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Research report

Diurnal effects of enriched environment on immediate early gene expression in the rat brain

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Abstract

Rodents housed in an enriched environment (EE) show increased neuronal plasticity with enhanced long-term potentiation and memory performance. We report an EE-induced increase in NGFI-A and Krox-20 mRNA expression exclusively during the dark period of the day. In addition, EE-housed rats showed considerable diurnal variation in NGFI-A, Krox-20, and NGFI-B mRNA expression which was absent in single-housed rats. Thus, EE-induced molecular changes are more evident during the dark phase when the rats have higher motor and exploratory activity. This is important to take into account in future studies of molecular mediators of experience-dependent neuronal plasticity.

Theme: Neural basis of behavior
Topic: Neural plasticity

Keywords: Environmental enrichment; Hippocampus; Plasticity; Inducible transcription factor

1. Introduction

Housing rodents in an enriched environment (EE) induce functional, neurophysiological, and structural brain plasticity, such as enhanced memory performance, increased brain weight and thickness of the cortex, enhanced dendritic branching, neurogenesis, synaptogenesis, and long-term potentiation (LTP) [12,20,21,23,24,33]. EE-induced plastic changes are often found in the visual cortex and the hippocampus, the latter in line with EE-enhanced cognitive function in healthy young, aged and brain lesioned rats [9,21,23,24,33]. Moreover, EE improves sensorimotor function and increase the number of dendritic spines in sensorimotor cortex after ischemic stroke in rats [15,25].

In the search of the molecular mechanisms of EE-induced neuroplastic effects, gene expression in rat brain has generally been studied after acute exposure to novelty and/or during the light phase of the day [26,30,32,37]. Considering that rats are night-active animals, many effects of a stimulating environment may be considerably more pronounced during the dark phase. Furthermore, information about the time course of EE-induced gene expression changes is limited, even though structural changes in the brain induced by EE are known to have different temporal profiles [10]. Thus, some effects may decrease due to habituation to the EE, while other effects may take a longer time to be established.

We have studied EE-effects on expression of the neuronal activity-dependent transcription factors NGFI-A (nerve growth factor induced-gene A; also known as egr1, krox-24, zif268, and TIS8), Krox-20 (known as egr-2), and NGFI-B (known as Nur77, N10, TIS1, or TR3). These immediate early genes (IEGs) act as third mes-

Abbreviations: DG, dentate gyrus; EE, enriched environment; IEG, immediate early gene; LTP, long-term potentiation; NGFI-A/B, nerve growth factor induced-gene A/B; NMDA, *N*-methyl-D-aspartate; PSD-95, postsynaptic density-95; SDS, sodium dodecyl sulphate; SSC, standard saline citrate

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sengers, and can alter cellular properties in response to extracellular stimuli through transcriptional regulation of specific target genes. These IEGs, as well as the NMDA-receptor-associated postsynaptic density protein PSD-95, have all been associated with LTP and/or memory formation [11,16,19,40]. Furthermore, NGFI-A, NGFI-B, and PSD-95 have all been reported to be upregulated after different periods of EE housing [26,31,37]. Notably, IEG gene expression may be influenced by the sleep/awake cycle [4,5] but a putative diurnal effect on expression of these genes has not been explored after environmental manipulation.

The aim of the study was to test the hypothesis that EE-induced IEG expression may vary between the light and dark period of the day and that EE-induced upregulation of IEG expression may decline with time in EE due to habituation.

2. Materials and methods

2.1. Animals and experimental design

The experimental protocol was approved by the Ethics Committee for Animal Research at Umeå University. Male Sprague–Dawley rats (B&K Universal Lab, Sollentuna, Sweden) were group housed 4–5 per cage in standard laboratory cages (595 × 380 × 200 mm) for 5 days prior to the start of the experiment, temp. 20–21 °C, with free access to water and food (standard rat chow).

7- to 8-week-old rats were randomized to enriched environment (EE) or deprived environment, i.e., individual housing in standard cages (425 × 266 × 185 mm). The EE consisted of a large cage (820 × 610 × 450 mm) with elevated horizontal and inclined boards and ladders and equipped with several objects, such as wooden tunnels, a chain, and a swing [7,14,25]. The objects were moved around once daily and some objects were exchanged with new ones. EE and deprived rats were housed in the same room, with lights on 06:00 to 18:00 h. Rats were sacrificed 8:00–9:00 h (AM groups) after 3, 7, 14, or 31 days of housing in EE (n = 4 at each time point), or 20:00–21:00 h (PM groups) after 7 or 31 days of EE housing (n = 4/group). Rats housed in the deprived environment for 31 days were sacrificed at corresponding AM/PM time points (n = 5/group) and were used as controls for all the EE-housed groups. All rats were sacrificed by decapitation within 30 s of initiation of contact. Trunk blood was collected and plasma was stored in −20 °C until corticosterone assay. The brains were removed, immediately frozen on dry ice and stored at −80 °C. Sagittal cryostat sections (10 μm, lateral 1.9 mm according to Paxinos and Watson [27]) from the right hemisphere were thaw-mounted onto Superfrost Plus microscope slides (Menzel-Gläzer, Braunschweig, Germany). Slides were stored at −80 °C.

2.2. Corticosterone assay

Plasma corticosterone was measured using a radioimmunoassay kit (Rat corticosterone ^{125}I assay system, Amersham Biosciences, Uppsala, Sweden) according to company instructions.

2.3. In situ hybridization

2.3.1. Preparation of riboprobes

Antisense and sense cRNA probes for NGFI-A, Krox-20, and NGFI-B were generated by in vitro transcription in the presence of [^{35}S]-UTP (Amersham Biosciences, Uppsala, Sweden), using nucleotides 184–414 of rat NGFI-A cDNA (GenBank #M18416), nucleotides 1142–1895 of mouse Krox-20 cDNA (GenBank #X06746), and nucleotide 1381–1528 of rat NGFI-B (GenBank # U17254) subcloned into pBSKS as templates. Plasmids were linearized with *Eco*RI (NGFI-A), *Bam*HI (Krox-20), or *Sma*I (NGFI-B) and then transcribed using T7 (NGFI-A and NGFI-B) or T3 (Krox-20) RNA polymerases.

2.3.2. Preparation of oligonucleotide probe

Antisense oligonucleotide probe for PSD-95 was commercially prepared (DNA Technology, Aarhus, Denmark) and the probe sequence was: 5′-CCCCTTTCCAATGT-GATCTCCTCATACTCCATCTCCCCCTCTGTT-3′. The oligonucleotide sequence showed no significant homology with other previously identified genes. The oligonucleotide was 3′ end labeled with [^{35}S]-dATP (Amersham Biosciences, Uppsala, Sweden) using terminal deoxyribonucleotidyl transferase (Roche Diagnostics GmbH, Mannheim, Germany), and then purified using QIAquick Nucleotide Removal Kit (QIAGEN, Hilden, Germany). The probe specificity for PSD-95 was proven by identical anatomical distribution pattern obtained using another probe complementary to bases 76–120 of rat PSD-95 (GenBank # M96853).

2.3.3. Pretreatment of slides and hybridization

Slides were fixed (4% paraformaldehyde in 0.1 mol/L phosphate buffer), acetylated (0.25% acetic anhydride in 0.1 mol/L triethanolamine) and dehydrated in graded ethanol before hybridization. For Krox-20 mRNA expression, sections were prehybridized for 2 h in 50 °C with 200 μL prehybridization buffer (50% formamide, 20 mmol/L NaCl, 50 mmol/L Tris pH 7.6, 25 mmol/L EDTA, 2.5 × Denhardt's solution, 0.625 mg/mL yeast tRNA), and briefly washed in 2 × standard saline citrate (SSC). All in situ hybridizations were then performed using 200 μL hybridization mixture (50% formamide, 0.6 mol/L NaCl, 20 mmol/L Tris–HCl pH 7.5, 1 × Denhardt's solution, 1 mmol/L EDTA, 0.2 mg/mL salmon sperm DNA, 10% dextran sulphate, 0.5 mg/mL yeast tRNA, and 0.1 mol/L dithiothreitol) per slide containing 1.0–3.0 × 10^6 cpm denatured riboprobe or 0.25 ng oligonucleotide probe, respectively.