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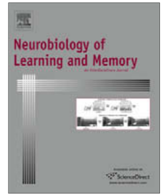

# A single exposure to an enriched environment stimulates the activation of discrete neuronal populations in the brain of the fos-tau-lacZ mouse

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ABSTRACT

Storage of experience, including learning and memory, is thought to involve plasticity within pre-existing brain circuits. One model for looking at experience-dependent changes is environmental enrichment (EE), which involves exposing animals to a complex novel environment. Animals exposed to EE have previously been shown to exhibit a variety of behavioural and structural alterations in the brain, including decreased stress, improved learning and memory, altered levels of immediate early genes and synaptic change in the visual cortex. We were interested in understanding what regions of the brain are activated during the initial stages of EE. We used *fos-tau-lacZ* (*FTL*) transgenic mice to examine changes in functional activation throughout the brain after a single exposure to EE. We found that there was a significant increase in *FTL* expression within particular morphologically identified neurons in a series of brain regions in the enriched group compared to control groups, indicating that multiple circuits were activated. These regions include the claustrum, infralimbic cortex, hippocampus, amygdala and the hypothalamus. The data suggest that EE stimulates an initial strong increase in activation of multiple functional circuits. These circuits are presumably involved in the initial response of the animal to the enriched environment.

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## 1. Introduction

Environmental enrichment (EE) is a popular model to study experience dependent change in the brain (Mohammed et al., 2002; Nithianantharajah & Hannan, 2006). In this model the animal is exposed to a novel environment containing many objects with different shapes, texture and colours. EE presumably involves many sorts of experiences, including sensory (including touch, smell, visual), emotional (stress, fear and other) and cognitive (novelty). Studies employing EE were the first to show experience-dependent synaptic plasticity (Turner & Greenough, 1985), with the demonstration of increased synaptic size in visual cortex and an overall increase in the thickness of the visual cortex.

Other effects of EE include a series of changes in the hippocampus, including increased synapse number and spine density (Rampon et al., 2000; Moser, Moser, & Andersen, 1994), altered synaptic transmission (Foster & Dumas, 2001; Green, McNaughton, & Barnes, 1990), increased neurotrophin levels (Ickes, Pham, Sanders, Albeck, Mohammed, et al., 2000) and enhanced neurogenesis (Kempermann, Kuhn, & Gage, 1997). EE has also delayed disease onset and progression in a mouse model of Huntington's disease (Hockly et al., 2002). Furthermore, there are significant behavioural effects on animals. Exposing animals to EE improves learning and memory (Rampon et al., 2000) and decreases stress (Nikolaev, Kaczmarek, Zhu, Winblad, & Mohammed, 2002). Our studies showed that EE results in changes in levels of pre- and post-synaptic proteins in multiple brain regions, throughout major cortical regions and in subcortical regions (Nithianantharajah, Levis, & Murphy, 2004). Thus, EE results in global changes in the brain.

Many of the studies of EE have focused on the effect over periods of weeks to months, as would be expected in the search for significant structural change in the brain. We were interested to look at regions of brain activation in the very first period of environmental enrichment, when the animal would be exposed to a novel and complex enriched environment for the first time. Such exposure might be similar to experiments involving simple exposure of an animal to a novel environment, albeit not an enriched environment. A number of other studies have looked for the effects on a novel environment on brain activation. Often these studies have used expression of the immediate early gene, c-*fos*, and other immediate early genes, as an indicator of activation in different regions of the brain (Herdegen & Leah, 1998; Herrera & Robertson, 1996; Wirtshafter, 2005). These studies show a number of different brain regions are activated following exposure to a novel environment, including infralimbic, anterior cingulate and retrosplenial cortices, hippocampus and other subcortical regions.

In our study, we have employed the *fos-tau-lacZ* (*FTL*) transgenic mouse (Wilson et al., 2002) to map brain regions which show c-*fos* related expression following initial exposure to an enriched

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environment. In the *FTL* mice, neurons that express c-*fos* express β-galactosidase (βgal) in their cell bodies, axons and dendrites, permitting direct visualization of their projections using a simple enzymatic assay (Wilson et al., 2002). Thus, the *FTL* transgenic mice have certain advantages in imaging functionally activated areas and circuits in the visual system, in that it can allow the identification of cell bodies and their projections. Our results confirm and extend findings from previous studies of EE and also allow us to characterise the cell morphology of the neurons activated in different regions of the brain; which is not possible using just the expression of immediate early genes such as c-*fos*.

## 2. Materials and methods

### 2.1. Animals and environmental enrichment

Mice were female transgenic *FTL* mice aged between 9 and 11 weeks. Female mice have been used successfully by us and others for enrichment experiments in the past (Frick & Fernandez, 2003; Nithianantharajah et al., 2004), as they have the advantage over males in these type of studies as they do not engage in fighting with either their siblings or unrelated females. This makes housing of the females much simpler compared with housing males. The mice were housed within the animal facility of the Biomedical Sciences Cluster of the University of Melbourne in a room with a 12 h light–dark cycle. Food and water were supplied *ad libitum*. One week prior to enrichment, all mice were moved to the behavioural laboratory where the EE experiments were performed, where they were housed in quiet (<60 dB) and low light (15–20 Lux) conditions. All experimental procedures adhered to and were approved by the Institutional Animal Care and Use Committee of the University of Melbourne.

Mice were exposed to an enriched environment for 1 h. The environment was as previously described (Nithianantharajah et al., 2004). Briefly, they were taken from their home cages and placed singly in a large plastic box ($45 \times 30 \times 35$ cm; 275–325 Lux) containing many objects (shredded paper, palette bedding, two large white foam pieces, three cardboard tubes, one cardboard box, two metallic toys) for 1 h. After that, the animals were returned to their home cages and left for another 2 h.

Two control groups were used: mice of the first group (cage control) were taken directly from their home cage. The other control group (handled control) were handled the same way as the enriched group except that they were placed in a new cage similar to their home cage for 1 h and exposed to relatively bright light (600–700 Lux), and then returned to their home cage for another 2 h.

### 2.2. Histochemical procedures

*FTL* mice were deeply anesthetized with an intraperitoneal overdose (100 μl) of Lethabarb (Virbac, Peakhurst, Australia), then perfused intracardially with 12 ml of 5% sucrose in water, followed by 24 ml of 4% paraformaldehyde (PFA) containing 0.005% gluteraldehyde in 0.1 M PBS buffer (pH 7.4). After 30 min, brains were dissected out of the cranium and post fixed in fresh 4% PFA for 15 min and then cryoprotected in 20% sucrose/PBS for 48–76 h at 4 °C. After cryoprotection the brains were frozen in OCT (Sakura Finetek, Torrance, CA, USA) and kept at −30 °C until cut. Coronal sections, 100 μm thick, were collected into wells of 24 well tissue culture plates (1–2 sections/well) containing PBS.

For detection of βgal enzymatic activity, PBS was aspirated and sections incubated in assay buffer (10 mM $MgCl_2$, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, and 1.5 mg/ml 5-Bromo-4-chloro-3-indolyl-B-D-galactopyranoside (X-gal; Astral, Gymea, Australia)) for 7 h at room temperature (RT) with agitating. After staining, sections were rinsed in PBS and stored in 4% PFA until mounting by placing into 0.5% gelatin in $H_2O$ before transferring to slides coated with 0.5% gelatin, 10% potassium chromium alum. Sections were allowed to dry, dehydrated through graded alcohols to histolene and coverslipped in DPX (VWR International, England). These procedures are more completely described elsewhere (Murphy, Greferath, & Wilson, 2007).

### 2.3. Microscopic examination and data analysis

Sections were analysed microscopically and areas which were βgal positive were identified by comparison with an atlas of the mouse brain (Paxinos & Franklin, 2001). Slide containing the serial sections of the brains were scanned using MIRAX SCAN (Zeiss, Germany) and studied using MIRAX viewer software to compare between the levels of activation between enriched and control conditions in different brain structures. Brain structures and areas which showed a qualitative difference in the level of activation were then examined quantitatively. Quantitative analysis was undertaken by counting $FTL^+$ cells in matched sections of enriched and both control groups of mice. The counting was performed in an area of the same shape and size for each brain region. Cell counts were done using an BX61 microscope (Olympus, Australia) at $400\times$ magnification and employing a red filter in the light path. For all areas where neurons were counted, the area of section was examined in detail by focussing at this high power throughout its thickness. All neurons which were stained within the section were thus examined and counted. The examination of the sections at this relatively high power and throughout its thickness enabled all neurons to be distinguishable and separable from each other, even when there was some overlap of stained neurons within the section. Only clearly stained cells were counted, where the whole cell body was uniformly stained (see Figs. 1, 3, 5 and 7 for examples of neurons). High power images of cells were taken with this microscope and in focus images of the neurons and their processes through the depth of the sections were obtained using the extended focal imaging application of AnalySIS software (Soft Imaging System, Olympus, Germany). Optical density measurements were undertaken using Image-Pro Plus software (Media Cybernetics, MD, USA) and according to previous published procedures (Mallard, Rees, Stringer, Cock, & Harding, 1998). Statistical significance was determined using ANOVA. Prism software (GraphPad Software, CA, USA) was used for performing statistical analysis.

## 3. Results

In order to examine the effect of short exposure to novel enriched environment on brain activation, *FTL* mice were placed individually into a large box filled with different objects and allowed to explore for 1 h. Following this, the mice were returned to their home cages and sacrificed and perfused 2 h later. The brains of the mice were subsequently processed and analysed for *FTL* expression. Two sets of controls were employed. The first was an unhandled cage control group. The second group, the handled control group, was handled in the same manner as the enriched group however they were not exposed to an enriched environment, but instead placed into a new, clean cage under relatively bright light, which is mildly stressful (Schweizer, Henniger, & Sillaber, 2009). This control was designed to give the mice a similar experience to the enriched mice, with the same level of handling and a degree of mild stress, but without the experience of an enriched environment.

Examination of sections throughout the rostro-caudal extent of the brain showed that most areas of the brain had similar levels of

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