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Taste aversion memory reconsolidation is independent of its retrieval

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ABSTRACT

Reconsolidation refers to the destabilization/re-stabilization memory process upon its activation. However, the conditions needed to undergo reconsolidation, as well as its functional significance is quite unclear and a matter of intense investigation. Even so, memory retrieval is held as requisite to initiate reconsolidation. Therefore, in the present work we examined whether transient pharmacological disruption of memory retrieval impedes reconsolidation of stored memory in the widely used associative conditioning task, taste aversion. We found that AMPA receptors inhibition in the amygdala impaired retrieval of taste aversion memory. Furthermore, AMPA receptors blockade impeded retrieval regardless of memory strength. However, inhibition of retrieval did not affect anisomycin-mediated disruption of reconsolidation. These results indicate that retrieval is a dispensable condition to undergo reconsolidation and provide evidence of molecular dissociation between retrieval and activation of memory in the non-declarative memory model taste aversion.

1. Introduction

Memories stabilize over time through a protein synthesis-dependent process named consolidation (McGaugh, 2000). However upon activation, consolidated memories return to a labile state and undergo a consolidation-like process referred to as reconsolidation (Nader & Einarsson, 2010). Mainly, reconsolidation is sustained by the now widely reported observation that after a memory trace is activated, it is susceptible to disruption by the same treatments that disrupt memory during consolidation, e.g., protein synthesis inhibitors (Nader & Einarsson, 2010). Memory reconsolidation is an opportunity to modify or even erase memories, making manipulation of reconsolidation a plausible therapeutic tool for treating people with anxiety disorders, like PTSD or phobias. Therefore, to describe the relevant parameters that trigger reconsolidation is of general interest in the field.

It has been considered that retrieval is one condition to be essential to initiate reconsolidation (Nader & Einarsson, 2010). However, no experimental evidence support this notion and, on the contrary, one work reported that indeed, reconsolidation occurs in the absence of retrieval (Ben Mamou, Gamache, & Nader, 2006). In that study CNQX, an AMPA receptor antagonist, was shown to disrupt retrieval of fear conditioning when infused in the amygdala of rats. Then, CNQX and the protein synthesis inhibitor anisomycin were infused together into the amygdala. Retrieval was again disrupted and anisomycin impaired memory reconsolidation, indicating that memory can be activated and turn labile without memory retrieval (Ben Mamou et al., 2006). To provide further evidence on this regard, we examined whether transient pharmacological disruption of memory retrieval impedes reconsolidation in the widely used associative conditioning task, taste aversion.

Taste aversion was obtained by intraperitoneal injection of LiCl after intake of a novel saccharin taste solution. As a result taste-aversion association is induced and, on the next presentation saccharin solution was recognized as an aversive tastant and consumption was reduced (Bermudez-Rattoni, 2004). To assess effects on reconsolidation, memory was strengthened by repeating taste-aversion training on the next day (Fig. 1, solid circles). Protocols that update memory by strengthening have successfully been used to evaluate reconsolidation (Garcia-DeLaTorre, Rodriguez-Ortiz, Arreguin-Martinez, Cruz-Castaneda, & Bermudez-Rattoni, 2009; Lee, 2008; Rodriguez-Ortiz, De la Cruz, Gutierrez, & Bermudez-Rattoni, 2005; Rodriguez-Ortiz, Garcia-DeLaTorre, Benavidez, Ballesteros, & Bermudez-Rattoni, 2008).

2. Materials and methods

2.1. Subjects

Male Wistar rats from Instituto de Fisiología Celular breeding colony weighing between 270 and 310 g at the beginning of experiment were housed individually in plastic cages on a 12 h/12 h light/dark cycle. All manipulations were performed during light cycle. Food was freely available throughout experiments. Every effort was made to reduce the number of animals used and all experi-

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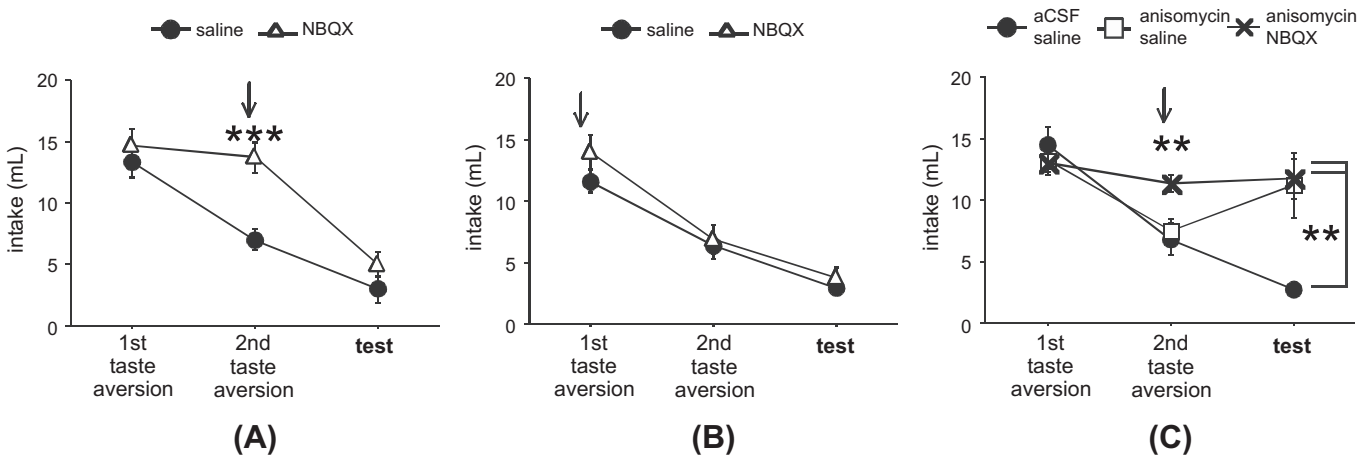

Fig. 1. Inhibition of protein synthesis impeded reconsolidation of taste aversion memory in the absence of its retrieval. (A) NBQX injection in the amygdala transiently disrupted taste aversion memory retrieval (NBQX, open triangles; saline, solid circles). (B) NBQX infusions spared memory when injected on the first acquisition trial. Similar consumptions were observed after NBQX or saline injections. (C) Contrary to controls (aCSF/saline, solid circles), anisomycin conjointly injected in the amygdala and insula hindered stabilization of stored memory (anisomycin/saline, open squares). Despite NBQX infusions, anisomycin impeded memory stabilization when injected on the 2nd taste aversion (anisomycin/NBQX, crosses). Results are presented as mean ± S.E.M. milliliters of intake. Arrows indicate intracerebral infusions. ** $p < 0.01$, *** $p < 0.001$ vs. respectively control on that trial.

ments were performed in accordance with the Ministry of Health of Mexico.

2.2. Surgery and microinjections

Animals under ketamine/xylazine (22/2.6 mg/rat) anesthesia were bilaterally implanted with stainless-steel guide cannulae in the amygdala or in amygdala and insular cortex. Rats recovered for at least 5 days before behavioral procedures.

For microinjections an injector was inserted into each guide cannula extending 2 mm below cannula tip. Coordinates of injections from bregma were in mm: amygdala, posterior 2.8, lateral ±5, ventral 8.5; insular cortex, anterior 1.2, lateral ±5.5 and ventral 6 (Paxinos & Watson, 1998). Drugs were infused over a minute and injectors were left for additional 30 s to avoid dragging along injection tracks. Anisomycin was dissolved in equimolar HCl and adjusted to 120 mg/mL, pH ~ 7.5 in aCSF solution (mM: NaCl 125, KCl 5, NaH2PO4.H2O 1.25, MgSO4.7H2O 1.5, NaHCO3 26, glucose 10, CaCl2 2.5). All anisomycin or aCSF injections (1 μL per hemisphere) were carried out bilaterally 2 h before behavior conjointly in the amygdala and insular cortex. NBQX (5 mg/mL, 0.5 μL per hemisphere) was dissolved in saline and injections were performed bilaterally in the amygdala 20 min before behavior. Rats were handled for several days before injections to minimize stress associated with the microinjection procedure.

2.3. Taste aversion protocol

Rats were water-deprived for 24 h. Then, they were allowed to drink water once a day. After three consecutive days of water intake, on day 4, rats received saccharin solution (0.1% w/v) followed by LiCl intraperitoneal injections (0.15 M, 10 mL/kg) 30 min after intake onset. On the next day, saccharin-LiCl association procedure was repeated after intracerebral injections unless indicated otherwise. For the experiments where we assessed drugs effects on asymptotic behavior conditions (Fig. 2A and B), saccharin-LiCl associations were performed for five consecutive days and intracerebral injections were carried out before the fifth association. All tests consisted on the presentation of saccharin solution (0.1% w/v).

To avoid dehydration, all saccharin intakes were followed by free water access 4:30 h later. All consumption periods were 15 min long and the volumes ingested were recorded.

2.4. Histology for injector tips placement

At the end of experiments, rats were injected a lethal dose of pentobarbital and perfused with physiological saline solution. Brains were removed and placed in 4% formaldehyde in phosphate buffered saline for one to four days. Afterwards, brains were transferred to 30% sucrose solution until they sank. Slices of 40 μm thick were obtained and stained with violet cresyl. Sites of injection were observed under light microscope (Fig. 3). Animals with misplaced cannula were discarded from further analysis.

2.5. Statistical analysis

Mixed ANOVAs with Bonferroni-corrected pairwise comparisons were performed to determine differences between groups on a particular trial. p-Value $\leqslant 0.05$ was considered significant.

3. Results

3.1. Retrieval is not necessary to initiate taste aversion memory reconsolidation

Previous work has shown that AMPA (αamino-3-hydroxyl-5-methyl-4-isoxazole-propionate) receptors inhibition in the amygdala of rats specifically disrupts retrieval of conditioned fear and conditioned taste aversion without affecting memory acquisition or consolidation (Ben Mamou et al., 2006; Yasoshima, Yamamoto, & Kobayashi, 2005). Accordingly, we infused NBQX (2,3-dioxo-6nitro-1,2,3,4-tetrahydrobenzo [*f*]quinoxaline-7-sulfonamide) in the amygdala of rats before acquisition of a second taste aversion. Fig. 1A shows that animals infused with NBQX drank significant more saccharin solution than the saline group on the injection session (2nd taste aversion). A mixed ANOVA revealed differences between groups [$F(1,12) = 7.64$, $p < 0.05$], trials [$F(2,24) = 61.51$, $p < 0.0001$] and interaction [$F(2,24) = 5.05$, $p < 0.05$]. A Bonferroni's corrected pairwise comparison showed differences between groups on the 2nd taste aversion [$p < 0.001$]. Nevertheless, NBQX