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Changes in the element concentration of the dorsal hippocampus CA1 region during memory consolidation and reconsolidation

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ABSTRACT

The concentration and distribution of Mg, P, Cl, K, Cu and Zn in the dorsal hippocampus CA1 region of rat brains were studied during memory consolidation and reconsolidation processes stimulated with inhibitory avoidance (IA) tests. Experimental rats were divided into four groups: i) group not submitted to inhibitory avoidance task (IA-N); ii) group submitted to inhibitory avoidance training session (IA-Y); iii) group submitted to inhibitory avoidance reactivation session but did not step down from the platform (IAR-N); and iv) group submitted to avoidance reactivation session and stepped down from the platform (IAR-Y). Elemental concentration and distribution in the CA1 hippocampus region were obtained through the Particle-Induced X-ray Emission (PIXE) technique. The results indicate that the concentration of Mg, P, Cl, K and Cu increased during memory consolidation. During the memory reconsolidation process, the concentrations of Mg, P, Cl and K increased, while Cu and Zn had no significant changes with respect to their basal condition. These results show that the major part of these elements may be engaged in memory consolidation could be also participating in memory reconsolidation. For all elements, the general trend related to their concentration did not change during reconsolidation regardless the presence of a novelty event, i.e. stepping down from the platform.

1. Introduction

Memory can be defined as the ability to acquire, store and recall information. The acquisition phase represents the time when new information is acquired. It involves the transport of elements through neuron's membrane and requires the participation of several signaling pathways in order to store the memory permanently (Lamprecht and Ledoux, 2004). The molecular activity related with early stages of memory takes place into the hippocampus and it is known as consolidation (Dudai, 2004). Memory consolidation is a complex process regulated by a series of biochemical reactions leading to progressive post-acquisition memory stabilization (Jobim, 2011). Consolidated memories become again labile and susceptible to intervention when reactivated. To keep the memory activated, it is necessary to go through a new process of stabilization called reconsolidation (Lee, 2008). The reconsolidation provides a window of opportunities for the maintenance and strengthening of the evoked mnemonic trace. Many treatments used to block consolidation can impair reconsolidation, which leads to the hypothesis that the reconsolidation leads mostly to the same molecular event that took place during consolidation. But even with some similarities, consolidation and reconsolidation processes are not identical (Alberini, 2005). As a matter of fact, some studies pointed out that some proteins are synthesized specifically during consolidation while some others are synthesized specifically during reconsolidation, thus revealing their different molecular profiles (Tronel et al., 2005). In addition, the timing and purpose of these two processes are also different since consolidation gives rise to new memories after acquisition while reconsolidation works as a memory update in a post-reactivation stage (Lee, 2010). There are also limiting factors for reconsolidation which may prevent it from happening (Lee, 2009).

Recent studies have shown the importance of chemical elements to the synapse plasticity and particularly to the memory consolidation. For instance, the influx of Ca into post-synaptic neurons through N-methyl-

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D-aspartate receptors (NMDAR) and voltage-gated calcium channels (VGCCs) triggers the synaptic plasticity (Byth, 2014). Another study showed that NMDAR blockage by Mg suppressed basal expression of a repressor isoform of CREB (cyclic adenosine monophosphate response element binding protein) during uncorrelated activities. This blockage has direct implications in long term memories (Miyashita et al., 2012). Moreover, Ca influx activates K channels (SK2), which is directly involved in contextual memories (Murthy et al., 2015). In a different approach, it has been shown that Na spikes are necessary for the induction of LTP (Long Term Potentiation) in the dendrites of CA1 pyramidal neurons (Kim et al., 2015). This induction may rely on an ionic mechanism mediated by Na flux during synaptic activation, which would affect the membranés potential and lead to Ca uptake by the cells (Araya et al., 2007). To brake this cycle, Cl is released and activates γ-aminobutyric acid receptors (GABAR) to create a reversal potential (Spitzer, 2010). Finally, other elements like Cu and Zn are usually associated with learning and memory formation processes (Lutsenko et al., 2010). Indeed, Cu and Zn are very likely to be present at synapses during these processes since they play an important role as cofactors for many proteins and enzymes, including various effectors of synaptic plasticity.

In order to understand how acquisition and reactivation processes involved in memory stabilization change the elemental concentrations and distributions in the brain, it is necessary an analytical tool with very good spatial resolution to scan portions of the brain and provide qualitative and quantitative results. In this context, Particle-Induced X-ray Emission (PIXE) (Johansson et al., 1995) has been applied to quantify elements in different organic matrix with a limit of detection of the order of 1 mg/kg or below. PIXE consists in the emission of characteristic X-rays induced by energetic protons interacting with target atoms, thus providing its elemental composition and concentrations. PIXE is a powerful analytical tool since it is relatively fast and requires virtually no sample preparation, thus avoiding contamination by chemicals (Santos et al., 2010). Moreover, its multielemental character and relatively low limit of detection makes it one of the most used ion beam techniques for applications in biomedical sciences including genotoxicity (de Souza et al., 2015) and biomonitoring (Zocche et al., 2014).

Recently, the element composition of cortex and hippocampus from rats submitted to inhibitory avoidance (IA) task at different stages of memory consolidation was measured by PIXE (Jobim et al., 2014). This study showed that the concentrations of Ca, Fe and Cu are higher in the hippocampus than in the cortical region during memory consolidation process. Moreover, significant differences were observed for the concentrations of Ca and Cu between the hippocampus and cortical region for those rats that learned the task.

In this context, the aim of the present work was to obtain information on the elemental concentration of the hippocampus during consolidation and reconsolidation process. To that end, particular protocols for sample preparation and step down IA essays were developed. Finally, PIXE was employed in order to obtain the concentration of the elements in brain tissues, including the analysis using a micrometric beam spot size (micro-PIXE). Despite this work deals with ionic species as far as physiological processes are concerned, we will refer to inorganic elements since the present technique employed in this work cannot differ from neutral elements and ions.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (340–440 g at the time of the experiments) from the State Health Research Foundation (FEPPS-RS, Porto Alegre, RS, Brazil) were housed in plastic cages (five per cage) with sawdust bedding. They were maintained on a 12 h light/dark cycle (lights turned on at 7 a.m.) at a room temperature of 22 ± 1 °C. The rats had access to standardized food pellets and water *ad libitum*. Considering the important role of the circadian rhythm in memory acquisition and recall events (Smarr et al., 2014), the behavioral procedures and the sample collection were conducted during the light phase of the cycle between 9 a.m. and 6 p.m. In more details, the IA behavioral task was performed between 9 a.m. and 11 a.m. After the IA training or test sessions, the animals were placed back in their home cages and stayed there during 3 h. The euthanasia and sample collection procedures took place from 12:00 p.m. to 6 p.m. All experimental procedures were performed in accordance with the Guide for Care and Use of Laboratory Animals (NIH publication N° 80–23, revision 1996) and all protocols were approved by the institutional research ethics and animal care committee (document number GPPG-HCPA 10-0071). All efforts were made to minimize the number of animals used in the experiments and their suffering.

2.2. Inhibitory avoidance (IA) protocols

IA apparatus was a 50 × 25 × 25 cm^3 acrylic box (Albarsch, Porto Alegre, RS, Brazil) with a floor consisting of parallel stainless steel bars (1 mm diameter) spaced 1 cm apart from each other. A 7 cm wide and 2.5 cm high platform was placed on the floor of the box against the left wall. On training sessions, rats were placed on the platform and their latency to step down on the grid with all four paws was measured with an automatic device. Immediately after stepping down on the grid floor, rats received a 0.7 mA footshock during 2 s and were removed from the apparatus right after that. Retention test sessions took place 24 h and 48 h after training sessions by placing the rats on the platform and recording their latencies to step down on the grid floor. No footshock was applied during retention test sessions. Step down latencies during the retention tests (maximum of 180 s) were used as a measure of IA memory retention.

Four main experimental groups with distinct number “n” of animals were considered: i) IA-N group (n = 7), which was not submitted to inhibitory avoidance task; ii) IA-Y group (n = 10), which was submitted to inhibitory avoidance training session; iii) IAR-N group (n = 8), which was submitted to inhibitory avoidance reactivation session but did not step down from the platform; and iv) IAR-Y group (n = 8), which was submitted to avoidance reactivation session and stepped down from the platform. In this case, however, they were gently pushed from the platform.

Finally, other groups were used as control: i) FS (footshock) group (n = 5) submitted to latent IA protocol in which the access to the platform was blocked and each rat stayed 3 h inside the IA box before the application of the footshock; ii) CO (context) group (n = 5) exposed to a 15 min IA box context without the footshock experience; iii) SA-N group (n = 7) received saline injection and was not submitted to IA training; iv) SA-Y group (n = 10) received saline injection right after the IA training; v) KE-N group (n = 7) received ketamine injection and was not submitted to IA training; and vi) KE-Y group (n = 10) received ketamine injection right after IA training. It is believed that protocol i) (FS group) separates the footshock experience from the contextual representation, while protocol ii) (CO group) selects those animals that received only the contextual information without the aversive stimulus to be paired with. Protocols iii) and iv) (SA-N and SA-Y groups respectively) tested for any possible influence of the vehicle (saline solution) in the IA protocols employing ketamine. Finally, protocols v) and vi) (KE-N and KE-Y groups respectively) checks whether the use of ketamine leads to any change in the element concentrations after the IA training. The description of all groups is summarized in Table 1.

2.3. Sample preparation

After IA training and testing, the animals returned to their cages and 3 h later, when a second protein synthesis wave was happening on the CA1 region of the dorsal hippocampus (Quevedo et al., 1999), they