NeuroImage 109 (2015) 190–198

Contents lists available at ScienceDirect

NeuroImage

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

Environmental enrichment is associated with rapid volumetric brain changes in adult mice

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ARTICLE INFO

Article history:
Accepted 8 January 2015
Available online 14 January 2015

Keywords:
Environmental enrichment
Mice
In-vivo MRI
Maze
DBM

ABSTRACT

Environmental enrichment is a model of increased structural brain plasticity. Previous histological observations have shown molecular and cellular changes in a few pre-determined areas of the rodent brain. However, little is known about the time course of enrichment-induced brain changes and how they distribute across the whole brain. Here we expose adult mice to three weeks of environmental enrichment using a novel re-configurable maze design. In-vivo MRI shows volumetric brain changes in brain areas related to spatial memory, navigation, and sensorimotor experience, such as the hippocampal formation and the sensorimotor cortex. Evidence from a second cohort of mice indicates that these plastic changes might occur as early as 24 h after exposure. This suggests that novel experiences are powerful modulators of plasticity even in the adult brain. Understanding and harnessing the underlying molecular mechanisms could advance future treatments of neurological disease.

Introduction

Experiences leave a permanent mark in the brain by altering its structure. To study the effect of experiences on the brain, researchers have used training regimes specifically aimed at improving a certain skill or a change in environment that provides a variety of novel stimuli. A number of sensory, motor, and cognitive training paradigms have been shown to be related to structural alterations in the adult human brain. Visual orientation discrimination training is associated with cortical thickness increases in several visual-related areas (Swisher et al., 2011). Motor training paradigms, such as juggling, have been shown to be effective in altering gray matter (Boyke et al., 2008; Draganski et al., 2004; Driemeyer et al., 2008) and white matter (Scholz et al., 2009) in occipito-parietal areas associated with vision and reaching. A balance task has been used to demonstrate that vestibulo-motor training is related to structural brain changes in frontal and parietal areas (Taubert et al., 2010). Finally, cognitive training, such as learning a second language is associated with structural alterations of frontal white matter tracts (Schlegel et al., 2012). In humans socio-economic status might be a measure of the stimulating quality of an individual's environment. Socioeconomic status has been found to be associated with improved cognitive performance and increased gray matter density in the hippocampal formation of children (Jednoróg et al., 2012). However, to date it remains unclear what environmental variations in humans are comparable to environmental enrichment paradigms in animal models.

Animal models of experience-related structural plasticity offer a number of advantages over human studies. Using genetically identical mice eliminates variance arising from differences in plasticity-related gene expression. Further, age, diet, and upbringing can be precisely controlled along with the specific parameters of the intervention. Rodent studies have employed specific training paradigms, such as the water maze (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011b), rotarod (Scholz et al., under review), reaching (Sampaio-Baptista et al., 2013), and fear conditioning (Ding et al., 2013) to demonstrate structural changes in the brain.

The most popular paradigm of increased plasticity in rodents is environmental enrichment, which has been used for the past 50 years to study the effect of experience on the brain (Diamond et al., 1964). Environmental enrichment refers to housing conditions that provide increased stimulation over standard or impoverished housing. Most enrichment protocols house larger groups of mice in larger cages furnished with running wheels and toys. Part of the enrichment is thought to stem from the complexity and novelty of the environment (Nithianantharajah and Hannan, 2006; van Praag et al., 2000). Complexity can be increased by providing a greater variety of opportunities to interact with the environment, while novelty can be enhanced by replacing toys and re-arranging the layout of the cage.

Past environmental enrichment studies have mostly relied on histology to investigate structural brain changes. Although histology is

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http://dx.doi.org/10.1016/j.neuroimage.2015.01.027
1053-8119/

selective for specific cell types and processes, it is usually applied to predetermined areas of interest and cannot be used to study the same individual over time. Based on histology, increases in cortical depth of the rat somatosensory and visual cortex have been observed in the earliest enrichment experiments (Diamond et al., 1964). The concomitant decreases in neuronal density have led to the hypothesis that the change in cortical depth must be due to dendritic changes. Indeed, subsequent research showed enrichment-related increases in dendritic branching and length, proliferation of dendritic spines and larger synapses (Faherty et al., 2003; Greenough et al., 1985; Greenough and Volkmar, 1973; Leggio et al., 2005; Turner and Greenough, 1985).

While the cross-sectional histological evidence is extensive, little is known about the time course of enrichment-induced brain changes and how they distribute across the whole brain. Thus, most histological studies of healthy wild type mice have focused on one or at most a few regions of interest within the brain, such as the dentate gyrus and the sensorimotor cortex (van Praag et al., 1999; Rossi et al., 2006; Zhu et al., 2009). Further, across studies enrichment protocols have varied considerably in number of mice, cage size and "furnishing". Enrichment periods have lasted from three weeks to several months (Table 2 in Nithianantharajah and Hannan (2006)). A study in gerbils used as many as 25 novel objects, including light bulbs, a sponge, and a miniature jungle gym (Rosenzweig and Bennett, 1969). Other studies added dietary variety by supplying enriched mice with extra treats, such as cheese, crackers, popcorn, and whole-grain nibble bars (Kempermann et al., 1997). Only recently efforts to standardize cages and enrichment have been made in mice (Sztainberg and Chen, 2010) and rats (Fares et al., 2013).

This study is set to rectify these short-comings by using in vivo MRI to observe the whole brain longitudinally before and after enrichment with a standardized and easily reproducible protocol in healthy wild-type mice. Using longitudinal measurements we aim to answer open questions of how early structural changes appear and whether they are maintained over the time course of enrichment. Using the same volumetric estimation methods as previous studies we can test whether the amplitude of the structural changes following enrichment is comparable to the amplitude of the changes following more targeted training paradigms, such as Morris water maze training (Lerch et al., 2011b). In other words, does continuous, multi-modal stimulation change brain structure more effectively than temporarily sparse training on a specific task?

Specifically, we predict that enrichment-induced cellular changes can be detected as local volume changes with in vivo MRI. We expect to observe structural brain changes in the hippocampal formation, due to the need to navigate through and adapt to the complex and dynamic maze layout of the enriched cage. The enrichment-related histological changes in the hippocampus have been described in detail before (Diamond et al., 1976; Faherty et al., 2003). Further, it has been shown that the rodent brain, and especially the hippocampal formation is altered by training on spatial memory tasks, such as the Morris water maze, to a degree detectable by MRI (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011b). We further anticipate that sensorimotor stimulation stemming from interacting with a complex environment is associated with volumetric changes in sensorimotor and visual cortices. Motor training by the physically demanding vertical traversal of the maze and exercising on the running wheel might be associated with changes in motor cortex and the cerebellum.

Previous studies suggest that about three weeks are needed to observe effects of enrichment (reviews in van Praag et al. (2000) and Nithianantharajah and Hannan (2006)). We thus subject mice to three weeks of enrichment to set an upper boundary on the time needed for the changes to take place. Because we anticipate experience-related changes in structural plasticity to be small (2–4% in Lerch et al. (2011b)), the aim of this study is to elicit MRI-detectable changes and not to differentiate the various components of enrichment, which individually might be associated with less pronounced structural alterations. Indeed, it has been suggested that physical and cognitive enrichment might be synergistic and produce larger changes than the sum of their individual effects (van Praag et al., 2000). Previous enrichment studies suggest plasticity processes operating in the order of days and weeks. However, following rotarod or reaching training some structural changes seem to occur rapidly within the first 24 h as evidenced by two-photon microscopy (Xu et al., 2009; Yang et al., 2009). To test whether there were rapid volumetric changes detectable with MRI, a separate cohort of mice was enriched for only 24 h. Finally, we test whether enrichment is associated with improved performance on the Barnes maze, a spatial navigation task. A number of previous rodent studies have reported that enrichment facilitates performance on spatial navigation tasks, such as the Morris Water Maze and radial maze task (Kempermann et al., 1997; Leggio et al., 2005). In summary, our study aims to systematically characterize the enrichment-related structural changes across the whole brain and how they develop across time using MRI in wild type mice. In addition we propose an easily reproducible enrichment method that will allow future studies to replicate and compare findings.

Methods

Mice

A total of 28 seven-week-old, male C57BL/B6 mice participated in the three-week environmental enrichment experiment. Mice were randomized into large cages that were fitted as described below. Each cage housed seven mice and contained grain, bedding, a water bottle, and one food hopper. The two social control cages were identical to the two enriched cages except for enrichment-related items. A second cohort of 24 male C57BL/B6 mice participated in a 24 h enrichment experiment. Fourteen mice were enriched as described below (seven mice per cage). Ten mice were housed in standard cages (five mice per cage). Mice had access to ad lib water and feed. Cages of both enriched and control mice were cleaned at the same day, every 3 days. All animal experiments were approved by the animal ethics committee of the Toronto Centre for Phenogenomics.

Environmental enrichment

Mice belonging to environmental enrichment and social control groups were housed in Double Decker Rat IVC Green Line cages (Tecniplast, Italy) with dimensions 462 × 403 × 404 mm (W × D × H) and a floor Area of 1862 cm^2. The cages were changed from their standard configuration by removing the second-level floor board. Water bottles were fitted with longer spouts to allow mice to drink from the ground level. The food hopper was fitted with a lid. Grain and bedding material was added to the floor. This base configuration was used for environmental enrichment and social control conditions.

For the environmental enrichment condition we added a dome, a running wheel and a three-level maze. The maze consisted of interlocking polycarbonate walls, ceilings/floors, and pipes (Fig. 1). The walls and pipes can be easily re-arranged to change the spatial layout of the maze and create new pathways. At every cage cleaning we altered the maze according to a sequence of pre-specified maze layouts (Supplementary Fig. 1).

Behavior

Following the initial three weeks of enrichment, enriched and control mice were trained on the Barnes maze, a test of spatial learning and memory (Barnes, 1979). Enriched mice continued to be housed in enriched cages during the behavioral testing period. Briefly, the Barnes maze consists of a circular table (diameter 1.5 m, 1 m tall) with 40 equidistant holes located at the periphery. Only one hole contains the 'escape box' which the mouse can enter to escape from the maze; the