-1464.

28. Sampedro-Piquero P, et al. Housing condition-related changes involved in reversal learning and its c-fos associated activity in the prefrontal cortex. Neuroscience. 2015;307:14-25.