Involvement of the anterior cingulate cortex in memory formation, consolidation, and reconsolidation of recent and remote contextual fear memory

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Supplementary Methods

Subjects

Adult male Sprague-Dawley rats (Charles River, Saint-Constant, PQ) were housed individually and maintained on a 12/12 light/dark cycle (lights on at 7 a.m.) with food and water provided ad libitum. The rats were handled on three consecutive days for ~3 min before start of training.

Surgery and histology

Under Ketamine (55 mg/kg), Xylazine (3.33 mg/kg) and Domitor (27 mg/kg) anesthesia, 26-gauge stainless steel cannulae were implanted bilaterally into the ACC, injector co-ordinates: AP: 2.6 mm relative to bregma; ML: ± 2.4 mm; DV: -1.6 mm to dura surface (Paxinos and Watson 2004). For motor cortex cannulation, 24-gauge were implanted bilaterally, injector co-ordinates: AP: 2.6 mm relative to bregma; ML: ± 0.7 mm; DV: -1.6 mm to dura surface. Rats were given a week to recover. In experiments testing recent memory (3d post-conditioning) rats were operated on before conditioning. In experiments testing remote memory (30 post-conditioning) rats were
operated on between conditioning and testing. At the end of the experiment, animals were transcardially perfused with physiological saline followed by 10% formal saline. Brains were sectioned at 50 µm thickness and stained with formol-thionin and examined by light microscopy for verification of cannula placement in the ACC and dorsal hippocampus. All procedures were in accordance with the CAA Guide, and were approved by the McGill University Animal Care and Use Committee.

Drugs and infusions

Drugs were infused slowly via infusion pump at a rate of 0.25µl/min. Following drug infusion, injectors were left in place for an additional minute to allow diffusion of the drug away from the injector tip. Ro25-6981 (Tocris, Ellisville, MO) was dissolved in artificial cerebrospinal fluid (ACSF) and infused at 2 µg / 0.5 µl per side. Anisomycin (Sigma, St. Louis, MO) was dissolved in equimolar HCl, diluted with artificial cerebrospinal fluid (ACSF), and adjusted to pH 7.4 with NaOH. The dose was 62.5 µg / 0.5 µl per side.

Apparatus

Conditioning was conducted in a Plexiglas rodent conditioning chamber with a metal grid floor (Model E10-10, Coulbourn Instruments) that was enclosed within a sound attenuating chamber (Model E10-20). The chamber was dimly lit with a single house light and scented with diluted vanilla in order to create a distinct context.

General behavioral procedures

Animals were trained using a contextual fear conditioning paradigm that has been shown to demonstrate a temporally graded retrograde amnesia after lesions of the dorsal hippocampus, and be sensitive to dorsal hippocampus cellular and systems reconsolidation of recent and remote memories (Debiec et al. 2002). In all of the experiments, rats were habituated to the conditioning chamber for 5 min one day before training. Rats were placed in the chamber and after 2 min
received 8 unsigned foot-shocks (1 sec, 1.5 mA) at 62 sec interval. The rats were left in the chamber for 30 sec after the termination of the procedure. For all tests, freezing (the complete absence of movement, except that of respiration) was scored with a time-sampling procedure in which each animal was observed as either freezing or not every 5 sec.

**Statistical analysis**

Independent groups t-tests were performed, in addition to one-way independent groups and two-way mixed-factor ANOVAs that were followed up with *Post hoc* tests where appropriate.

**Supplementary References**

Supplementary Figure 1. Schematic diagrams showing placements of cannula tips. (A) Placement of cannula tips in experiment presented in Figure 1A targeting the ACC. Open circle: vehicle group; closed circle: Ro25-6981 group. (B) Placement of cannula tips in experiment presented in Figure 1B targeting the ACC. Open circle: vehicle group; closed circle: anisomycin group. (C) Placement of cannula tips in experiment presented in Figure 2A targeting the ACC. Open circle: vehicle group; closed circle: anisomycin group. (D) Placement of cannula tips in experiment presented in Figure 2B targeting the ACC. Open circle: vehicle group; closed circle: anisomycin group. (E) Placement of cannula tips in experiment presented in Figure 2C targeting the ACC. Open circle: vehicle group; closed circle: anisomycin group. (F) Placement of cannula tips in experiment presented in Figure 2D targeting the primary / secondary motor cortices. Open circle: vehicle group; closed circle: anisomycin group. Numbers on the right indicate stereotaxic coordinates relative to bregma. Adapted from Paxinos and Watson (2004), used with permission from Elsevier.