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RETRIEVAL AND RECONSOLIDATION OF OBJECT RECOGNITION MEMORY ARE INDEPENDENT PROCESSES IN THE PERIRHINAL CORTEX

I. BALDERAS,[†] C. J. RODRIGUEZ-ORTIZ[†] AND F. BERMUDEZ-RATTONI[*]

División de Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, A.P. 70-253, México D.F. 04510, Mexico

Abstract—Reconsolidation refers to the destabilization/restabilization process upon memory reactivation. However, the parameters needed to induce reconsolidation remain unclear. Here we evaluated the capacity of memory retrieval to induce reconsolidation of object recognition memory in rats. To assess whether retrieval is indispensable to trigger reconsolidation, we injected muscimol in the perirhinal cortex to block retrieval, and anisomycin (ani) to impede reconsolidation. We observed that ani impaired reconsolidation in the absence of retrieval. Therefore, stored memory underwent reconsolidation even though it was not recalled. These results indicate that retrieval and reconsolidation of object recognition memory are independent processes.

Key words: reconsolidation, updating, retrieval, object recognition memory, perirhinal cortex, rats.

INTRODUCTION

Memories turn stable in the long term through a protein synthesis-dependent process known as consolidation (McGaugh, 2000). However, after consolidation, memories can become labile (Nader et al., 2000). Reconsolidation theory posits that upon retrieval, consolidated memories are destabilized and once again require a protein synthesis-dependent process to be retained in long-term storage (Nader and Einarsson, 2010). Nevertheless, some results indicate that inhibition of retrieval in the amygdala does not impede fear or taste-conditioned memory to undergo reconsolidation (Yasoshima et al., 2005; Ben Mamou et al., 2006; Rodriguez-Ortiz et al., 2012). Since it has been observed that different types of memory differ on the conditions that render them labile (Finnie 2012), the aim of the present study was to evaluate whether retrieval is indispensable for declarative memory to reconsolidate.

The object recognition task is useful to evaluate declarative memory. This task relies on rodents' natural tendency to explore for a longer time novel than familiar objects when presented together (Ennaceur and Delacour, 1988). It has been suggested that this task maintains a close analogy to recognition memory tasks used in humans to evaluate impairments in declarative memories (Reed and Squire, 1997). Previous studies have demonstrated that the perirhinal cortex is specifically involved in long-term formation of object recognition memory (Brown and Aggleton, 2001; Mumby et al., 2007; Balderas et al., 2008; Winters et al., 2008; Tinsley et al., 2011; Brown et al., 2012). Therefore, the present study focused on retrieval and reconsolidation processes taking place in the perirhinal cortex during object recognition memory reactivation.

By infusing the $GABA_A$ receptor agonist muscimol (musc), previous reports have shown that temporal inactivation of brain structures, like the hippocampus and prefrontal cortex, effectively reveals whether these structures are mandatory for the retrieval process of different kinds of memories (Moser and Moser, 1998; Holt and Maren, 1999; Blum et al., 2006; Jo et al., 2007). Moreover, it has been shown that musc infusions into the hippocampus impaired retrieval of spatial memory without affecting new learning processing (Moser and Moser, 1998; Holt and Maren, 1999; Blum et al., 2006; Jo et al., 2007). Consequently, in order to assess retrieval we used musc to temporally inactive the perirhinal cortex and, anisomycin (ani) was applied to unveil effects over reconsolidation.

EXPERIMENTAL PROCEDURES

Subjects

The general protocol used for animal manipulation and object recognition memory task have been described in detail elsewhere [see (Balderas et al., 2012)]. Briefly male Wistar rats from our Institute breeding colony, (270–310 g) were used. All manipulations were carried out in accordance to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (eighth edition). The institutional Bio Safety and Ethics Committee approved these protocols, aimed to minimize the number of animals used and their suffering. All experiments were carried out in independent groups (for the detailed number of subjects in each group please see the figure legends).

*Corresponding author. Tel: +525-556225626; fax: +525-556225607.
E-mail addresses: fbermude@ifc.unam.mx, bermudez@unam.mx (F. Bermudez-Rattoni).
[†] These authors contributed equally to this work.
Abbreviations: ani, anisomycin; ANOVA, analysis of variance; ACSF, artificial cerebrospinal fluid; musc, muscimol; sal, saline; veh, vehicle.

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Surgery

Animals were bilaterally implanted with stainless-steel guide cannulae aimed to the perirhinal cortex: posterior 3.0, lateral ± 6.5 ventral 7.0 [coordinates of infusion sites from Bregma (Paxinos and Watson, 1998)]. Rats recovered for 10 days before the beginning of behavioral procedures.

Infusions

Saline (sal) 0.9% or musc (2.4 mg/mL, Sigma, St. Louis, MO, USA) was infused 30 min before the objects were presented and, artificial cerebrospinal fluid (ACSF) (mM: NaCl 125, KCl 5, $NaH_2PO_4{\cdot}H_2O$ 1.25, $MgSO_4$ $7H_2O$ 1.5, $NaHCO_3$ 26, glucose 10, $CaCl_2$ 2.5; pH ~7.5) or ani (120 mg/ml, Sigma, St. Louis, MO, USA) was microinjected immediately after exploration of objects. In all cases, infusions were 1 μL in volume over a minute and the injector was left in place for an additional minute to allow complete diffusion. All experiments were carried out in independent groups.

Experimental design

A square arena placed in a dim-light illuminated room and made of gray-painted wood (40 × 40 × 60 cm) with the floor covered with sawdust was used. The objects to be discriminated were white glass bulbs (6 cm in diameter and 11 cm length), transparent glass jars (5.5 cm in diameter and 5 cm in height) and spiral glass bulbs (11 cm length). Objects were fixed to the floor at the back corners of the arena (10 cm from walls) with Velcro to prevent them to be displaced by the rats. To avoid olfactory cues, objects were thoroughly cleaned with 70% ethanol and sawdust was stirred after each trial. For all experiments, objects and their relative positions were counterbalanced.

The general protocol was as follows. For 5 consecutive days, animals were handled for a minute and immediately after, posited into the open-field arena without any objects for 3 min. In the sample phase, rats were placed in the arena facing the wall opposite to two identical objects and were allowed to freely explore for 5 min. The reactivation session was carried out 24 h later where two objects were presented during 5 min. To test memory reconsolidation 24 h later, rats were allowed to explore freely one copy of the object presented in the sample phase together with a new object for 1 min. Recognition index was calculated as follows: time spent exploring one of the objects divided by the total exploration time. A recognition index equal to 0.5 indicates no preference for any of the objects and, an index significantly higher than 0.5 indicates preference for that particular object. A detailed schematic representation of the experiments can be found above each figure.

In the experiments presented in Fig. 1 three different protocols were tested to elucidate the conditions that trigger object recognition memory reconsolidation. In the three protocols rats explored two identical objects (A1 and A2) in the sample phase. On reactivation of protocol 1, rats were presented two different copies of the same object shown the day before (A3 and A4). On reactivation of protocol 2, rats were exposed to two copies of a second object different from the one shown the day before (B1 and B2). Finally, on reactivation of protocol 3, rats were presented one copy of the object presented the day before together with a new object (A3 and B1). In all cases, rats were infused with ACSF or ani immediately after reactivation and memory was tested by presenting one copy of the object presented in the sample phase together with a new third object (A5 and C1).

For the experiments presented in Fig. 2, two identical objects (A1 and A2) were presented in the sample phase. On reactivation, rats were exposed to one copy of the object presented the day before together with a new object (A3 and B1). In all cases, rats were infused with sal or musc before the reactivation session, and ACSF or ani immediately after. Test consisted of the presentation of one copy of the object shown in the sample phase together with a new third object (A4 and C1).

Experiments in Fig. 3 were aimed to identify musc effects on mnemonic processes other than retrieval. To do so, rats were infused with sal or musc before two identical objects (A1 and A2) were presented. On the next day, rats were exposed to one copy of the object shown the day before together with a new object (A3 and B1).

Histology for injector tips placement

At the end of the experiments the rats were injected a lethal dose of pentobarbital and perfused with sal 0.9%. 40-μm-thick slices were cut and stained with violet cresyl. Sites of infusion were observed under a light microscope. All animals included in the analysis had the needle tips in the cerebral region of interest between 3.14 and 3.60 mm posterior to Bregma (see Fig. 4).

Statistical analysis

Mean ± SE recognition indexes were used for comparisons between groups. One-sample *t*-tests were used to determine whether recognition indexes were significantly different from 0.5 (“chance” level, i.e., no preference between objects). One-way analysis of variance (ANOVA) was used to determine differences in total exploration times between groups in the test phase. Two-way ANOVAs (phase x treatment) were used to determine differences between recognition indexes for novel objects. A p-value < 0.05 was considered significant. The first minute of exploration was used for statistical analysis (reactivation and test phases), since it has been reported that novel objects discrimination is more evident during that period of time (Dix and Aggleton, 1999; Mumby et al., 2002; Winters et al., 2011).

RESULTS

Ani in the perirhinal cortex disrupted reconsolidation of object recognition memory only when new related information was presented

A previous report showed that ani impaired object recognition memory reconsolidation when administered