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Infusion of protein synthesis inhibitors in the entorhinal cortex blocks consolidation but not reconsolidation of object recognition memory

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ABSTRACT

Memory consolidation and reconsolidation require the induction of protein synthesis in some areas of the brain. Here, we show that infusion of the protein synthesis inhibitors anisomycin, emetine and cycloheximide in the entorhinal cortex immediately but not 180 min or 360 min after training in an object recognition learning task hinders long-term memory retention without affecting short-term memory or behavioral performance. Inhibition of protein synthesis in the entorhinal cortex after memory reactivation involving either a combination of familiar and novel objects or two familiar objects does not affect retention. Our data suggest that protein synthesis in the entorhinal cortex is necessary early after training for consolidation of object recognition memory. However, inhibition of protein synthesis in this cortical region after memory retrieval does not seem to affect the stability of the recognition trace.

1. Introduction

Recognition memory allows to distinguish familiar from novel entities (Squire, Wixted, & Clark, 2007). Functional integrity of the medial temporal lobe is essential for encoding and expression of this type of information (Clark, Zola, & Squire, 2000; Ennaceur & Delacour, 1988; Logothetis & Scheinberg, 1996; Riesenhuber & Poggio, 2002). Indeed, the anterograde amnesia observed in several patients with medial temporal lobe damage is characterized by the loss of recognition memory (Scoville & Millner, 1957). However, different areas of the medial temporal lobe seem to deal with different aspects of recognition memory processing (Balderas et al., 2008). Thus, while the hippocampus is essential for remembering contextual details and the temporal order of previous experiences, the perirhinal cortex appears to be mainly involved in familiarity detection (Brown & Aggleton, 2001; Myskiw et al., 2008; Rossato et al., 2007; Suchan, Jokisch, Skotara, & Daum, 2007).

The entorhinal cortex (EC) plays a crucial role in the communication between the hippocampus and sensory/association cortical areas. Indeed, the EC is the main source of projections to the hippocampus and also the primary output structure of the hippocampal formation (Canto, Wouterlood, & Witter, 2008). The most prominent entorhinal output is directed to the perirhinal cortex which, in turn, regulates transmission of neocortical inputs to the EC (Pinto, Fuentes, & Pare, 2006), suggesting that most hippocampal–cortical connections are controlled by a relay involving entorhinal–perirhinal interactions (Insausti et al., 1997). However, although inactivation of the EC impairs different types of hippocampus-dependent memories, including those induced by spatial, contextual and aversive learning (Bevilaqua et al., 2007; Eijkenboom, Blokland, & Van Der Staay, 2000; Kopniczky et al., 2006; Miwa & Ueki, 1996; Parron & Save, 2004a; Ramirez et al., 1988; Ueki, Miwa, & Miyoshi, 1994), and it has been demonstrated that excitotoxic lesion of the EC impairs recognition (Galani, Weiss, Cassel, & Kelche, 1998; Mumby & Pinel, 1994; Parron & Save, 2004b) little is known about the participation of the EC in object recognition (OR) memory. Considering that long-term memory (LTM) requires experience-dependent protein synthesis in areas of the brain relevant for information processing (Barondes & Cohen, 1967; Davis & Squire, 1984; Dunn & Leibmann, 1977; Flexner, Flexner, De La Haba, & Roberts, 1965; Flexner, Flexner, Stellar, De La Haba, & Roberts, 1962; Flood, Bennett, Orme, & Rosenzweig, 1975; Glassman, 1969; Gold, 2008; Luft, Buitrago, Ringer, Dichgans, & Schulz, 2004; Matthies, 1974; Rudy, 2008; Squire & Barondes, 1972) we examined whether induction of protein synthesis is necessary in the entorhinal cortex for consolidation of OR LTM. Because evidence suggests that after retrieval OR LTM may briefly return to a fragile state and in order to persist must undergo a protein synthesis-dependent reconsolidation process (Akirav & Maroun, 2006; Bozon, Davis, & Laroche, 2003; Kelly, Laroche, &

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Davis, 2003; Maroun & Akirav, 2008; Rossato et al., 2007) we also analyzed whether post-retrieval inhibition of protein synthesis in the EC affects OR memory retention.

2. Materials and methods

2.1. Subjects, surgery and drug infusion

Naive male Wistar rats (3-month-old 280–300 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 (lights on at 7:00 AM). The animal's room temperature was maintained at 22–24 °C. Rats were bilaterally implanted with 27-gauge stainless steel cannulas into the entorhinal cortex under thiopental anesthesia (30–50 mg/kg). Coordinates were (in mm) 6.8 posterior to bregma, 5.0 lateral to the midline, and 8.1 ventral to the skull surface (Paxinos & Watson, 1986). Rats were given at least 4 days to recover before the experimental procedures. At the time of drug delivery, 30-gauge infusion cannulas were fitted into the guides. Infusions (1 μl/side) were carried out over 60 s using an infusion pump (KDS-200; kdScientific, USA). Placement of the cannulas was verified postmortem: 2–4 h after the last behavioral test, 1 μl of a 4% methylene-blue solution was 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 injected. Only data from animals with correct 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

Anisomycin (ANI), emetine (EME) and cycloheximide (CHX) were purchased from Sigma (St Louis, MO, USA). EME and CHX were dissolved in DMSO. ANI was dissolved in 1 M HCl, diluted in saline and the pH adjusted to pH 7.2–7.5 with NaOH. All drugs were stored in a light-proof container at −20 °C. Immediately before use, aliquots were thawed and diluted to working concentration with saline.

2.3. Object recognition task

The object recognition task was conducted in an open-field arena (60 × 40 × 50 cm) built of polyvinyl chloride plastic, plywood and transparent acrylic. Before training the 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 objects to the arena's floor. The role (familiar or novel) and the relative position of the two stimulus objects were counterbalanced and randomly permuted for each experimental animal. All objects were behaviorally irrelevant and equally conspicuous for the rats as determined in pilot experiments and in previous reports (Clarke et al., 2008; Myskiw et al., 2008; Rossato et al., 2007). The open-field arena and the stimulus objects were cleaned thoroughly between trials to ensure removal

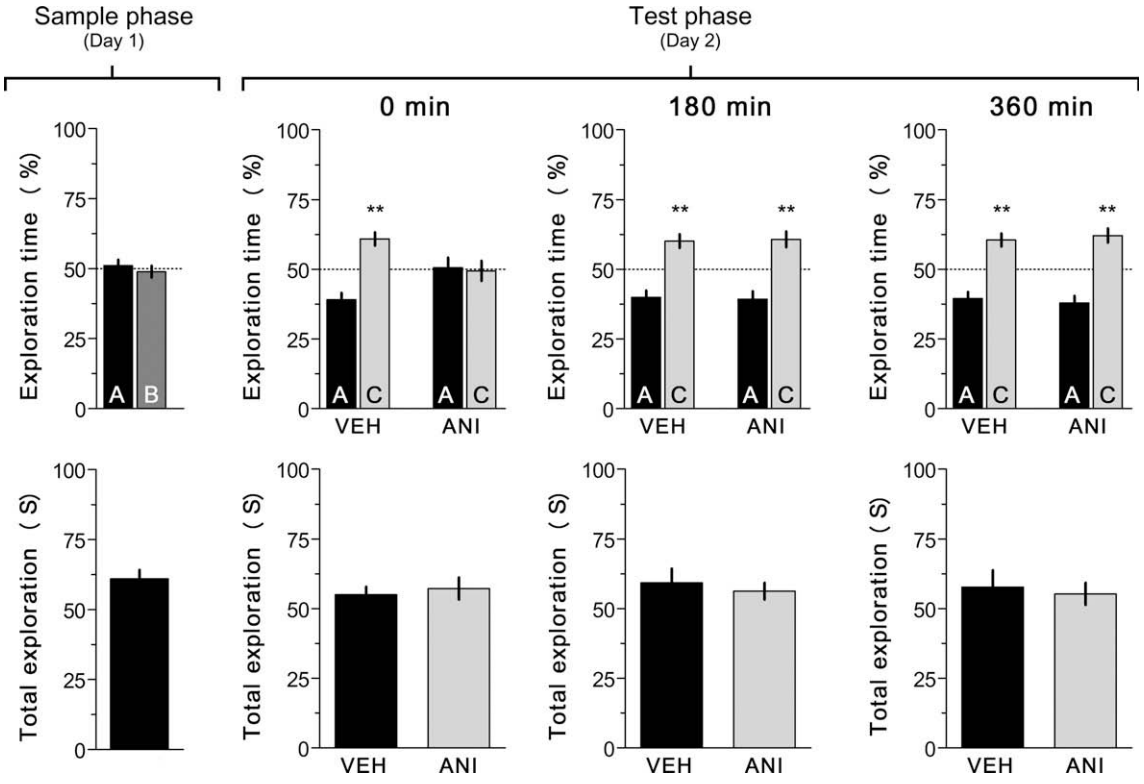

Fig. 1. Post-training infusion of anisomycin in the entorhinal cortex blocks consolidation of object recognition long-term memory. On day 1 (sample phase) rats (n = 54) were exposed to two different objects (A and B) for 5 min and, at different times after that (0, 180 or 360 min), received bilateral infusions (1 μl/side) of vehicle (VEH) or anisomycin (ANI; 160 μg/side) in the entorhinal cortex. On day 2 (test phase) the animals were exposed to a familiar (A) and a novel object (C) for five additional minutes to assess OR LTM retention. (Top panel) Data are presented as mean (±SEM) of the percentage of time exploring a particular object over the total time of object exploration. $^{**}p < 0.005$ in one-sample Student's t-test with theoretical mean = 50; n = 9 per group. Note that the animals that received ANI immediately after the sample phase spent the same amount of time exploring objects A and C during the test phase (Day 2; 0 min – ANI). (Bottom panel) Also note that total exploration time was not affected by ANI infusion