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Short communication

Differential involvement of glutamatergic and catecholaminergic activity within the amygdala during taste aversion retrieval on memory expression and updating

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HIGHLIGHTS

- Exposure to an aversive-conditioned stimulus during retrieval, enhances glutamate, norepinephrine and dopamine signaling within the amygdala.
- The AMPA receptors in the amygdala are involved in the behavioural expression of CTA but not in memory updating.
- Dopaminergic D1 and NMDA receptors are not involved in behavioural expression but are neccesary for CTA memory updating.
- β-adrenergic receptors subserve behavioural expression and CTA memory updating.

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ABSTRACT

During memory retrieval, consolidated memories are expressed and destabilized in order to maintain or update information through a memory reconsolidation process. Despite the key role of the amygdala during memory acquistion and consolidation, the participation of neurotransmitter signals in memory retrieval is poorly understood. Hence, we used conditioned taste aversion and *in vivo* microdialysis to evaluate changes in glutamate, norepinephrine and dopamine concentrations within the amygdala during memory retrieval. We observed that exposure to an aversive-conditioned stimulus induced an augmentation in glutamate, norepinephrine and dopamine levels within the amygdala, while exposure to a familiar and safe stimulus did not induce changes in these neurotransmitters levels. Also, we evaluated the amygdalar blockade of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl-D-aspartate (NMDA), β-adrenergic and dopamine D1 receptors in memory retrieval and updating. Results showed that during retrieval, behavioural expression was impaired by intra-amygdalar blockade of AMPA and β-adrenergic receptors, whereas NMDA, D1 and β-adrenergic receptors blockade hindered memory updating. In summary, during conditioned taste aversion retrieval there was an increase in the extracellular levels of glutamate, norepinephrine and dopamine within the amygdala, and their receptors activity were differentially involved in the behavioural expression and memory updating during retrieval.

In conditioned taste aversion (CTA), animals associate a novel taste with gastric malaise; this association produces taste aversion measured as a decrease in the consumption of the taste in further presentations [3]. The amygdala is highly involved in the acquisition, consolidation and retrieval of CTA (see Ref. [18]. We have demonstrated that during the acquisition of CTA, exposure to a novel taste stimulus induces an increase in the extracellular levels of norepinephrine (Guzmán-Ramos et al. [9], whereas induction of gastric malaise promotes an augmentation of extracellular levels of glutamate and norepinephrine within the amygdala [22,13,9]. Although, there is scarce information about amygdala neurotrasmitters relase during CTA retrieval, it has been shown that exposure to aversive-conditioned saccharin induces an augmentation of glutamate within the amygdala [22]. However, it remains

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unknown whether catecholamines could follow similar extracellular augmentations as glutamate during memory retrieval.

Memory is consolidated over time through a protein synthesis-dependent process. Furthermore, during memory retrieval, consolidated memories become labile and require similar protein-synthesis processes to maintain or update memories, a process named memory reconsolidation [16,5]. However, it has been shown that inhibition of protein synthesis in the amygdala has no effect on CTA memory reconsolidation when a single acquisition trial is used [1]. Thus, we have proposed that multi-trials protocol induces strong CTA memory and its extinction is delayed on subsequent presentations, facilitating the observation of memory reconsolidation process and updating [20,5]. Using this model, we have demostrated that AMPA receptor antagonists impair behavioural expression during retrieval without affecting memory reconsolidation [20,6]. Whereas infusions of protein synthesis inhibitors or an NMDA receptor antagonist in the amygdala disrupt CTA memory reconsolidation without affecting behavioural expression on retrieval [20,6]. In regard of catecholaminergic activity within the amygdala, there is scarce information about memory retrieval. Nevertheless it has been demonstrated that norepinephrine antagonists impaired reconsolidation of aversive memories induced by morphine [23], and dopaminergic activity seems to be required for taste-rewarded memory reconsolidation [12].

In this study, we used *in vivo* microdialysis in order to evaluate changes in glutamate, norepinephrine and dopamine levels within the amygdala during memory retrieval. Moreover, to analyse the functional role of NMDA, AMPA, β-adrenergic and D1 receptors in CTA memory retrieval and updating, we used selective antagonists in the amygdala before a second CTA acquistion trial.

Adult male Wistar rats from the Instituto de Fisiología Celular were anesthetized with a ketamine–xylazine mixture (100–10 mg/kg) and a unilateral guide cannula (CMA Microdialysis. Stockholm, SE) aiming at the amygdala was implanted using standard stereotaxic procedures, right an left hemispheres were counterbalanced. The guide cannula was implanted with coordinates from Bregma (AP—2.8 mm; L 4.8 mm; DV—7.5 mm) [17], and was fixed to the skull using two screws and dental acrylic cement. All procedures were approved by the institutional committee for the care and use of laboratory animals of the Instituto de Fisiología Celular (FBR25-14) which is based on National Institutes of Health Guide for the Care and Use of Laboratory Animals. Behavioural scheme began 6 days after surgery allowing the animals to recover. Rats were then water-deprived 24 h prior behavioural scheme. Animals were habituated in a microdialysis chamber once a day for 1 h and were allowed to drink 30 mL of tap water from a graded bottle during 15 min, water baseline consumption intake was established over 6 days. A second drinking session in the afternoon served to prevent dehydration. On the seventh day, rats were separated in two groups, an aversively conditioned group (Aversive, n = 10), which was exposed for 15 min to 30 mL of a 0.1% (wt/vol) sodium saccharin solution (Sigma-Aldrich. Missouri, US) and fifteen minutes later rats received an i.p. LiCl (Baker. New Jersey, US) injection (0.2 M, 7.5 mL/kg, this concentration induces a robust CTA, see Ref. [19]. In the control group (Non-Aversive, n = 8), rats drank the same saccharin solution, paired with an i.p. NaCl (Sigma-Aldrich. Missouri, US) injection (0.2 M, 7.5 mL/kg). NaCl does not cause gastric malaise and therefore no taste aversion would be developed. Three days after training, microdialysis procedure was performed in both groups by the insertion of 1 mm length membrane dialysis probe (CMA 12 MD Probe, CMA Microdialysis. Stockholm, SE) connected to the micro-infusion pump system. (CMA Microdialysis. Stockholm, SE). The pump perfused the probe continuously at a rate of 0.8 μL/min with Ringer solution (NaCl 118 mM, KCl 4.7 mM, $NaH_2PO_4H_2O$ 1.2 mM, $MgSO_47H_2O$ 1.2 mM, $NaHCO_3$ 19 mM, $CaCl_2$ 2.5 mM, glucose 3.3 mM). After probe insertion, the first hour of sampling was discarded due to fluid stabilization; samples were collected every 5 min (4 μL/sample) in vials containing 1 μL of antioxidant mixture (0.25 mM ascorbic acid, Na_2EDTA 0.27 mM, 0.1 M acetic acid). The first three samples were used to calculate the basal concentration of extracellular neurotransmitters; afterwards, a graded bottle with 0.1% (wt/vol) sodium saccharin solution was placed in the microdialysis chamber for 15 min and consumption intake was measured.

Neurotransmitter concentrations were determined by capillary electrophoresis as described in Ref. [8]. Capillary electrophoresis-based separations with laser induced fluorescence detection were used for the analysis (Beckman-Coulter PACE/MDQ, Glycoprotein System). In order to identify glutamate, norepinephrine and dopamine, we matched the obtained electropherograms with a spiked sample. Samples were corrected by relating the area under the curve of the unknown sample with the area under the curve of the internal standard. Analyses were performed with the Karat System Gold software (Beckman Coulter. California, US). Results are expressed as percentage of baseline concentration (% Baseline concentration = analyte concentration × 100/mean of the three first samples).

A repeated measures ANOVA indicated a significant interaction between time and group ($F_{(7,98)} = 2.171$ $p < 0.05$) in the extracellular levels of glutamate. The Non-aversive group remained without changes among time in glutamate levels within the amygdala (p = NS), whereas the Aversive group showed significant differences when the conditioned stimulus was present at the 25 min fraction ($p < 0.01$). Exposure to the aversive-conditioned gustatory stimulus induced a significant augmentation of glutamate within the amygdala at the 25 min fraction between Aversive and Non-Aversive group ($p < 0.05$).

During CTA retrieval, a repeated measures ANOVA showed a significant interaction between time and group ($F_{(7,63)} = 2.121$ $p < 0.05$) in the concentration of norepinephrine (Fig. 1B) within the amygdala. *Post hoc* tests indicated that in the Aversive group, there were differences in norepinephrine levels compared to baseline extracellular levels ($p < 0.05$) and also there were differences between groups ($p < 0.05$) at minutes 20 and 25 when the conditioned stimulus is present.

We also observed a significant main effect of the group on the extracellular levels of dopamine (Fig. 1C) within the amygdala during CTA retrieval ($F_{(1118)} = 4.172$ $p < 0.05$). Furthermore, we did not observe changes in extracellular levels of dopamine between time ($F_{(7118)} = 0.838$ p = NS), neither a time-group interaction ($F_{(7118)} = 1.222$ p = NS). The *post hoc* test revealed that the Aversive group showed changes in dopamine levels compared to baseline concentration ($p < 0.01$) and also compared to Non-Aversive groups at minute 25. Student's non-paired statistical analysis showed that the Aversive group produced a significant and clear taste aversion, while the Non-Aversive group failed to elicit reliable CTA ($t = 4.343$, $p < 0.01$; Fig. 1D).

According to these results, when animals are exposed to saccharin previously paired with gastric malaise, there is a reduction in saccharin consumption and there is an augmentation in the extracellular levels of glutamate, norepinephrine and dopamine in the amygdala compared to Non-Aversive group animals.

In order to evaluate the functional role of NMDA, AMPA, β-adrenergic or D1 receptors in the behavioural expression and memory updating during CTA memory retrieval, rats were implanted bilaterally with 12 mm long stainless-steel guide cannulae (23 gauges) directed to the amygdala (AP—2.8 mm, L ± 4.8 mm, DV—6.5 mm relative to Bregma). Animals were deprived of water for 24 h and baseline water consumption intake was established over 6 days. Animals were handled for 3 min a day until the infusion day to diminish handling-associated stress. During CTA acquisition, rats were exposed to a 0.1% (wt/vol) saccharin solu-