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On the participation of mTOR in recognition memory

Jociane C. Myskiw, Janine I. Rossato, Lia R.M. Bevilaqua, Jorge H. Medina, Iván Izquierdo, Martín Cammarota *

Centro de Memória, Instituto de Pesquisas Biomédicas, Pontifícia Universidade Católica do Rio Grande do Sul, Av. Ipiranga 6690, Porto Alegre, RS 90610–000, Brazil
Laboratorio de Neuroreceptores, Instituto de Biología Celular y Neurociencias ''Prof. Dr. Eduardo de Robertis'', Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155 3° Piso, Ciudad Autónoma de Buenos Aires, CP 1121, Argentina

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Abstract

Evidence indicates that activation of the neuronal protein synthesis machinery is required in areas of the brain relevant to memory for consolidation and persistence of the mnemonic trace. Here, we report that inhibition of hippocampal mTOR, a protein kinase involved in the initiation of mRNA translation, immediately or 180 min but not 540 min after training impairs consolidation of long-term object recognition memory without affecting short-term memory retention or exploratory behavior. When infused into dorsal CA1 after long-term memory reactivation in the presence of familiar objects the mTOR inhibitor rapamycin (RAP) did not affect retention. However, when given immediately after exposing animals to a novel and a familiar object, RAP impaired memory for both of them. The amnesic effect of the post-retrieval administration of RAP was long-lasting, did not happen after exposure to two novel objects or following exploration of the training arena in the absence of other stimuli, suggesting that it was contingent with reactivation of the consolidated trace in the presence of a behaviorally relevant and novel cue. Our results indicate that mTOR activity is required in the dorsal hippocampus for consolidation of object recognition memory and suggest that inhibition of this kinase after memory retrieval in the presence of a particular set of cues hinders persistence of the original recognition memory trace.

Keywords: Memory; Object recognition; Consolidation; Reconsolidation; mTOR; Rapamycin; Hippocampus; Protein synthesis; Learning

1. Introduction

New memories are initially fragile but, over time, consolidate through a protein synthesis-dependent process that makes them resilient to disruption (Izquierdo & McGaugh, 2000; McGaugh, 2000; Izquierdo, Bevilaqua, Rossato, Bonini, Da Silva, Medina and Cammarota, 2006). Notwithstanding that, upon retrieval, many consolidated memories are rendered again vulnerable to the action of metabolic blockers, notably protein synthesis inhibitors, suggesting the existence of a protein synthesis-dependent process that operates to re-stabilize the reactivated trace (Judge & Quartermain, 1982; Milekic & Alberini, 2002; Nader, Schafe, & Le Doux, 2000; Przybyslawski & Sara, 1997; Tronson & Taylor, 2007). Therefore, signaling pathways controlling mRNA translation are likely to be involved in memory consolidation as well as in persistence of the trace after retrieval (Jacinto & Hall, 2003; Matthies, 1989; Raught, Gingras, & Sonenberg, 2001; Steward & Schuman, 2001; Bekinschtein, Cammarota, Igaz, Bevilaqua, Izquierdo and Medina, 2007a).

The mammalian target of rapamycin (mTOR) is a serine–threonine protein kinase that acts as a downstream mediator of the PI3K/Akt pathway, modulating

* Corresponding author. Fax: +55 51 3320 3312.
E-mail address: mcammaro@terra.com.br (M. Cammarota).

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cell growth and proliferation via phosphorylation of p70S6K and the eukaryotic initiation factor 4E-binding protein (4EBP), both critically involved in the initiation of mRNA translation (Hay & Sonenberg, 2004). mTOR has been profusely studied mainly because of its role in cellular differentiation and tumor progression (Huang & Houghton, 2003; Mamane, Petroulakis, LeBacquer, & Sonenberg, 2006). Recent data, however, revealed that in the central nervous system mTOR is activated by several signaling cascades involved in synaptic plasticity, including those mediated by *N*-methyl-D-aspartic acid receptors (NMDAr; Gong, Park, Abbassi, & Tang, 2006; Huang, Kang, Tian, Liu, Luo, Hester and Snyder, 2007) and brain derived neurotrophic factor (BDNF; Takei, Inamura, Kawamura, Namba, Hara, Yonezawa and Nawa, 2004; Inamura, Nawa, & Takei, 2005) suggesting that it might also play an important role in memory processing. In fact, it has been shown that rapamycin, a selective inhibitor of mTOR (Schreiber et al., 1991; Ferrari, Pearson, Siegmann, Kozma, & Thomas, 1993; Pearson, Dennis, Han, Williamson, Kozma, Wettenhall and Thomas, 1995; Sugiyama, Papst, Gelfand, & Terada, 1996), prevents NMDAr- and BDNF-dependent long-term potentiation (LTP) in the rat hippocampus (Tang, Reis, Kang, Gingras, Sonenberg and Schuman, 2002; Horwood, Dufour, Laroche, & Davis, 2006), blocks long-term facilitation in *Aplysia* (Casadio, Martin, Giustetto, Zhu, Chen, Bartsch, Bailey and Kandel, 1999) and impairs retention of spatial and aversive memories in the rat (Dash, Orsi, & Moore, 2006; Bekinschtein et al., 2007a).

Object recognition memory confers the ability to discriminate between novel and familiar entities. Neuropsychological analysis of amnesic patients and behavioral experiments in laboratory animals suggest that the functional integrity of the temporal lobe, including the hippocampal formation, is essential for acquisition and/or storage of this type of information (Clark, Zola, & Squire, 2000; Ennaceur & Delacour, 1988; Logothetis & Sheinberg, 1996; Riesenhuber & Poggio, 2002). In agreement with these findings, recent pharmacological experiments indicate that consolidation of object recognition memory requires protein synthesis in different structures of the temporal lobe and suggest that non-reinforced retrieval makes the recognition trace susceptible to pharmacological interventions once again (Akirav & Maroun, 2006; Kelly, Laroche, & Davis, 2003; Rossato Bevilaqua, Myskiw, Medina, Izquierdo and Cammarota, 2007). However, very little is known about the mechanism that regulates protein synthesis during object recognition memory processing.

Here, we examine whether mTOR signaling is required in the CA1 region of the rat hippocampus for consolidation of object recognition memory and analyze whether inhibition of this kinase after memory retrieval affects the persistence of an already consolidated recognition trace.

2. Materials and methods

2.1. Subjects, surgery and drug infusion

Naive male Wistar rats (2- to 3-month-old, 250–280 g) raised in our own facilities or bought at FEPPS (Fundação Estadual de Produção e Pesquisa em Saúde do Rio Grande do Sul, Porto Alegre, Brazil) were used. The animals were housed 5 to a cage and kept with freely access to food and water under a 12/12 light/dark cycle, with light onset at 7:00 AM. The temperature of the animal's room was maintained at 22–24 °C. To implant them with indwelling cannulae, rats were deeply anesthetized with thiopental (30–50 mg/Kg, i.p.) and 27-gauge cannulae stereotaxically aimed to the CA1 region of the dorsal hippocampus, in accordance with coordinates (*A* −4.0, *L* ±3.0, *V* 1.8) taken from the atlas of Paxinos and Watson (1986). Animals were allowed to recover from surgery for 4 days before submitting them to any other procedure. At the time of drug delivery, 30-gauge infusion cannulae were tightly fitted into the guides. Infusions (0.8 μl/side) were carried out over 60 s and the cannulae were left in place for 60 additional seconds to minimize backflow. The placement of the cannulae was verified postmortem: 2–4 h after the last behavioral test, 0.8 μl of a 4% methylene-blue solution were infused as described above and the extension of the dye 30 min thereafter was taken as an indication of the presumable diffusion of the vehicle or drug previously given to each animal. Only data from animals with correct cannulae implants were analyzed. All procedures were conducted in accordance with the "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1996). Every effort was made to reduce the number of animals used and to minimize their suffering.

2.2. Drugs

Rapamycin was purchased from Sigma (St. Louis, MO, USA), dissolved in DMSO and kept protected from light at −20 °C. Immediately before use, aliquots were thawed and diluted to working concentration with saline.

2.3. Object recognition task

When rats are exposed to familiar and novel objects, they explore preferentially the novel objects. This characteristic behavior was exploited to design a learning paradigm known as object recognition task, which has been employed to successfully evaluate recognition memory (Ennaceur & Delacour, 1988). The object recognition task was conducted in an open-field arena (50 × 50 × 50 cm) built of polyvinyl chloride plastic, plywood and transparent acrylic. Before training all animals were habituated to the experimental arena by allowing them to freely explore it 20 min per day for 4 days in the absence of stimulus objects. The stimulus objects were made of metal, glass or glazed ceramic. There were several copies of each object, which were used interchangeably. Glued to the base of each object was a rounded piece of Velcro, which was used to fix the object to the arena's floor. The role (familiar or novel) as well as the relative position of the 2 stimulus objects were counterbalanced and randomly permuted for each experimental animal. The open field arena and the stimulus objects were cleaned thoroughly between trials 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 turning around the objects was not considered exploratory behavior. A video camera was positioned over the arena and the rats' behavior was recorded using a video tracking and analysis system for later evaluation. The experiments were performed by an observer blind to the treatment condition of the animals. Data are expressed as percentage of the total exploration time.

2.4. Object recognition memory acquisition protocol

On day 1, rats were placed in the open field arena containing 2 different objects and left to freely explore them for 5 min. The test session was performed either 180 min (to analyze short-term memory retention) or 24 h