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Two waves of proteasome-dependent protein degradation in the hippocampus are required for recognition memory consolidation

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ABSTRACT

Healthy neuronal function and synaptic modification require a concert of synthesis and degradation of proteins. Increasing evidence indicates that protein turnover mediated by proteasome activity is involved in long-term synaptic plasticity and memory. However, its role in different phases of memory remains debated, and previous studies have not examined the possible requirement of protein degradation in recognition memory. Here, we show that the proteasome inhibitor, lactacystin (LAC), infused into the CA1 area of the hippocampus at two specific time points during consolidation, impairs 24-retention of memory for object recognition in rats. Administration of LAC after retrieval did not affect retention. These findings provide the first evidence for a requirement of proteasome activity in recognition memory, indicate that protein degradation in the hippocampus is necessary during selective time windows of memory consolidation, and further our understanding of the role of protein turnover in memory formation.

1. Introduction

The formation and storage of new memories involve synapse remodeling. It is currently well established that gene expression and protein synthesis triggered by learning events mediate changes in synapse structure and activity associated with memory formation (Alberini, 2009; Costa-Mattioli, Sossin, Klann, & Sonenberg, 2009; Kandel, Dudai, & Mayford, 2014; McGaugh, 2000). However, normal neuronal function and synaptic modification depend not only on synthesis, but also on the degradation of proteins (Bingol & Sheng, 2011). Thus, increasing attention has been devoted to understanding the role of protein turnover in synaptic plasticity and memory. Protein turnover in neurons is regulated at multiple levels. One major mechanism is the attachment of multiple ubiquitin molecules to lysine residues in a protein, which signals for protein degradation by the proteasome, a proteolytic organelle (Bingol & Sheng, 2011; Yi & Ehlers, 2005). Proteasome inhibition leads to protein accumulation, ultimately resulting in alterations in intracellular signaling and synapse function and reorganization (Ehlers, 2003; Patrick, 2006).

A growing number of studies have shown a role for the ubiquitin–proteasome system (UPS) in mammalian synaptic plasticity and memory. The proteasome inhibitor MG132 inhibits the early and late phases of long-term potentiation (LTP) in the CA1 area of the rat dorsal hippocampus (Karpova, Mikhaylova, Thomas, Knöpfel, & Behnisch, 2006), whereas another, more specific inhibitor, lactacystin (LAC), enhances early-phase but blocks late-phase hippocampal LTP (Dong, Upadhya, Ding, Smith, & Hegde, 2008; Fonseca, Vabulas, Hartl, Bonhoeffer, & Nägerl, 2006). LAC given after behavioral training in rats impairs the consolidation of memory for inhibitory avoidance when infused into the CA1 area (Lopez-Salon et al., 2001), taste aversion when administered into either the amygdala or the insular cortex (Rodriguez-Ortiz, Balderas, Saucedo-Alquicira, Cruz-Castañeda, & Bermudez-Rattoni, 2011), cued and contextual fear conditioning when given into the amygdala (Jarome, Werner, Kwapis, & Helmstetter, 2011), and trace fear conditioning when infused into the prefrontal cortex (Reis, Jarome, & Helmstetter, 2013). In addition, LAC administration into the CA3 hippocampal region hinders the consolidation of spatial memory in

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mice (Artinian et al., 2008). The levels of ubiquinated synaptic proteins increase in different brain areas after either inhibitory avoidance training (Lopez-Salon et al., 2001) or fear conditioning (Jarome et al., 2011; Reis et al., 2013) in rats.

In spite of this evidence, there is still controversy regarding the need for proteasome activity in the formation of new memories. One study found that LAC infusion into the CA1 area of the hippocampus blocked the extinction of fear conditioning and prevented the memory-impairing effect of the protein synthesis inhibitor anisomycin when given after retrieval, but did not affect memory formation when administered after training. Thus, it was proposed that protein degradation plays a specific role in the destabilization of preexisting memories, allowing their modification by reconsolidation or extinction, but may not be required for the consolidation of memories for novel learning experiences (Lee et al., 2008). A role for protein degradation in memory extinction and reconsolidation is also supported by other studies (Artinian et al., 2008; Lee et al., 2012; Ren et al., 2013). Thus, the characterization of the involvement of protein degradation in memory consolidation, as opposed to its modification after retrieval, warrants further investigation.

Moreover, previous studies have not examined the possible role of protein degradation in recognition memory. Over the past years, the object recognition task has been extensively used in the investigation of the biological basis of memory in healthy rodents, as well as in studies focusing on memory dysfunction associated with brain disorders and the effects of potential therapeutic agents (Dere, Huston, & De Souza Silva, 2007; Ennaceur, 2010; Ennaceur & Delacour, 1988; Lyon, Saksida, & Bussey, 2012). Formation of memory for object recognition requires the dorsal hippocampus (Broadbent, Gaskin, Squire, & Clark, 2010; Clark, Zola, & Squire, 2000; Cohen et al., 2013; de Lima, Luft, Roesler, & Schröder, 2006; Gaskin, Tremblay, & Mumby, 2003), however the molecular pathways involved remain relatively poorly described. Here, we examined the requirement of proteasome activity for the consolidation of object recognition.

2. Methods

2.1. Subjects

Adult male Wistar rats (225–340 g at time of surgery) were obtained from the institutional breeding facility (CREAL, ICBS, UFRGS). Animals were housed five per cage in plastic cages with sawdust bedding, and maintained on a 12 h light/dark cycle at a room temperature of 22 ± 1 °C. The rats were allowed *ad libitum* access to standardized pellet food and water. All experiments took place between 9 AM and 6 PM. All experimental procedures were performed in accordance with the Brazilian Guidelines for the Care and Use of Animals in Research and Teaching (DBCA, published by CONCEA, MCTI) and were approved by the institutional animal care committee (CEUA-HCPA).

2.2. Surgery

Animals were implanted under anesthesia with ketamine (75 mg/kg) and xylasine (25 mg/kg) with bilateral 14-mm or 9.0-mm, 23-gauge guide cannulae aimed 1.0 mm above the CA1 area of the dorsal hippocampus, as described in previous studies (de Lima et al., 2006; Jobim et al., 2012). Coordinates anteroposterior, −4.3 mm from bregma; mediolateral, ±3.0 mm from bregma; ventral, −2.0 mm from skull surface were obtained from the atlas of Paxinos and Watson (2007). Animals were allowed to recover at least 7 days after surgery.

2.3. Drug infusions

General procedures for intrahippocampal infusions were described in previous reports (de Lima et al., 2006; Jobim et al., 2012). At the time of infusion, a 30-gauge infusion needle was fitted into the guide cannula. The tip of the infusion needle protruded 1.0 mm beyond the guide cannula and was aimed at the CA1 area. LAC or vehicle (VEH; 2% dimethylsulfoxide, DMSO, in saline) were infused during a 30-s period. The infusion needle was left in place for an additional minute to allow diffusion of the drug away from the needle tip.

Immediately after, 1.5, 3, or 6 h after object recognition memory training, or immediately after retrieval, rats received a bilateral 1.0-μl infusion of VEH or LAC (200 μM; Sigma–Aldrich, St. Louis, USA). The dose of LAC was chosen on the basis of previous studies (Artinian et al., 2008; Rodriguez-Ortiz et al., 2011). Drug solutions were freshly prepared before each experiment.

2.4. Object recognition

Training and testing took place in a 40 cm × 50 cm open field surrounded by 50 cm high walls made of plywood with a frontal glass wall. The floor was covered with sawdust. The objects used for exploration were made of plastic Duplo Lego Toys and had a height of about 10 cm (de Lima, Laranja, Bromberg, Roesler, & Schröder, 2005; de Lima et al., 2006; 2011; Dornelles et al., 2007). Objects presented similar textures, colors and sizes, but distinctive shapes. The different objects and their positions were counterbalanced across experiments and behavioral trials, and all objects had a height of about 10 cm. The objects were washed with a 70% ethanol solution between trials. Exploration was defined as sniffing or touching the object with the nose and/or forepaws, sitting on the object was not considered exploration. General training and test procedures followed the methods described in previous reports (de Lima et al., 2005; 2006; 2011; Jobim et al., 2012; Reolon et al., 2011). Rats were left to explore the empty arena for 5 min in the first day (habituation). Twenty-four hours after habituation, training was conducted by placing individual rats into the field, in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 10 cm from the walls. Animals were left to explore the objects during 5 min and the time exploring each object was recorded. On memory retention test trials given 24 h after training, rats explored the open field for 5 min in the presence of one familiar (A) and one novel (B) object. In the experiment using post-retrieval infusions, the 24-h test trial (Test 1) was used as a memory reactivation session, and a second test trial (Test 2) was given 24 h after reactivation. Rats were exposed to a novel object C during Test 2 (Jobim et al., 2012).

2.5. Histology

Twenty-four to 72 h after behavioral testing, a 1.0-μl infusion of a 4% methylene blue solution was given into the dorsal hippocampus. Rats were sacrificed by decapitation 15 min later, and their brains were removed and stored in 10% formalin for at least 72 h. The brains were sectioned and examined for cannulae placement in the hippocampus. The extension of the methylene blue dye was taken as an approximation of diffusion of the drugs given to each rat. Animals included in the final analysis (123 rats) had bilaterally placed cannula in the intended sites. Infusion placements into the dorsal hippocampus, as revealed by the diffusion of methylene blue, was similar to those described in previous reports (de Lima et al., 2006; Jobim et al., 2012) (data not shown).