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Dynamic functional brain networks involved in simple visual discrimination learning

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ABSTRACT

Visual discrimination tasks have been widely used to evaluate many types of learning and memory processes. However, little is known about the brain regions involved at different stages of visual discrimination learning. We used cytochrome c oxidase histochemistry to evaluate changes in regional brain oxidative metabolism during visual discrimination learning in a water-T maze at different time points during training. As compared with control groups, the results of the present study reveal the gradual activation of cortical (prefrontal and temporal cortices) and subcortical brain regions (including the striatum and the hippocampus) associated to the mastery of a simple visual discrimination task. On the other hand, the brain regions involved and their functional interactions changed progressively over days of training. Regions associated with novelty, emotion, visuo-spatial orientation and motor aspects of the behavioral task seem to be relevant during the earlier phase of training, whereas a brain network comprising the prefrontal cortex was found along the whole learning process. This study highlights the relevance of functional interactions among brain regions to investigate learning and memory processes.

1. Introduction

It has been suggested that memories might be gradually reorganized over time (Frankland & Bontempi, 2005; Ribot, 1982) and that brain regions dynamically interact at different stages of the learning process. This is in agreement with recent research that emphasizes the relevance of functional interactions between different brain regions (Henson & Gagnepain, 2010; Vann & Albasser, 2011) in memory processes. The functional connectivity in local and distal anatomical brain pathways has mostly emerged from neuroimaging studies in humans (Guye, Bartolomei, & Ranjeva, 2008). However, dynamic interactions between brain regions during the learning process have also been found in rodents analyzing correlations in neuronal oxidative metabolism (Conejo, Gonzalez-Pardo, Gonzalez-Lima, & Arias, 2010). In this context, studies in humans and rodents indicate that interactions among cortical and non cortical brain regions are necessary during memory processing (Zimmer, 2008). Moreover, there is currently a vigorous debate about the traditional concept of 'systems memory consolidation' stating that declarative memory is initially hippocampus-dependent but it is later represented by a distributed cortical network independent of the hippocampus (Lesburgueres et al., 2011; Takashima et al., 2009; Winocur, Moscovitch, & Bontempi, 2010). Although several studies show a dissociation between different memory systems related to the striatum (procedural or habit learning) and those related to the hippocampus (declarative or spatial learning), there is also evidence suggesting the involvement of both brain regions in procedural learning tasks involving the use of visual cues (Fidalgo, Conejo, Gonzalez-Pardo, & Arias, 2012).

In the present study, we evaluated the contribution of different brain networks during visual discrimination learning in a water-T maze. The role of the hippocampus and the striatum together with anatomically related brain regions on discrimination learning is still unclear. For this purpose, cytochrome c oxidase (CO) activity was analyzed in the dorsal hippocampus, the dorsal striatum, the prefrontal, parietal, and temporal cortices as well as the nucleus accumbens and the amygdala complex. We used quantitative CO histochemistry as a metabolic brain mapping technique because it has a high anatomical resolution and it provides a measure of steady or sustained changes in oxidative metabolism associated with brain function (Wong-Riley, 1989). CO histochemistry has been successfully used in previous studies to map changes in brain oxidative metabolism involved in several learning tasks in rats (Arias, Fidalgo, Felipo, & Arias, 2014; Bruchey & Gonzalez-Lima, 2008; Leger et al., 2012). In addition, the CO method can be also used to investigate the functional interactions between brain regions. In this context, it has been previously demonstrated that

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brain regions that are functionally coupled show dynamical and coordinated changes in their metabolic capacity, expressed as changes in the strength of correlation in CO activity between regions (Arias, Mendez, Arias, & Arias, 2012; Fidalgo, Conejo, González-Pardo, Lazo, & Arias, 2012; Puga, Barrett, Bastida, & Gonzalez-Lima, 2007; Sakata, Coomber, Gonzalez-Lima, & Crews, 2000).

2. Material and methods

2.1. Animals

A total of 68 male Wistar rats weighing between 150 and 250 g were used in this experiment. The animals were obtained from the University of Oviedo central vivarium (Oviedo, Asturias, Spain) and were randomly housed in groups of five under standard conditions (12-h light/dark cycle with lights on from 08:00–20:00 h) at constant room temperature of 21 ± 2 °C with *ad libitum* access to food and water. Care and use of laboratory animals were done in accordance with the European Communities Council Directive (2010/63/UE) and the Spanish legislation (RD 1201/2005). All efforts were made to minimize the number of animals used and their suffering.

2.2. Apparatus

Rats were trained in a water T-maze made of black fiber-glass filled with tap water (23 ± 1 °C). The main alley (100 × 20 × 40 cm) was connected to two side arms (right and left) measuring 45 × 20 × 40 cm. A submerged platform made of Plexiglas (15 × 18 cm) was placed in one of the two arms always near a visual intra-maze cue. The position of the platform and the associated visual cue (a rectangular yellow card with a printed horizontal thick black line in the middle) changed from side to side (left or right arm) following a pseudorandom sequence. The visual cue was attached to the end wall of the goal arm. Each trial was recorded and swim paths of the animals were analyzed later using a computerized video-tracking system (Ethovision Pro, Noldus Information Technologies, Wageningen, The Netherlands).

2.3. Behavioral procedure

In order to discard possible motor and sensory deficits, animals were tested in a neurological assessment battery. The neurological tests used include the following tests: abduction response of hindlimbs, grasping reflex, extension and flexion reflexes, hearing and vestibular responses, head shaking reflex, pupillary reflex, negative geotactic response, and righting reflex (Bures, Buresova, & Huston, 1976). No animals were discarded due to abnormal neurological responses.

The visual discrimination task was performed between 09:30 and 13:30 h. After daily handling during five days, rats were randomly assigned to four different experimental groups. Animals were trained in a visual discrimination task using a water T-maze. During the habituation day, animals were gently immersed in the water T-maze for 1 min, without any escape platform available. Training proceeded during the following six days in which each rat received a single daily 12-trial session. During this training session, a hidden escape platform was placed underneath the water level and it was associated with a visual cue attached to the wall of the arm where the platform was located. In each trial, rats were allowed to swim to locate the platform or they were placed on it after 60 s, where they remained for 15 s before returning them to the cage for 30 s. The trial was considered as correct when the rat reached the platform whereas introducing the head in the incorrect arm was counted as error. The position of the escape platform and the adjacent visual cue was randomly changed between trials. The learning criterion used for the 6-*day group* was eight or more correct trials out of 12 trials.

2.4. Design

Three experimental groups of animals were used in this experiment that were sacrificed at different time points of the behavioral training, after 1 day (1-*day group; n* = 10), 4 days (4-*day group; n* = 10), or 6 days (6-*day group; n* = 9) of training respectively. In addition, three free-swimming groups (one of each experimental group) were composed of rats that were placed in the maze the same number of times and days as compared to their respective experimental groups but without escape platform available. The 1-day swim control group (*SC-1-day group; n* = 10), the 4-day swim control group (*SC-4-day group; n* = 10) and the 6-day swim control group (*SC-6-day group; n* = 9). The free-swimming control groups swam during an amount of time equivalent to the mean daily escape latencies recorded for the experimental groups. In addition, one group of animals was only gently handled (*handling only group*; *n* = 10) and it was decapitated 90 min later.

2.5. Tissue preparation

90 min after finishing the behavioral procedure the animals were decapitated. Their brains were quickly removed, frozen in isopentane at −70 °C (Sigma–Aldrich, Madrid, Spain) and stored at −40 °C to preserve the brain tissue and enzyme activity. Next, 30 μm-thick coronal sections were obtained from the brain tissue using a cryostat microtome (MicromHM 505 E, Heidelberg, Germany). These sections were mounted on slides and stored at −40 °C until processing with quantitative CO histochemistry. Some sections from a few subjects could not be used as a result of tissue processing, although the final number of sections available for histochemistry was enough in all cases.

2.6. CO histochemistry

We used a modified version of the method originally described by Wong-Riley, 1989, based on the quantitative CO histochemical method developed by Gonzalez-Lima and Cada (1994). To control staining variability across different baths, sets of brain tissue homogenate standards of known CO activity from Wistar rat brain were cut at different thicknesses (10, 30, 50 and 70 μm) and included with each bath of slides. In brief, slides were fixed for 5 min with a 0.5% glutaraldehyde solution, rinsed three times in phosphate buffer and preincubated 5 min in a solution containing 0.05 M Tris buffer, pH 7.6, with 275 mg/l cobalt chloride, 10% (w/v) sucrose, and 5 ml dimethyl-sulfoxide. Once the sections had been rinsed in phosphate buffer (pH 7.6; 0.1 M), they were incubated at 37 °C for 1 h in the dark and with continuous stirring in a solution containing 50 mg 3,3′-diaminobenzidine, 15 mg cytochrome c (Sigma, St. Louis, MO, USA), 4 g sucrose per 100 ml phosphate buffer (pH 7.4; 0.1 M). The reaction was stopped by fixing the tissue in buffered formalin for 30 min at room temperature with 10% (w/v) sucrose and 4% (v/v) formalin. The slides were dehydrated, cleared with xylene 10 min and coverslipped with Entellan (Merck, Darmstadt, Germany). CO histochemical staining intensity was measured by densitometric analysis using a computer-assisted image analysis workstation (MCID, InterFocus Imaging Ltd., Linton, England) composed of a high precision illuminator, a digital camera and a computer with specific image analysis software. A total of twelve measurements were taken per region. These measures were averaged to obtain one mean per region for each animal and were expressed as arbitrary units of optical density (OD) in the prefrontal cortex (anterior cingulate, prelimbic and infralimbic areas),