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RAPID REPORT

RETRIEVAL MEDIATED BY HIPPOCAMPAL EXTRACELLULAR SIGNAL-REGULATED KINASE/MITOGEN-ACTIVATED PROTEIN KINASE IS REQUIRED FOR MEMORY STRENGTHENING

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Abstract—Established memories can be strengthened by additional learning and rehearsal. However, the brain processes enabling memories to be updated by further information is unclear. We found that blockade of retrieval of a stabilized memory by inhibition of the extracellular signal-regulated kinase/mitogen-activated protein kinase signaling pathway in the hippocampus prevented memory enhancement induced by an additional learning trial in rats. The findings indicate that retrieval is critical for memory strengthening.

Key words: hippocampus, mitogen-activated protein kinase, memory retrieval, fear memory.

Previously consolidated memories can be strengthened by repeated learning and rehearsal (Ebbinghaus, 1913). However, brain processes underlying this form of modification of a stabilized memory remain to be understood. One obvious question is whether expression of an established memory at the time of relearning is required to enable its strengthening. One hypothesis is that remembering memories for past learning events is critical for allowing their integration with new information. Alternatively, memories for the original and the additional training could be processed by parallel and independent events in the absence of reactivation of the original memory, and the different traces could then be integrated to produce an updated, enhanced memory. These hypotheses have been difficult to examine because the use of experimental interventions that specifically block retrieval without affecting memory formation is required.

As retrieval of hippocampus-dependent fear memory can be selectively blocked in rats by inhibition of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling pathway in the CA1 area of the dorsal hippocampus (Szapiro et al., 2000; Chen et al., 2005), we asked whether retrieval is required for memory strengthening produced by additional learning.

EXPERIMENTAL PROCEDURES

Animals

Adult male Wistar rats (220–350 g at time of surgery) from the State Health Research Foundation (FEPPS-RS, Porto Alegre, RS, Brazil) were housed five to a cage in a temperature-controlled colony room with food and water available *ad libitum*, and maintained on a 12-h light/dark cycle (lights on at 7:00 A.M.). Behavioral procedures were conducted during the light phase of the cycle between 10:00 A.M. and 5:00 P.M. All procedures were conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH publication No. 80-23 revised 1996). Experimental protocols were approved by the institutional research ethics and animal care committee (document number GPPG-HCPA 05–519). All efforts were made to minimize the number of animals used and their suffering.

Surgery

Animals were implanted under thionembutal anesthesia (30 mg/kg i.p.) with bilateral 9.0-mm, 23-gauge guide cannulae aimed 1.0 mm above the CA1 area of the dorsal hippocampus as described in previous studies (Quevedo et al., 1999, 2004). Coordinates (anteroposterior, −4.3 mm from bregma, mediolateral, ±3.0 mm from bregma, ventral, −1.4 mm from dura) were obtained from the atlas of Paxinos and Watson (2007). Animals were allowed to recover at least 7 days after surgery.

Behavioral procedures

We used the single-trial step-down inhibitory avoidance (IA) conditioning as an established model of fear-motivated, hippocampus-dependent memory (Izquierdo and Medina, 1997; Taubenfeld et al., 1999). In IA training, animals learn to associate a location in the training apparatus with an aversive stimulus (foot shock). The IA behavioral training and retention test procedures were described in previous reports (Quevedo et al., 1999, 2004). The IA apparatus was a 50×25×25-cm acrylic box (Albarsch, Porto Alegre, RS, Brazil) whose floor consisted of parallel caliber stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7-cm wide, 2.5-cm high platform was placed on the floor of the box against the left wall. On the training trial, rats were placed on the platform and

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Abbreviations: ERK, extracellular signal-regulated kinase; IA, inhibitory avoidance; MAPK, mitogen-activated protein kinase; PD98059, 2-(2′-amino-3′-methoxyphenyl)-oxanaphthalen-4-one.

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their latency to step down on the grid with all four paws was measured with an automatic device. Immediately after stepping down on the grid, rats received a mild foot shock and were removed from the apparatus immediately afterward. In experiments aimed at examining memory strengthening by additional learning, rats were given two consecutive training trials (Training 1 and Training 2, 0.4 mA foot shock for 2 s) separated by 24 h as previously described (Quevedo et al., 1999). For control experiments examining drug effects on memory of a single training trial, rats were given a single training (0.5 mA for 2 s). A retention test trial was carried out either 24 or 48 after the last training trial. The retention test trial was procedurally identical to training, except that no foot shock was presented. Step-down latencies (s) on the retention test trial (maximum 180 s) were used as a measure of IA retention (Quevedo et al., 1999, 2004).

Drugs and infusion procedures

Intrahippocampal infusion procedures were as described in previous reports (Quevedo et al., 1999, 2004). At the time of infusion, a 30-gauge infusion needle was fitted into the guide cannula. The tip of the infusion needle protruded 1.0 mm beyond the guide cannula and was aimed at the CA1 area of the dorsal hippocampus. Ten minutes before IA training trials, immediately after training trials, or 10 min before retention test trials, the ERK/MAPK inhibitor PD98059 (50 μM per side in 0.5 μl; Calbiochem, San Diego, CA, USA) was infused bilaterally into the hippocampus as previously described (Quevedo et al., 2004). PD98059 [2-(2′-amino-3′-methoxyphenyl)-oxanaphthalen-4-one] acts on the ERK/MAPK pathway by inhibiting MAPK kinase (MEK) (Dudley et al., 1995; Alessi et al., 1995). Control animals were given a 0.5-μl bilateral infusion of vehicle (5% dimethyl sulfoxide, DMSO, in saline). PD98059 or vehicle was infused during a 30-s period, and the infusion needle was left in place for an additional minute to allow diffusion of the drug away from the needle tip. The dose of PD98059 was based on previous studies (Quevedo et al., 2004; Chen et al., 2005). Drug solutions were freshly prepared before each experiment.

Histology

Twenty-four to 48 hours after behavioral testing, 0.5 μl of a 4% Methylene Blue solution was infused into the hippocampus as described above, animals were killed by decapitation 10 min later, and their brains were removed and stored in 5% formalin for at least 72 h. The brains were sectioned and examined for cannula placement and spread of the dye in the dorsal hippocampus as previously described (Quevedo et al., 1999, 2004). The extension of the dye was taken as indicative of diffusion of the drugs previously given to each rat.

Statistics

Data are presented as mean±standard error of the mean (SEM) retention test latencies to step-down (s). Because a ceiling was imposed on the retention test values, nonparametric statistics was used to analyze the data (Quevedo et al., 1999, 2004). Statistical analyses were performed using a Mann–Whitney *U*-test for pairwise comparisons between groups and a Wilcoxon test for pairwise comparisons between behavioral sessions within the same group. In all comparisons, $P<0.05$ was considered to indicate statistical significance.

RESULTS

In the first set of experiments (Fig. 1), rats were initially given a first training trial in IA, and were then given a second training trial 24 h later. In the first experiment, we bilaterally infused PD98059 or vehicle into area CA1 of the hippocampus 10 min before the second training trial (Fig. 1A, B). The second trial induced a significant enhancement of memory retention in controls: rats given two training trials and treated with vehicle before training 2 showed significant differences both between the first and second training trial and between the second training and retention test (*P*s <0.01). Rats treated with PD98059 showed a blockade of retrieval of memory for training trial 1 during trial 2 compared to controls ($P<0.01$), and showed a significant difference between the second training and test (*P*s <0.02), but not between the first and second training trials ($P=0.86$). In addition, they had significantly memory impairment compared to controls when tested for retention 24 h after the second training trial (*P*s <0.05). However, the original memory for training 1 was not permanently affected by PD98059 infusion; PD98059-treated rats showed retention levels during the retention test trial comparable to those of control rats during training trial 2 (comparison between latencies of rats treated with PD98059 in the retention test and latencies shown by control animals at training 2, $P=0.97$). These findings indicate that suppression of ERK/MAPK activity in the hippocampus transiently impaired memory retrieval and prevented memory strengthening induced by additional learning, but did not affect the original memory induced by the first learning trial.

In several additional control experiments, the specificity of disrupted retrieval by PD98059 infusions was confirmed. First, we found that treatment with PD098059 did not prevent memory strengthening when infused immediately after the second training trial (Fig. 1C, D), indicating that the impairing effect was not related to a blockade on consolidation of memory for the second trial. Rats given two training trials and infused with either PD98059 or vehicle after training 2 showed significant differences in latencies between the first and second training trials (all *P*s<0.01) and between the second training and retention test (vehicle, $P<0.01$; PD98059, $P<0.05$). There were no significant differences between groups in training 1 ($P=1.00$), training 2 ($P=0.55$), or test ($P=0.84$). In addition, PD98059 did not affect memory retention when given 24 h after the first training trial in the absence of a second training trial (no retrieval controls; Fig. 1E, F). Rats receiving either PD98059 or vehicle showed significant differences in latencies between training and test (*P*s<0.05). There were no significant differences between groups in training ($P=0.37$) or test ($P=0.48$).

Intrahippocampal infusion of PD98059 10 min before (Fig. 2A, B) or immediately after (Fig. 2C, D) a single training trial did not affect learning or retention of IA memory. However, memory was impaired by PD98059 infused 10 min before a retention test carried out 24 h after a single training trial (Fig. 2E, F), supporting the view that the drug infusion induced a selective impairment of retrieval, and not of memory formation. Rats given a single training trial and infused with either PD98059 or vehicle before training showed significant differences in latencies between training and test (*P*s<0.01). There were no significant differences between groups in training ($P=0.36$) or test ($P=0.78$). Rats given a single training trial and infused with