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Spatial short-term memory in rats: Effects of learning trials on metabolic activity of limbic structures

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ABSTRACT

The oxidative metabolism was assessed in the hippocampus and related regions in rats that were trained in a spatial short-term memory task in the water maze following distinct training schedules. The cytochrome oxidase (COx) histochemistry was evaluated in groups of rats that received a daily session made up of either two or three learning trials. An untreated group was added to determine baseline levels of COx. We found that the 3-Trials group exhibited a better performance concerning the differences in latency between trials 1 and 2. Trained groups showed higher COx activity than the untreated group in the medial prefrontal cortex, dentate gyrus, CA1, and the mammillary region. However, a decrease in COx activity was found in the dentate gyrus, CA1, and supramammillary region of the 3-Trials group. In addition, COx activity levels found in this group were similar to those of the untreated group in some thalamic nuclei. Most of the regions that presented significant correlations between COx activity and behavioral scores were found in the 3-Trials group. These findings suggest an influence of task difficulty in the oxidative metabolism of brain regions involved in spatial learning.

The water maze is a learning paradigm for assessment of spatial memory in rodents [18] in which animals use visible cues from the environment to find a hidden platform. Within this context, the evaluation protocol for a short-term memory task involves the temporal storage of a spatial location to use within a training session, but not between sessions [5,16,17].

Some studies have shown that differences in the training schedule can influence learning of the water maze task [2,22,24,28]. For example, an improvement in spatial memory was found by separating trial blocks [2,24], reducing the retention interval [2,28], and increasing the number of training sessions [2,22]. Furthermore, some of these studies have shown changes in the number and morphology of hippocampal neurons as a consequence of modification in training variables [2,24].

Studies examining the neural substrates of memory have found that spatial learning is related to the functioning of a neuronal circuit composed of cortical, hippocampal, and diencephalic brain regions [1]. Hence, learning impairment in the water maze has been found after lesions of the dorsal hippocampus [19], medial prefrontal cortex [14], anterior thalamus [27] and mammillary region [23,25].

The cytochrome oxidase (COx) is a mitochondrial enzyme involved in the oxidative phosphorylation process in which ATP is generated and this metabolic energy is necessary to sustain neuronal functions [27]. Histochemical labelling of COx [7] has been extensively used in studies to evaluate the metabolic activity of limbic structures associated with spatial training in the water maze [3,15–17]. Interestingly, it has been shown that this technique can detect significant changes in metabolic activity over training days [3].

In the present study, we examined the effect of changes in the training schedule on brain metabolic activity of subjects trained in a matching-to-position task developed for the assessment of short-term spatial memory in the water maze. The COx activity of the dorsal hippocampus, medial prefrontal cortex, anterior thalamic nuclei, and mammillary body region was examined by quantitative COx histochemistry in rats submitted to a daily session made up of either two or three learning trials. In addition, correlations between COx activity and behavioral scores were also calculated.

Twenty-one male Wistar rats (240–270 g; *vivarium* of the University) maintained under standard conditions (21 ± 1 °C temperature, 65–70% relative humidity and 12 h light–dark cycle) with *ad libitum* access to food and water, were used. The experimental procedures were carried out according to the National legislation (Royal Decree no. 1201/2005), which is in compliance with the European Community Council Directive 86/609/EEC for the use and care of laboratory animals.

Fourteen rats were trained in the water maze [18] and the remaining rats were used as a control group (CO, $n = 7$) to determine basal COx activity. These rats remained in their home cage. The maze consisted of a cylindrical tank (150 cm diameter, 40 cm

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deep) of water at 22 ± 1 °C and a hidden platform. The pool was surrounded by panels on which the spatial clues were placed (a square, a horizontal line and a vertical line) and was divided into four quadrants to locate the platform and the start positions. Animals were trained on a matching-to-position task that was developed in three consecutive days (one session daily) and received a habituation session one day prior to the initial test [16,17]. Each daily session involved two identical trials for the "2-Trials group" ($n = 7$) and three identical trials for the "3-Trials group" ($n = 7$). The trials consisted of releasing the animal from one starting point and letting it swim until it reached the platform or 60 s had elapsed. If the animal had not reached the platform in this time, it was placed on the platform and kept there for 15 s. The intertrial interval was 5 s. The task demands a recall of the position occupied by the platform during the first trial. The locations of the start positions and of the hidden platform were the same during one session but varied on the different days in a pseudorandom order. The escape latencies were recorded. The mean escape latency was calculated statistically for each animal in the trials 1, 2 and 3 (in the case of the 3-Trials group), grouping all sessions carried out. Also, the difference in escape latency scores between trials 1 and 2 of the last session was used as performance index. This difference was defined as saving in escape latency to platform.

Ninety minutes after the end of the behavioral task, the animals were decapitated. The CO group was decapitated at the same time as the trained groups. Brains were removed intact, frozen in isopentane (Sigma–Aldrich, Spain) and stored at −40 °C. Coronal sections (30 μm) of the brain were processed with quantitative COx histochemistry [7]. Briefly, sets of tissue homogenate standards from Wistar rat brain were cut at different thickness (10, 30, 40 and 60 μm) and mounted on a slide. Previously, COx activity of the homogenate was spectrophotometrically assessed [8]. Sets of tissue homogenate standards of known COx activity were used as calibration standards in each staining batch. Sections were fixed with 0.5% (v/v) glutaraldehyde and 10% (w/v) sucrose in phosphate buffer (pH 7.6; 0.1 M). After this, the sections were rinsed in 0.1 M phosphate buffer with 10% (w/v) sucrose. Then 0.05 M Tris buffer, pH 7.6, with 275 mg/l cobalt chloride, 10% (w/v) sucrose, and 0.5% (v/v) dimethylsulfoxide, were applied. Subsequently, they were rinsed and incubated in a solution of 0.06 g cytochrome c (Sigma–Aldrich), 0.016 g catalase, 40 g sucrose, 2 ml dimethylsulfoxide and 0.4 g diaminobenzidine tetrahydrochloride in 800 ml of phosphate buffer (pH 7.6; 0.1 M). Finally, the tissue was fixed in 4% (v/v) buffered formalin with 10% (w/v) sucrose, dehydrated and coverslipped.

Quantification of COx histochemical staining intensity was done by densitometric analysis using a computer-controlled microscope (Leica Q550IW) with a light source (Leica DM-RHC) connected to a camera CCD (Cohu, Japan) and an image analysis software (Leica Q-Win). The mean optical density (OD) of each structure was measured on the right side of bilateral structures using three consecutive sections in each animal [16]. In each section, four, non-overlapping readings were taken using a square-shaped sampling window that was adjusted for each region size. A total of 12 measurements were taken in each region per animal and averaged to obtain a mean. To control staining variations between different staining baths, measurements were taken from stained brain homogenate standards. Regression curves between section thickness and known COx activity measured in each set of standards were calculated for each incubation bath [8]. OD values were then converted to COx activity units (μmol cytochrome c oxidized/min/g wet tissue at 23 °C) using the regression curves calculated.

The regions of interest were the infralimbic cortex (IL) and prelimbic cortex (PL); the anterodorsal thalamic nucleus (ADT), anteroventral thalamic nucleus (AVT) and anteromedial thalamic nucleus (AMT); the dentate gyrus (DG), CA1 and CA3 subfields

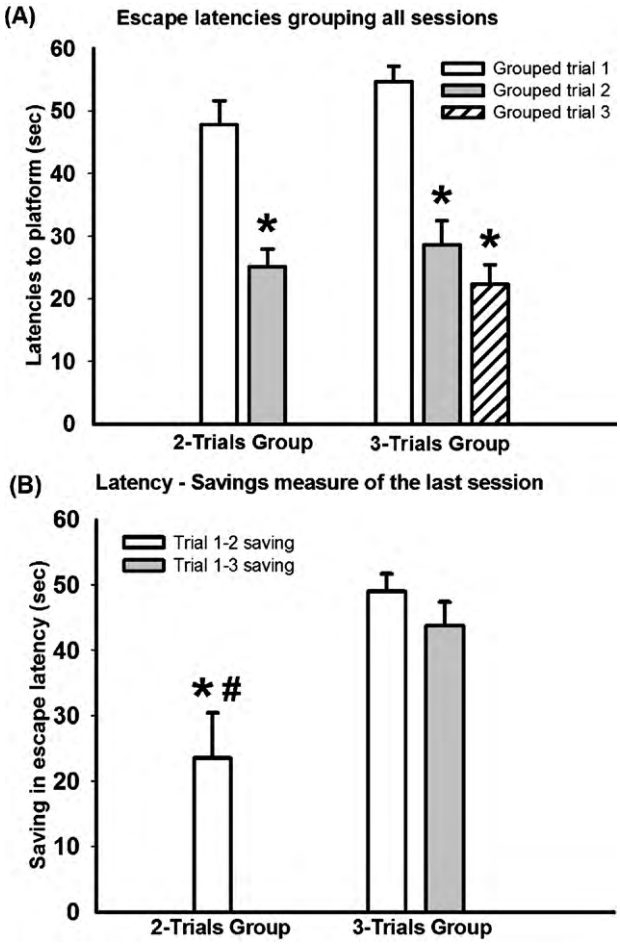

Fig. 1. (A) Escape latencies to platform (mean ± S.E.M). Significance of differences between trials, grouping all sessions carried out, $^{*}p \leq 0.001$. (B) Difference in latency scores between trials (mean ± S.E.M) within the third session. Significant difference between groups in the 1–2 saving measure, $^{*}p = 0.005$. Significantly different with respect to the 1–3 saving measure of the 3-Trials group, $^{\#}p = 0.02$.

of the hippocampus; and the medial mammillary nucleus (MM), lateral mammillary nucleus (LM) and supramammillary nucleus (SuM). The stained coronal sections corresponded to Bregma levels [20]: +3.24 mm for IL and PL; −1.92 mm for ADT, AVT and AMT; −3.84 mm for DG, CA1 and CA3; and −4.44 mm for MM, LM and SuM.

A paired-sample *t*-test was used to describe differences between trials in escape latencies, grouping all training sessions (Fig. 1A). A reduction of escape latencies was showed for both trained groups in trial 2 ($t_6 \geq 6.81$, $p \leq 0.001$). In addition, shorter escape latencies were found during trial 3 in the 3-Trials group ($t_6 = 9.08$, $p \leq 0.001$). Also, saving from trial 1 to trial 2 within the last session was compared between groups using a *t*-test. As shown in Fig. 1B, this saving was lower in the 2-Trials group ($t_{12} = -3.47$, $p = 0.005$). Similarly, this saving was significantly lower than the trials 1–3 saving of the 3-Trials group within the last session ($t_{12} = -2.639$, $p = 0.02$; Fig. 1B).

The results of the COx activity measures obtained from the different brain regions studied are illustrated in Table 1. COx activity of the studied groups was compared using a one-way analysis of variance and Tukey's test was applied as post hoc test. COx values taken in the IL, CA3 hippocampal subfield, and AMT were similar between groups ($F_{2,18} \leq 2.52$, $p \geq 0.10$). However, differences between groups were found in the PL, MM and LM ($F_{2,18} \geq 6.83$, $p < 0.001$), where an increase in COx activity was found in the trained groups ($p < 0.05$). Also, an increase in COx activity was found in the trained groups in the DG, CA1 and SuM ($F_{2,18} \geq 20.79$,