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Research report

The relationship between protein synthesis and protein degradation in object recognition memory

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HIGHLIGHTS

- UPS inhibition did not affect consolidation and reconsolidation of OR memory.
- Protein synthesis inhibition impairs consolidation and reconsolidation of OR memory.
- UPS inhibition reversed the effect of Ani intra-CA1 on reconsolidation of OR memory.
- There is a direct link between protein synthesis and degradation on reconsolidation.

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ABSTRACT

For decades there has been a consensus that *de novo* protein synthesis is necessary for long-term memory. A second round of protein synthesis has been described for both extinction and reconsolidation following an unreinforced test session. Recently, it was shown that consolidation and reconsolidation depend not only on protein synthesis but also on protein degradation by the ubiquitin-proteasome system (UPS), a major mechanism responsible for protein turnover. However, the involvement of UPS on consolidation and reconsolidation of object recognition memory remains unknown. Here we investigate in the CA1 region of the dorsal hippocampus the involvement of UPS-mediated protein degradation in consolidation and reconsolidation of object recognition memory. Animals with infusion cannulae stereotaxically implanted in the CA1 region of the dorsal hippocampus, were exposed to an object recognition task. The UPS inhibitor β-Lactacystin did not affect the consolidation and the reconsolidation of object recognition memory at doses known to affect other forms of memory (inhibitory avoidance, spatial learning in a water maze) while the protein synthesis inhibitor anisomycin impaired the consolidation and the reconsolidation of the object recognition memory. However, β-Lactacystin was able to reverse the impairment caused by anisomycin on the reconsolidation process in the CA1 region of the hippocampus. Therefore, it is possible to postulate a direct link between protein degradation and protein synthesis during the reconsolidation of the object recognition memory.

1. Introduction

Memory consolidation is a time-dependent process by which labile new memories are stabilized into long-term memories (LTMs [1–5]. There is considerable evidence that the consolidation process depends on gene expression, *de novo* protein synthesis, and the formation of new synaptic connections [6–11]. This is supported by the fact that several pharmacological experiments using different species and learning and memory paradigms, have been shown that the administration of gene transcription or protein synthesis inhibitors into different brain regions impairs LTM formation [6,10–21].

When reactivated during retrieval a consolidated memory can transiently return to a labile state and undergoes a second protein synthesis wave in order to be re-stabilized, a process known as reconsolidation [22–28]. The destabilization and restabilization of pre-existing memories has been considered as a dynamic and active process that provides an opportunity for updating the original memory with new information [24,26–32].

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A growing amount of evidence has demonstrated that consolidation and reconsolidation of a LTM depend not only on protein synthesis [22,28] but also on protein degradation through the ubiquitin-proteasome system (UPS) [21,33,34]. In the UPS, proteins are targeted for degradation by covalent attachment of a small protein called ubiquitin [35].

Drugs able to inhibit the UPS alter long-term potentiation (LTP) in the hippocampus [36,37] and long-term facilitation in *Aplysia* [38,39]. These results suggest that protein degradation is critical for both forms of synaptic plasticity.

Also, it was reported that the levels of ubiquitinated synaptic proteins increased in the hippocampus after inhibitory avoidance [40] and retrieval of fear memory [25]. Additionally, it has been demonstrated that UPS-mediated protein degradation is also involved in consolidation and reconsolidation of spatial memory [6,20], inhibitory avoidance [40], fear conditioning [40,41], cocaine-associated place preference [42] and aversion taste memory [43].

However, the involvement of UPS in consolidation and reconsolidation of object recognition memory remains unstudied. This task relies on integrity of that structure [44] and probably on LTP in it [45]. Here we investigate in the CA1 region of the dorsal hippocampus the involvement of UPS-mediated protein degradation on the consolidation and reconsolidation of object recognition memory, as well the interaction between protein synthesis and protein degradation in the processing of this memory.

2. Materials and methods

2.1. Subjects

Male Wistar rats (3-month-old, 300–330 g) purchased from Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil were used. Animals were housed 4 to a cage and kept with free access to food and water under a 12-h light/dark cycle (lights on at 7:00 a.m.). The temperature of the animals' room was maintained at 22–24 °C. All of the experimental procedures were performed in accordance with guidelines of the USA National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committees of the Pontifical Catholic University of Rio Grande do Sul.

2.2. Surgery

Under deep anesthesia (75 mg/kg ketamine plus 10 mg/kg xylazine; intraperitoneal) animals were bilaterally implanted with stainless steel 22-gauge guide cannulae aimed 1 mm above of the CA1 region of the dorsal hippocampus. The coordinates were based on the atlas of Paxinos and Watson [46]: (anterior, −4.2 mm; lateral, ±3.0 mm; ventral, −1.8 mm). The guide cannulae were fixed to the skull with dental acrylic. Animals were allowed 7 days to recover from surgery prior to experimental procedures. Animals were handled once daily for 3 consecutive days and all behavioral procedures were conducted between 8:00 and 11:00 a.m.

2.3. Drug administration

The drugs (purchased from Sigma–Aldrich; St. Louis, MO, USA) and the doses used were the inhibitor of ribosomal translation, Anisomycin (375 nmol/side) and the proteasome-ubiquitin blocker, Clasto-Lactacystin β-lactone (200 nmol/side). The doses were chosen based on previous work in which their effects were established [20,47]. The volume of the drugs infused was 1.0 μl per side into the dorsal CA1 area of the hippocampus. At the time of the bilateral microinfusion a tight fitting 30-gauge injection needle, connected to a Hamilton microsyringe by polyethylene tubing, was introduced into the guide cannula. The injection needles extended 1.0 mm beyond the cannulae tip. Infusion drugs were carried out over 60 s and at the end the injection needle was kept in place for 60 additional seconds to maximize diffusion and to prevent backflow of drug into the cannula. Control groups received equal volumes of sterile saline (0.9%).

2.4. Object recognition task

The experimental apparatus used was an open field arena (60 × 40 × 50 cm) placed in a dimly illuminated room [18,48]. The objects to be discriminated were made of glass and varied in shape and texture and were chosen basis on previous observations that demonstrated a lack of preferential exploration for one object over the other. The objects were secured to the floor of the arena with Velcro tape. The open field arena and the stimulus objects were thoroughly cleaned with 70% ethanol after each animal to ensure the absence of olfactory cues. Exploration was defined as sniffing or touching the stimulus object with the nose and/or forepaws. Sitting on or moving around the objects was not considered exploratory behavior. During sample phase, reactivation phase and test phase stimuli objects combinations, as well as their relative position, were counterbalanced and randomly permuted.

Before sample phase, animals were habituated to the experimental apparatus by allowing them to freely explore it for 20 min per day for consecutive 4 days. No stimuli object was placed inside the arena during habituation. For the sample phase, animals were individually placed in the open field arena with two different objects (A and B) and left to freely explore them for 5 min. Twenty-four hours later, animals were placed again in the same apparatus for a 5-min reactivation phase, with a familiar object and a novel object (A and C). After 24 h, animals were placed again in the same apparatus for a 5-min retention test phase, with a familiar object and a novel object (A and D, B and D or C and D). The microinjections into CA1 were carried out immediately after the reactivation phase.

2.5. Cannula placement

Correct cannulae placements were verified 2–4 days after the end of the last behavioral procedure. Animals were infused with a 4% methylene blue solution over 30 s into the CA1 region of the dorsal hippocampus (1.0 μl/side) at the coordinates mentioned above. After 30 min, the animals were sacrificed by excess anesthesia and the brains were removed and kept in 10% formalin. The extension of the spread of the dye was considered to represent an estimate of the amount of drug infused. Cannula placement was considered correct when the spread was ≤1 mm from the intended infusion site; this occurred in 98% of the animals [49,50].

2.6. Statistical analyses

All data from the object recognition task were converted in percentage of total exploration time, expressed as mean and standard error and analyzed using one-sample Student's *t* -test to asses differences to the theoretical mean of 50%. One-way ANOVA followed by Bonferroni's Multiple Comparison Test was performed to assess differences between percentage of exploration time of objects on the test phase. An unpaired *t*-test was performed to assess differences between drug and control group in the exploration time in seconds (Table 1). Data were analyzed using the Graphpad Prism software.