Supplementary Information for Dopamine controls whether new declarative information updates reactivated memories through reconsolidation

María Carolina Gonzalez[§], Janine I. Rossato[§], Andressa Radiske, Lia R.M. Bevilaqua, and Martín Cammarota[*]

Memory Research Laboratory - Brain Institute/UFRN, Brazil. [§]Equally contributed. *Corresponding author.

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Dataset S1

Extended Methods

Subjects.

We used a total of 640 adult male Wistar rats (3-month-old; 300-350 g) to test our hypotheses. Rats were housed in groups of 5 per cage with free access to food and water and kept on a 12-h light/dark cycle (lights on at 06:00 AM) at 23°C in the institutional vivarium. Experiments were performed during the light phase of the cycle in accordance with the USA National Institutes of Health Guidelines for Animal Care and were approved by the local institutional ethics committee (Comissão de Ética no Uso de Animais – Federal University of Rio Grande do Norte) and conducted by researchers blinded to the animals' treatment.

Stereotaxic surgery for cannula implants.

Rats anesthetized with ketamine (80 mg/kg)/xylazine (10 mg/kg) were bilaterally implanted with 22-gauge stainless steel guide cannulas aimed to the CA1 region of the dorsal hippocampus (AP −4.2 mm; LL, ±3.0 mm; DV, −3.0 mm from Bregma). Guides were fixed to the skull with dental acrylic. At the end of surgery, the animals received subcutaneous meloxicam (0.2 mg/kg) as analgesic and were allowed a recovery period of at least 1 week, during which they were handled daily for 1-2 min.

Drugs and microinfusion procedures.

Drug doses were based on previous studies and pilot experiments. Anisomycin (ANI; 160 µg/µl), myristoylated autocamptide-2 related inhibitor peptide (AIP; 5 nmol/µl), myristoylated scrambled AIP, (sAIP; 5 nmol/µl), myristoylated zeta-inhibitory peptide (ZIP; 1 nmol/µl), myristoylated scrambled ZIP (sZIP; 1 nmol/µl) and SCH-23390 (SCH; 1.5 µg/µl) were dissolved upon arrival, aliquoted and stored at −20 °C. Stock aliquots were diluted to working concentration in sterile saline (VEH; NaCl 0.9%) on the day of the experiment. For microinjections, infusers were fitted into the guide cannulas. Microinjections (1 µl/side at a rate of 0.5 µl/min) were carried out using a Hamilton syringe

coupled to an infusion pump. Infusers were left in place for one additional minute to minimize backflow and ensure drug diffusion. Injection procedures were performed in a room separated from the experimental room.

Novel object-recognition task.

The animals were transported from the vivarium to an experimental anteroom where they remained for one hour before the beginning of the experimental sessions. The animals were then handled for 1 min and habituated to the training arena (a 60 cm x 60 cm x 60 cm grey plywood open field) in the absence of stimuli objects for 20 min/day during 4 days. One day after the last habituation session, the animals were trained in a novel object-recognition memory task. During the training session, the animals explored two different novel stimuli objects for 5 minutes. Memory reactivation was conducted by re-exposing the animals to a familiar object together with a novel one for 5 minutes in the training arena (Reactivation session). Object recognition memory retention was evaluated 1 day after the reactivation session by exposing the animals to a familiar and novel object for 5 minutes (Test Session). Behavioral procedures were carried out in a dim-light illuminated room acclimatized at 23-24°C. Stimuli objects were made of metal, glass, or glazed ceramic and had no innate significance for rats [1]. The open field arena and the stimuli objects were cleaned thoroughly before each trial to eliminate olfactory cues. Object exploration was defined as sniffing or touching the stimuli objects with the muzzle and/or forepaws. Animals' behavior was tracked using digital video cameras fixed above the training arenas and connected to an automatic video tracking system (ObjectScan software, CleverSys; RRID: SCR_017141). Video data were acquired at 30 frames/s.

Data analysis.

Animals' behavior was analyzed using the ObjectScan software. A discrimination index (DI = [Novel object exploration time - Familiar object exploration time] / Total exploration

time) was used as a measure of discrimination between familiar and novel objects. DI varies between −1 and +1. Positive DI scores indicate the rats' preference for novel objects. DI scores close to zero indicate similar amount of time exploring each object, suggesting no discrimination. Statistical analyses were performed using GraphPad Prism 6 software (RRID:SCR_002798). Significance was set at $P < 0.05$. Data were analyzed using one-sample t-test with theoretical mean=0 or unpaired Student's t-test.

Dataset S1. Object exploration time during training, reactivation and test sessions for experiments presented in Figure 1.

SI References

1. J.I. Rossato, M.C. Gonzalez, A. Radiske, G. Apolinário, S. Conde-Ocazionez, L.R. Bevilaqua, M. Cammarota. PKMζ Inhibition Disrupts Reconsolidation and Erases Object Recognition Memory. *J Neurosci*. 39, 1828-1841 (2019).