Environmental enrichments as therapeutic alternative: studies with animal models

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Abstract: Analysis of results of research based on enriched environment with animal models can elucidate differences between genders, ages, environments configuration and elements that shape it. Individually or interlinked, these factors constitute a range of sensory stimulation that exerts effects on animal welfare, being reason of attention to discuss. Enriched environment stimulates neurogenesis and plasticity parameters in rodent´s nervous system, promotes recovery of the nervous system damaged or diseased, reduces stress and improves learning. The application of enriched environment is still heterogeneous, since still doesn´t decipher the effect of auditory stimuli u odors during his confinement to be used as a therapeutic tool. The aim of this review is to characterize the effect of enriched environment on the biochemical and morphological modification of brain regions that promotes learning and improves behavior in the laboratory rat. The enriched environment in laboratory animals can be used as a tool for treatment of behavioral disorders such as anxiety and stress or to promote learning.
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Introduction

Hebb, 1947 placed a group of rats in standard laboratory boxes and another group of animals in pet boxes, the group of rats that grew in pet boxes executed a learning task in less time about rats grew in laboratory standard boxes. Subsequent studies indicated that box size where animals grew and presence of objects such as tunnels, corridors and inclined planes facilitated mobility of animals to be related to rat´s behavior in adulthood (Bingham y Griffits, 1952). In the 60's, it was indicated that environment in which an animal grows and develops, produces biochemical and morphological changes in brain (Rosenzweig *et al.,* 1962), which helped create of "enriched environment" (EE) concept as a method to induce measurable changes in animals exposed brain.

Standard conditions during growth of laboratory rodents vs. enriched environment

Laboratory studies mostly use rodents as the ideal animal model raised and distributed under standard laboratory conditions according to their living space and weight. This isolation reduces reuptake of dopamine and serotonin in prefrontal cortex (PC) and nucleus accumbens (NAc), and induces increased locomotor activity and startle behavior (Konkle *et al*., 2010). Increased sensory stimulation by increasing environment and social interaction (sensory-motor) is an EE that is achieved by placing 6 to 8 animals in boxes whose dimensions are greater than standard boxes and placing toys, tunnels, construction materials for burrows, food in different places and wheels for exercise voluntarily (Nithianantharajah and Hannan, 2006; Patel, 2012; Girbovan and Plamondon, 2013; Figure 1).

Enriched environment and learning

Animals that grow influenced by EE improve execution of tasks learned as adults (kobayashi *et al*., 2002). For example, if animals are maintained since weaning (day 21 after birth) in EE for 8 weeks and then assessed at 2.5 months of age using the Hebb-Williams maze, animals execute in less time learning test regarding animals raised in standard conditions (Hymovitch, 1952). This result is not observed in animals exposed to EE at age 15 or 25 months, suggesting that EE does not have the same effects on aging brain (Segovia *et al*., 2006; Patel, 2012). However, can attenuate age-related effects, for example, synaptic density in rat´s hippocampus decreases with age and is maintained when old animals are housed in enriched conditions (Saito *et al*., 1994). Suggesting that effect of EE on brain in subjects who learned a task is directly over DNA, increasing histone acetylation and promotes gene transcription (Patel, 2012).

In some mice strains, there is a spatial memory deficits emerging from 18 months of age (Frick *et al*., 2003). This impairment in spatial memory is associated with a decrease in protein levels that constitute pre-synaptic vesicles such

synaptophysin (Nakamura *et al*., 1999; Frick *et al*., 2003). When these mice are exposed to an EE at 18 months, spatial memory deficit is significantly reduced. In rats, it has been reported that EE increases the number of pre-synaptic vesicles in prefrontal cortex (Nakamura *et al*., 1999) as well as hippocampus and neocortex synaptophysin expression (Saito *et al*., 1994). Additionally it has been observed that activity of glutamic acid decarboxylase enzyme (GAD) (GABA-synthesizing enzyme) is increased in hippocampus of animals exposed to EE (Frick *et al*., 2003). Elevation on synaptophysin levels has been correlated with an improvement in spatial memory. However, there is not the same correlation between GAD levels and spatial memory, resulting uncertain the role of GAD.

To a morphological level has been reported that animals that grown under EE conditions show a significant increase in dendritic arborization and dendritic spines density in brain regions directly related to learning and memory consolidation. Such regions include pyramidal neurons of layer III of parietal and occipital cortex, as well as neurons of hippocampal CA1 region (Leggio *et al*., 2005); these effects have also been reported in diabetic mice (Beaquis *et al*., 2010). The animals exposed to EE increase neurogenesis rate in dentate gyrus of hippocampus and proliferation in rostral migratory route in rats exposed to EE of 1 to 3 weeks (Martonciková *et al*., 2011). Increased neurogenesis in this brain region may be associated with a better execution on learning tests (Lledo *et al*., 2006).

Enriched environment and restoration of damaged nervous system

After exposing animals with an injury nervous system to an EE, their partial recovery includes cognitive functions and movement in affected members (Johnson *et al*., 2013, Galeano *et al*., 2015). The sensory-motor stimulation induced by EE promotes recovery of motor activity of laminectomized animals of thoracic spinal segment 8 (Lankhorst *et al*., 2001). Rats with parietal cortex injury or bilateral injury of hippocampus, recover their spatial, motor and memory deficit when exposed for 14 days to EE compared to animals under standard laboratory conditions, suggesting that EE can function as therapeutic alternative (Sozda *et al*., 2010).

Microinjection of kainic acid in rat hippocampus induces epileptic seizures. Kainic acid is an excitatory of glutamatergic receptors and an over-stimulant of seizures, neuronal damage and epileptic crisis. Animals injected with kainic acid and EE exposed for 3 weeks showed an elongation at latency of first seizure appearance, suggesting that EE has a neuroprotective effect on neurons of hippocampal CA1 region (Young *et al.,* 1999). In animals under induced ischemia by occlusion of middle cerebral artery, and submitted to EE with rehabilitation therapy, recover motor function of forelimbs (Biernaskie and Corbett, 2001), it appears that females are more susceptible to effects of enrichment and recovering faster than males (Saucier *et al*., 2010). It has been suggested increased dendritic arborization in pyramidal neurons of motor cortex layer V of contralateral hemisphere to ischemic damage in animals exposed to EE, suggesting that sensorimotor stimulation induces plastic events that generate compensatory mechanisms to mitigate damage by ischemia (Biernaskie and Corbett, 2001).

Moreover, it has been demonstrated that EE significantly increased brain levels of nerve growth factor (NGF) in hippocampus and entorhinal cortex (Dahlqvist et al., 2003) of neuronal factor derived from brain (BDNF), NMDA receptor subunit 1 (Sun *et al*., 2010) and neurotrophin-3 (NT3). All these factors are involved in processes of neuronal plasticity, neuronal survival and differentiation (Torasdotter *et al*., 1998). Therefore it has been suggested that EE aid recovery of damaged region because it facilitates expression and synthesis of these growing factors (Dahlqvist *et al*., 2003).

In knockout mice for fragile X gene, a mental retardation model has been observed recovery of cognitive deficits after exposure to an EE (Grossman *et al.,* 2001). Mice with partial trisomy 21 (Ts65Dn), rats with attention deficit and hyperactivity disorder (ADHD) and knockout mice for NMDA receptor subunit 1 in CA1 region of hippocampus show a significant recovery in olfactory discrimination and object recognition after being exposed three hours a day for two months to enrichment conditions (Martínez-Cué *et al*., 2002, Pamplona *et al*., 2009; Baamonde *et al*., 2011). These reports suggest that EE attenuates genetic deficits and could be considered in studies of degenerative or congenital diseases.

Enriched environment and its relation with behavior and hormone levels in laboratory animal models

Conditions of environmental enrichment impact emotional behaviors such as stress, fear, anxiety and depression (Porsolt *et al*., 1978, Zubedat *et al*., 2015). Animals exposed to enriched conditions are less "emotional" than animals grown under standard laboratory conditions in impoverished conditions or isolated (1 animal per box) (Brenes *et al*., 2006). A series of studies were designed to evaluate anxiety levels in rats and mice at different stages of life through implementation of a plus maze, demonstrating EE effect (Table 1).

Effect of EE on hormone levels

EE reduces basal plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) in male and female rats. Females show higher plasma levels of CORT than males (Belz *et al.,* 2003). However, when females are manipulated under stress conditions (i.p. injection of saline solution) ACTH plasma levels were significantly reduced compared to males. This result agrees with findings of other research, in which females have a reduced defensive behavior to a predator compared with males (Klein *et al*., 1994). These results together suggest that exposure to EE induces significant changes on hormone levels of CORT and ACTH, which may be affecting animals behavior.

In male mice, not significant differences in plasma levels of CORT among which were exposed to conditions of EE and standard conditions. However, animal's exposure to a predator odor (cat feces) increases CORT plasma levels in animals in standard condition, but not in animals in enriched condition. Under stress conditions, dopamine and acetylcholine release in prefrontal cortex is reduced in animals placed in EE (Segovia *et al*., 2010). These results suggest that EE promotes adaptation mechanisms to stress more efficiently compared with animals raised in standard conditions.

Moreover, when a rat is placed in a cylinder with water from which it cannot escape, forcing her to swim and has repeatedly behavioral immobility (no swimming), is considered to have a greater degree of hopelessness or like-depression, compared with another rat present fewer immobilities. EE has an anti-immobility effect in rodents by decreasing the number of registered immobilities in forced swim test (Porsolt *et al*., 1978), previous reports show that EE can modify hormone levels and can be a tool to be considered in studies where conditions of stress or depression are important.

Conclusion

The EE can be used as a tool for treatment of behavioral disorders such as anxiety and stress or to promote learning. While most laboratories standard boxes are used to keep animals, it is important to consider that many of evaluations that we do may be influenced by this condition and sometimes the behavior interpretations we do could be biased by environmental factor. The effect of EE on damaged or diseased nervous system demonstrates that biochemical or morphological changes caused by increase in sensory-motor stimulation is critical in individuals recovery, suggesting that clinically this should be exploited for treatment of some neurological pathologies order since it has been demonstrated that EE stimulate learning and improvement behavior of animal under study. Nevertheless, more studies are needed to determine which components are most important in enrichment for each case or disease model.

Table 1. Effects of environmental enrichment on behavior in animal models.

	Experimental model	Effects
Klein *et al*. (1994)	Two groups of rats, G_1 in EE, G_2 in standard conditions for 8 weeks. They were exposed to a predator (domestic cat) to characterize behaviors that indicate stress such as freezing or defensive behaviors.	G_1 animals showed reduced immobility times (freezing behavior) and more activity next to predator. Females of both groups showed greater closeness and less defensive behaviors or freezing compared to males, so EE reduces stress and the effect is greater in females.
Fox *et al.,* 2006	Two groups of mice on growing conditions strain BALB/c, G_1 under EE conditions and G_2 under normal laboratory conditions. Were evaluated to determine anxiety degree.	Mice that grew in EE, showed less anxiety about group under standard conditions.
Friske *et al*., 2005	Two groups of female mice housed for six weeks (stage of pregnancy and up to two days post-partum). G_1 under EE conditions and G_2 under normal laboratory conditions. Were evaluated EPM at six days and three months post-partum. Progeny of both groups remained for days in same conditions, were evaluated with EPM at three months post-birth.	Female mice showed more entries into the open arms of EPM, indicating a lower level of anxiety than females maintained under standard conditions. Suggesting that effects of EE may be dependent on gender. On progeny the same results were observed with their mothers. This suggests that behaviors such as grooming mothers to the newborn pups or cleanup conduct, known to affect behavior and physiology of the offspring as adults, may have influenced, i.e. there appears to be an epigenetic effect of EE on mice´s progeny.

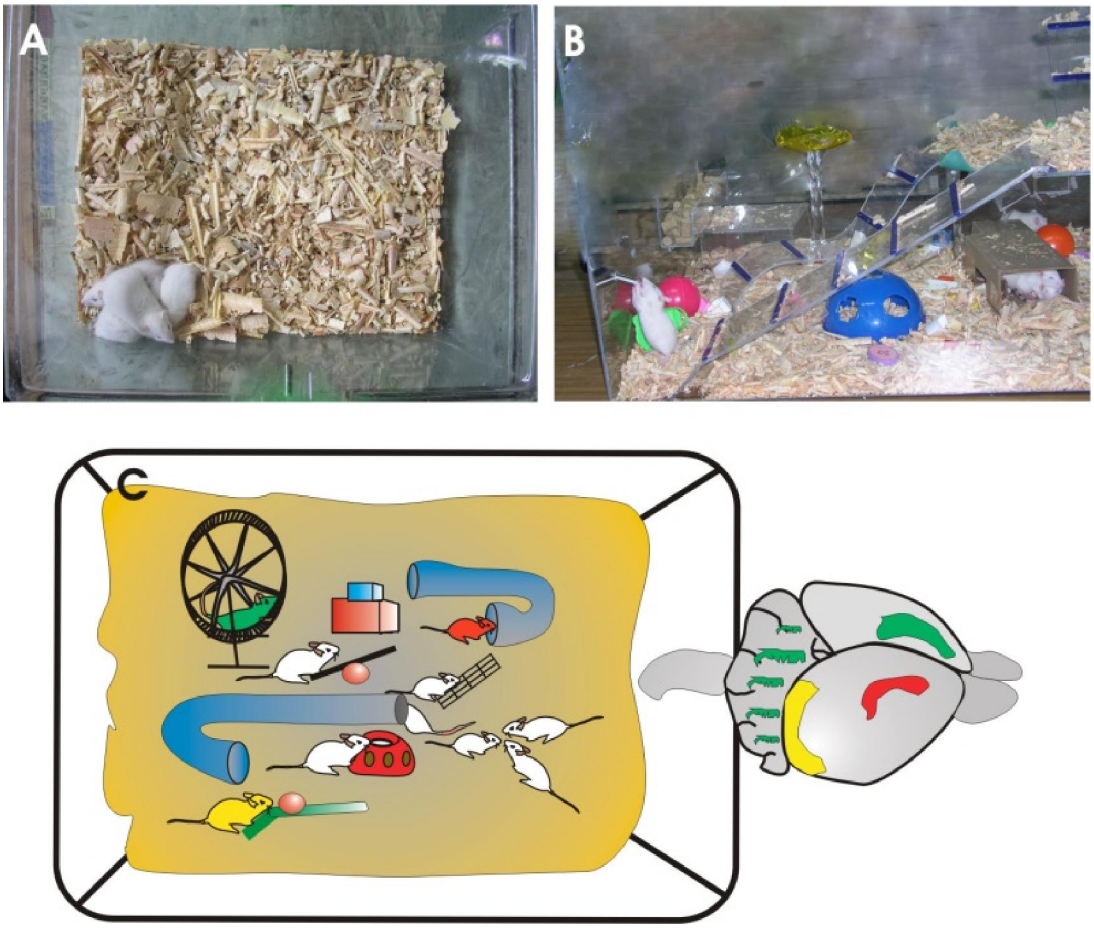

Figure 1. Environmental enrichment conditions. Figure illustrates different environmental conditions where an animal (rat) can grow in a laboratory. Panel A shows standard conditions. The panel B and C illustrate an enriched environment conditions. Dimensions of cage in panel A are 47X33X19 cm and panel B are 75X60X60 cm. Note that an enriched environment cages have several levels and objects that animals can manipulate freely. Also the number of animals that live in each of them is greater (n=8) compared to standard condition cages (n=4). Furthermore, panel C illustrates that environment configuration is very important, since different objects have a differential impact on animal's brain. For example, color rats that are represented on each schema object illustrates stimulations of different regions of the brain, visual region (yellow rat), motor (green rat) or cognitive regions like hippocampus (orange rat).

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