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Research report

Environmental enrichment improves recent but not remote memory in association with a modified brain metabolic activation profile in adult mice

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ABSTRACT

Environmental enrichment is known to improve learning and memory in adult rodents. Whereas the morphological changes underlying these beneficial effects are well documented, few studies have addressed the influence of this housing condition on the neuronal networks underlying memory processes. We assessed the effects of environmental enrichment on behavioural performances and brain metabolic activation during a memory task in mice. Adult mice were housed in standard (SC) or enriched (EC) conditions for 3 weeks. Then, recent and remote memory performances were measured in the passive avoidance test. After testing, brain metabolic activation was assessed through cytochrome oxidase (CO) activity. EC improved recent memory, in association with an increased metabolic activation in the frontal and prefrontal cortices and a decreased activation in the baso-lateral amygdala and the hippocampus. EC did not improve remote memory, and globally decreased CO activity. Our findings suggest the involvement of regions of pivotal importance during recent memory, such as the frontal cortex, in the beneficial effects of EC.

1. Introduction

Environmental enrichment consists in complex housing conditions that enhance sensory-motor activities, social interactions and cognitive stimulations [1], and therefore stimulate brain plasticity. In rodents, enriched condition (EC) is well known to reduce aged-related cognitive deficits and to alleviate those occurring in models of neurodegenerative disorders [for review, see 2,3]. Although less studied, EC also improves cognitive abilities in healthy adult mouse. Indeed, it is associated to an improvement of long-term spatial [4–7] and non-spatial memory performances [6,7]. Depending on the duration of memory trace, long-term memory is classically divided into recent (days to years in human) and remote memory (decades to entire life in human). Such dissociation, based on functional neuroimaging studies showing different activation patterns between both types of memory [for review, 8], is also valuable in laboratory animals [for review see, 9]. Whereas the beneficial effects of enriched environment on recent memory have been reported in several studies [4,6,7,10], the effects of EC on remote memory still remain poorly documented.

Brain morphological changes induced by EC have been extensively studied and many reports show an increase in cortical weight and thickness, as well as hippocampus volume, neurogenesis and synaptogenesis [5,6,11,12]. Moreover, an increase in synaptic transmission efficiency has been reported after exposure to EC, as attested by an increased long term potentiation (LTP) in CA1 area of the hippocampus [10,13]. Modulation of synaptic plasticity by EC is also associated with changes in synaptic proteins mRNA expression, as for instance an increase in postsynaptic protein PSD-95 mRNA levels [14]. Moreover, an increased concentration of growth factors such as BDNF and NGF has also been reported in the hippocampus of enriched rodents [15]. Overall, these data show EC-related functional changes in brain regions that are fundamental for learning and memory processes.

Whether EC influences the neuronal networks recruited during a memory task and whether this neuronal plasticity could be part of the mechanisms underlying the beneficial effects of EC on memory is not known. Therefore, we aimed to assess the effects of EC on recent and remote memory in a passive avoidance test. To this end, we first performed a preliminary experiment in which we measured the duration of the memory trace in mice subjected to standard condition (SC); inter-session intervals (ISI) varied from 24 h to 12 weeks. Then, we assessed the effects of EC on performances using two different ISI, 24 h or 8 weeks, to dissociate recent and remote memory, respectively. Neuronal networks activated by the memory task were investigated by cytochrome oxidase

Abbreviations: EC, enriched condition; SC, standard condition; CO, cytochrome oxidase.

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histochemistry (CO). Being a key enzyme in the cellular oxidative energy metabolism, CO is an endogenous neuronal marker that reveals brain metabolic changes related to memory tasks such as those based on aversive stimulus [16–19].

2. Materials and methods

2.1. Animals

NMRI male mice aged 10 weeks at the beginning of the experiments were used (local breeding facility, F1 from Centre d'Elevage René Janvier, Le Genest, France). All animals were maintained in a room with reversed 12 h light–dark cycle (20:00–8:00), and a constant temperature (21 °C) and humidity (55%). Water and food were available *ad libitum*. All experiments were carried out in accordance with the European Communities Council Directive (86/809/EEC) regarding the care and use of animals for experimental procedures, and approved by the regional ethical committee (agreement number: 01-10-09/17/07-12).

2.2. Environmental enrichment

For each experiment, mice were randomly divided in two groups and affected to SC ($n = 18$) or EC ($n = 18$). SC mice were maintained in transparent polycarbonate cages ($42 \times 29 \times 15\,cm^3$, 6 mice per cage) containing nesting material and cardboard tube. Mice from the EC groups were continuously housed in environmental enrichment consisting in large polycarbonate cages ($80 \times 60 \times 60\,cm^3$, 18 mice per cage), provided with various objects of different shapes, sizes, colours, textures and material (wood, plastic and metal). Additionally, climbing ladders joined to an elevated platform, ropes, chains, swings, wooden blocks, plastic tubes of different diameters, nesting material and a large running wheel were placed inside the cage. To ensure novelty, most of the objects and their location were renewed twice a week. To limit inter-individual male agonistic behaviours [20], some objects were neither changed nor cleaned, and a piece of soiled sawdust was placed in the cage at each cage cleaning. EC mice were placed and maintained in these conditions 3 weeks before the start of behavioural testing and until the end of the experiment.

2.3. Behavioural experiments

Behavioural experiments were conducted between 8:30 and 12:30. Mice were placed in the experimental room 30 min before the beginning of the experiments.

2.3.1. Passive avoidance

Passive avoidance is the ability to inhibit innate preference for a dark and confined space after a single association with an aversive stimulus [21,22]. The apparatus consisted of two compartments with an electric grid floor, separated by an automated sliding door (LETICA LE 812, Bioseb, France). For acquisition session, the mouse was placed in the illuminated white compartment ($20 \times 21 \times 20\,cm^3$, 1000 lux), with the door closed. After a 30 s delay, the door opened and elapsed time before mouse entrance into the smaller dark compartment ($7.3 \times 7.5 \times 14\,cm^3$, 10 lux) was measured (maximum latency: 50 s). Immediately after entering, the door was closed and the mouse received an inescapable electric foot shock (0.4 mA; 2 s) before being placed back in its home cage. For the retention session, the experimental procedure was the same as for acquisition except that mice did not receive the electric shock (maximal latency to enter the dark compartment: 300 s). The latency to enter at the retention session was used as an index of memory performances. In case of a difference between groups in latencies at the acquisition session, a normalisation obtained by the ratio of retention/acquisition latencies has been used.

2.3.2. Experimental procedures

2.3.2.1. Effect of increased ISI on passive avoidance performances in SC mice. A first experiment was conducted to assess the effects of increasing ISI on passive avoidance task performances. Adult mice maintained in standard housing conditions were used. Each group was used to assess one of the following ISI: 24 h ($n = 12$), 3 weeks ($n = 12$), 5 weeks ($n = 12$), 8 weeks ($n = 12$), and 12 weeks ($n = 12$).

2.3.2.2. Effects of housing condition on recent and remote memory performances. To assess the effects of environmental enrichment on memory, mice were housed in SC or EC, and used to assess passive avoidance performances with either 24 h ISI ($n = 12$ per housing condition) or 8 weeks ISI ($n = 12$ per housing condition) (Fig. 1). These two ISI were chosen according to the results of the first experiment. In order to control for non-memory aspects of the behavioural task, such as locomotor activity or context arousal [23], additional SC and EC mice were exposed to the same behavioural procedure, except the electric foot shock during the acquisition session. These control groups are mostly used in addressing brain activation studies with aversive tasks using electric foot shock as stimulus [18,24,25]. The same ISI were used for these control groups: 24 h ISI ($n = 6$ per housing condition) or 8 weeks ISI ($n = 6$ per housing condition).

2.4. Cytochrome oxidase histochemistry

Immediately after the end of the retention session, mice of both test and control conditions were euthanised by cervical dislocation. The brains were rapidly removed, frozen in isopentane (−40 °C) and stored at −80 °C until cytochrome oxidase histochemistry was processed (Fig. 1).

Brain coronal sections (20 μm thickness) were obtained by cryo-cutting. The distance between sections was 100 μm along the whole brain, except for the frontal cortex for which the distance was 40 μm. Sections were mounted on gelatinised slides and stored at −80 °C until processing.

According to the method described by Wong-Riley [26], slides were incubated in the dark for 1 h at 37 °C in a solution of 50 mg diaminobenzidine, 20 mg horse heart cytochrome *c* (Sigma–Aldrich, Saint-Louis, MO, USA), 4 g sucrose and 18 mg catalase per 90 ml in 0.1 M phosphate buffer. Slides were thereafter rinsed in 10% sucrose in 0.1 M phosphate buffer, and then in 0.1 M phosphate buffer (twice for 5 min). Finally, they were dehydrated in successive increasing concentrations of ethanol solutions, immerged in Clearify™ (American Master Tech Scientific, Inc., Lodi, CA, USA), and coverslipped with R.A. Mounting Medium™ (Richard-Allan Scientific, Kalamazoo, MI, USA).

After being scanned (Nikon SUPER COOLSCAN 8000 ED, 6.3 μm resolution), sections were analysed in a blind manner with an image analysis software (ImageJ 1.6® software). The mean grey level of 8 sections of each region was measured, converted into optical density (OD). OD was then averaged for each structure reflecting the mean CO activity of the structure. According to the Paxinos and Franklin's atlas [27], several brain regions were selected: cortical regions (frontal, prelimbic, infralimbic, motor, parietal, anterior cingulate, enthorinal, perirhinal and visual cortices), hippocampal regions (dentate gyrus, CA3 and CA1 subfields, subiculum), baso-lateral amygdaloid nucleus, thalamic nucleus (ventral posterolateral and posteromedial), accumbens nucleus (core), striatum and medial septal nucleus (Fig. 2). The whole brain CO activity was measured by taking OD values of every two sections (45 sections per brain) and averaged for each animal. The mean OD value of each region of interest was then normalised by dividing by the mean OD value of the whole brain. This ratio was taken as a measure of the relative metabolic activity of the structure [16,28]. This relative CO activity of each test animal was expressed as a percentage of the corresponding mean CO activity of control animals for this region (SC or EC, 24 h or 8 weeks ISI). Because glial cells depend for the most part of anaerobic glycolysis rather oxidative metabolism [29] the CO activity is classically considered as an endogenous marker of neuronal metabolic activity [see for review, 30].

2.5. Statistical analysis

Values are expressed as mean ± SEM. Statistical analyses were processed with Statview 5.0® software. Analyses of variance (ANOVA) using as factor treatment (SC or EC) and repeated measurements (latency to enter the dark compartment during the acquisition and the retention sessions; CO activity of brain structures) were performed. When appropriate, *post hoc* Student–Newman–Keuls (SNK) multiple range tests were used. Data that were not normally distributed (Kolmogorov–Smirnov), such as the ratio of latency between the acquisition and the retention sessions, were analysed with the non-parametric Mann–Whitney U-test. A Spearman's rank correlation was used to test for correlations between CO activity and behavioural performances in selected brain regions for which a modulated metabolic activation by EC was found. A significant difference was considered when the *P* value was lower than 0.05.

3. Results

3.1. Effect of increased ISI on passive avoidance performances

In a preliminary experiment, ISI had a significant influence on mice performances in the passive avoidance test (Fig. 3). The latency to enter the dark compartment was globally increased between acquisition and retention sessions (ANOVA with repeated measurements: ISI effect $F_{(4,49)} = 3.78$, $P < 0.01$; session effect $F_{(1,49)} = 66.15$, $P < 0.001$, ISI × session interaction $F_{(4,49)} = 4.57$, $P < 0.01$). For each ISI, the retention latency differed from the acquisition latency (SNK multiple range test: $P < 0.05$), except for the 12 weeks ISI. Furthermore, with a 24 h ISI, the latency at the retention session was significantly higher compared with 12-week-ISI (SNK multiple range test: $P < 0.05$). The effects of housing conditions were then investigated with 24 h- (recent memory) and 8-week-ISI (remote memory).