Memory retrieval of inhibitory avoidance requires histamine H_1 receptor activation in the hippocampus

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Contributed by Ivan Izquierdo, March 30, 2016 (sent for review March 2, 2016; reviewed by Michel Baudry and Benno Roozendaal)

Retrieval represents a dynamic process that may require neuromodulatory signaling. Here, we report that the integrity of the brain histaminergic system is necessary for retrieval of inhibitory avoidance (IA) memory, because rats depleted of histamine through lateral ventricle injections of α-fluoromethylhistidine (a-FMHis), a suicide inhibitor of histidine decarboxylase, displayed impaired IA memory when tested 2 d after training. a-FMHis was administered 24 h after training, when IA memory trace was already formed. Infusion of histamine in hippocampal CA1 of brain histamine-depleted rats (hence, amnesic) 10 min before the retention test restored IA memory but was ineffective when given in the basolateral amygdala (BLA) or the ventral medial prefrontal cortex (vmPFC). Intra-CA1 injections of selective H_1 and H_2 receptor agonists showed that histamine exerted its effect by activating the H_1 receptor. Noteworthy, the H_1 receptor antagonist pyrilamine disrupted IA memory retrieval in rats, thus strongly supporting an active involvement of endogenous histamine; 90 min after the retention test, c-Fos–positive neurons were significantly fewer in the CA1s of a-FMHis–treated rats that displayed amnesia compared with in the control group. We also found reduced levels of phosphorylated cAMP-responsive element binding protein (pCREB) in the CA1s of a-FMHis–treated animals compared with in controls. Increases in pCREB levels are associated with retrieval of associated memories. Targeting the histaminergic system may modify the retrieval of emotional memory; hence, histaminergic ligands might reduce dysfunctional aversive memories and improve the efficacy of exposure psychotherapies.

memory | retrieval | inhibitory avoidance | histamine | H1 receptor

Memory determines the uniqueness of our personal history and is decisive for each individual to survive and prosper. It is a multistate process that includes acquisition, consolidation, and retrieval (1). Whereas considerable advancement has been made toward understanding the specific brain structures (e.g., amygdala, prefrontal cortex, and hippocampus) and molecular mechanisms (receptors and signaling pathways) that underlie acquisition and consolidation (2, 3), the understanding of retrieval has lagged behind, although it is ultimately the only possible measure of memory (4–6). Indeed, retrieval is not simply a static readout of stored information; rather, it represents a dynamic process that can be studied separately from either acquisition or consolidation, with which it shares similar mechanisms (1, 7, 8). Furthermore, retrieval can elicit specific processes that modify the recalled memory (9). In this regard, protein synthesis, a necessary step in the transfer of a labile short-term memory into a stable long-term memory (LTM) (2), is required to enable retrieval, because infusion of protein synthesis inhibitors in the amygdala 10 min before retrieval impaired fear memory expression (10). An interesting question is whether neuromodulatory signaling is required for not only memory acquisition and consolidation (11) but also, retrieval. There is evidence that adrenergic signaling is required for the retrieval of various types of hippocampus-dependent memory in mice (12) as well as in humans (13). Intrahippocampal blockade of metabotropic glutamate or alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid (AMPA) receptors immediately before retrieval impaired LTM expression of an inhibitory avoidance (IA) response (7), thus suggesting that glutamatergic signaling in hippocampus is necessary for retrieval of an aversive memory. In contrast, cholinergic and dopaminergic signaling contributes to acquisition and/or consolidation but is generally not required for retrieval (6). The histaminergic system modulates memory consolidation in many cognitive tasks from IA to fear conditioning and object recognition (14–16). We recently showed that rats were able to consolidate an IA memory only when the brain histaminergic system was intact (17). Histamine within the brain is synthesized from histidine by histidine-decarboxylase solely in hypothalamic tuberomamillary nucleus neurons (18), which are organized into functionally distinct circuits (19), display selective control mechanisms (19, 20), and impinge on different brain regions, including amygdala, prefrontal cortex, and hippocampus, that are responsible for many forms of learning (21).

Based on the above findings, it is conceivable that histaminergic signaling is required for memory retrieval as well. Consequently, this study was specifically designed to address this question using an IA task, an associative learning paradigm that has largely contributed

Significance

Several neurotransmitters contribute to memory formation by modulating selectively acquisition, consolidation, and/or retrieval. Integrity of the brain histamine system is necessary for the consolidation of inhibitory avoidance (IA) memory. Here, we report that cerebral histamine depletion also impairs retrieval of IA in rats and blunts retrieval-induced c-Fos activation and cAMP-responsive element binding protein phosphorylation in the CA1 region of the hippocampus. Histamine infusion into the CA1 restores IA retrieval in histamine-depleted rats by targeting brain histamine H_1 receptors. Our study uncovers previously unidentified mechanisms involved in memory retrieval and may offer possible targets for eventual pharmacotherapies to treat dysfunctional aversive memories, including phobias, panic attacks, and posttraumatic stress disorders, as well as improve the efficacy of exposure psychotherapies.

Author contributions: R.F., C.R.G.F., M.B.P., I.I., J.d.C.M., and P.B. designed research; R.F., C.R.G.F., G.P., E.B., C.B., and J.d.C.M. performed research; R.F., C.R.G.F., M.B.P., I.I., J.d.C.M., and P.B. analyzed data; and M.B.P., I.I., J.d.C.M., and P.B. wrote the paper.

Reviewers: M.B., Western University of Health Sciences; and B.R., Donders Institute, Radboud University.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1604841113/-/DCSupplemental.

to the understanding of memory processes (22). We first examined whether brain histamine depletion through administration of the histidine-decarboxylase suicide inhibitor α-fluoromethylhistidine (a-FMHis) (23) in the lateral ventricle (LV) 24 h after training affected IA retrieval. Then, we tested whether the local infusion of histamine in the basolateral amygdala (BLA), the ventral medial prefrontal cortex (vmPFC), or the CA1 region of the dorsal hippocampus overcame a-FMHis–elicited impairment of retrieval, and we characterized the type of histamine receptor involved. We also investigated changes in neuronal activity by assessing after retrieval the pattern of c-Fos expression as well as cyclic adenosine monophosphate (cAMP)-responsive element binding protein (CREB) phosphorylation in BLA, vmPFC, and CA1 region of histamine-depleted rats. Finally, we investigated the ability of histamine to restore retrieval after administration of protein synthesis inhibition in the CA1 region.

Results

Depletion of Histamine Impairs Retrieval Independently of Consolidation. We recently showed that intra-LV infusion of a-FMHis (5 μg/μL) quickly and fully suppressed the release of brain histamine measured by microdialysis, which was restored to control levels after about 48 h (17). Thus, to investigate the role of endogenous histamine in retrieval, we examined the performance of rats infused into the LV with a-FMHis 24 h after the IA training session. The retention test was carried out 48 h after training. Latencies were compared with those of rats treated with a-FMHis 24 h before training and animals given equivalent infusions of saline (controls). Latencies of all groups during IA training did not differ. Fig. 1 shows the stepdown latency during testing of rats treated with a-FMHis and control. One-way ANOVA performed at the retention test revealed a significant difference across groups ($F_{2,41} = 62.89$; $P < 0.0001$). Additional analysis with Bonferroni's multiple comparisons tests (MCT) showed that latencies of all rats treated with a-FMHis either 24 h before or 24 h after IA training were significantly shorter than those of controls. Further analysis with Bonferroni's MCT showed that latencies of all rats treated with a-FMHis, either 24 h before or after IA training, were significantly shorter than those of controls. As a-FMHis effect on histamine synthesis persists for about 48 h (22), it is conceivable that rats receiving a-FMHis prior to IA training lacked integrity of the histaminergic system during the consolidation process, whereas those treated 24 h after training, during the retrieval. These findings confirm that histamine depletion impairs the consolidation of IA memory (17) and suggest that it also worsens IA-LTM expression by influences on memory retrieval mechanisms. To further test these hypotheses, we investigated whether intracerebral administration of histamine reversed the amnesic effect of histamine depletion caused by a-FMHis.

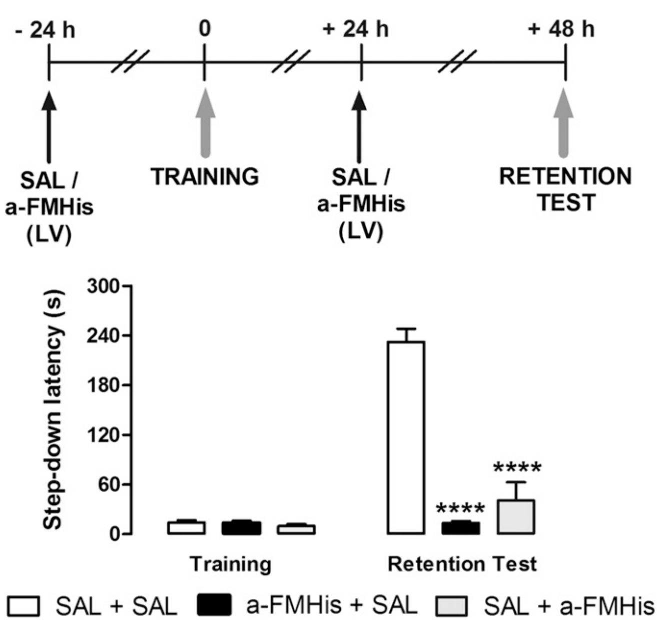

Fig. 1. Effect of histamine acute depletion through a-FMHis administration on IA task. The schematic drawing shows the sequence of behavior procedures and treatments. Rats were implanted with an infusion cannula in the LV and distributed to three groups: one group received saline (SAL) 24 h before and after IA training (control), a second group received a-FMHis 24 h before IA training and SAL 24 h after training, and the third group received SAL 24 h before IA training and a-FMHis 24 h after training. Data are expressed as means ± SEMs of 10–12 animals for each group. ****$P < 0.0001$ vs. control (one-way ANOVA and Bonferroni's MCT).

Effects of Histamine Infusion into the BLA, vmPFC, or CA1 on a-FMHis–Induced Amnesia. Histamine (1 μg/μL) was infused bilaterally into the BLA (Fig. 2*A*), the vmPFC (Fig. 2*B*), or the CA1 (Fig. 2*C*) 10 min before the retention test of rats given a-FMHis 24 h before or after IA training. The retention test was carried out 48 h after IA training. Controls received equivalent infusions of saline. Latencies of all groups during IA training did not differ. One-way ANOVA performed at the retention test revealed a significant difference across groups (BLA: $F_{2,24} = 58.76$; $P < 0.0001$; vmPFC: $F_{2,40} = 41.05$; $P < 0.0001$; CA1: $F_{2,27} = 34.11$; $P < 0.0001$). Additional analysis with Bonferroni's MCT showed that rats treated with a-FMHis before training and histamine in the BLA (Fig. 2*A*), the vmPFC (Fig. 2*B*), or the CA1 (Fig. 2*C*) displayed latencies significantly shorter than respective controls. Also, latencies of rats infused with a-FMHis 24 h after training and histamine into the BLA (Fig. 2*A*) or the vmPFC (Fig. 2*B*) were significantly shorter than those of corresponding controls. Conversely, latencies of rats treated with a-FMHis intra-LV 24 h after training and intra-CA1 histamine did not differ from their controls (Fig. 2*C*). Taken together, these results indicate that histamine never rescued IA-LTM of rats given a-FMHis before training. When infused into the BLA or the vmPFC of animals treated with a-FMHis after training, histamine did not restore IA-LTM. Conversely, it antagonized amnesia when given into the CA1 region. These findings suggest that animals treated with a-FMHis after IA training formed a memory of this experience and support a crucial role for histamine neurotransmission in the CA1 during retrieval.

Intra-CA1 Infusion of Histamine Did Not Reverse Amnesia of IA-LTM Elicited by Intra-CA1 Administration of Anisomycin. To verify whether the amnesia induced by a-FMHis given 24 h before IA training shared features with the memory impairment of rats unable to consolidate memory, we investigated IA-LTM in animals that received bilateral infusion of anisomycin (80 μg/μL), a protein synthesis inhibitor, in the CA1 immediately after training, saline or a-FMHis intra-LV 24 h after training, and saline or histamine (1 μg/μL) into the CA1 10 min before the retention test (Fig. 2*D*). Controls received comparable infusions of saline. Latencies of all groups during the IA training did not differ. One-way ANOVA performed on the retention test revealed a significant group effect ($F_{3,55} = 98.10$; $P < 0.0001$). As shown in Fig. 2*D*, Bonferroni's MCT analysis showed no difference among the three groups of rats given anisomycin independently of the subsequent treatments, but all displayed latencies significantly shorter than controls. Thus, anisomycin-treated rats showed a substantial memory impairment that was not reversed by histamine administration in the CA1.

Histamine H_1 Receptor Activation Is Required for IA-LTM Retrieval. Histaminergic fibers innervate the hippocampus through the fornix and a caudal route (24), and histamine modulates hippocampal functions, including memory processes, through interactions

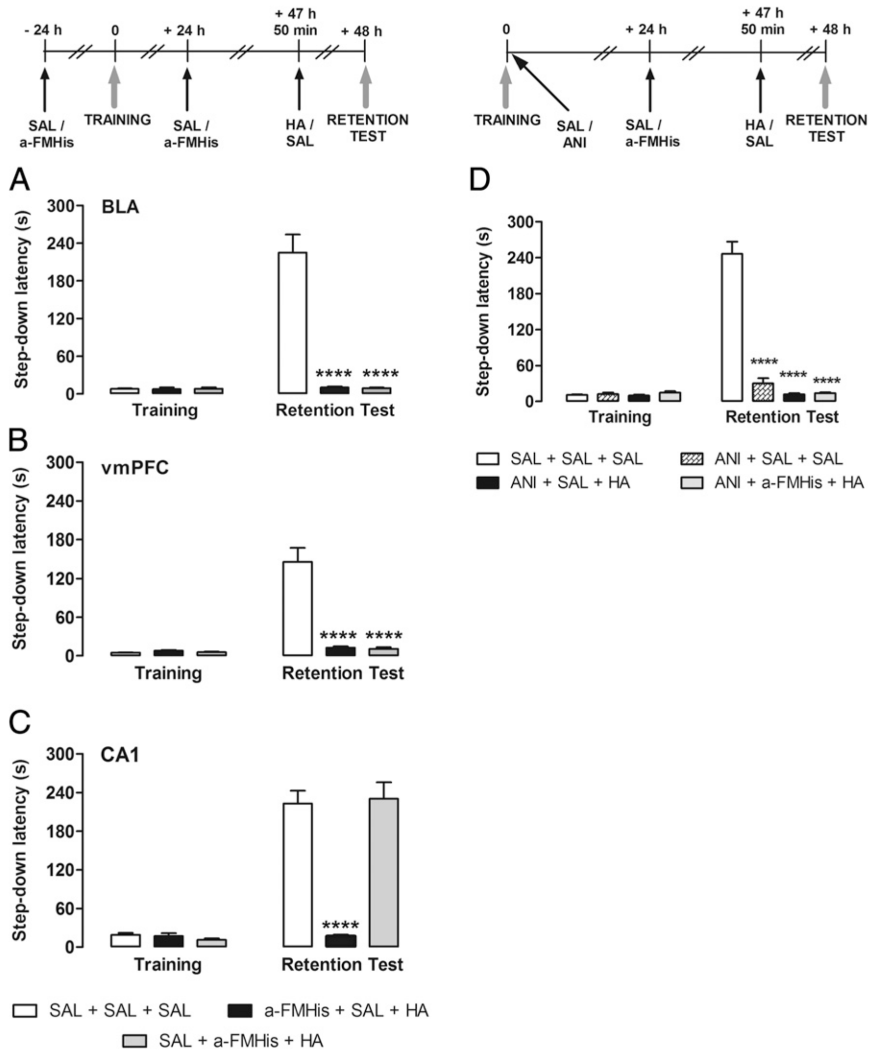

Fig. 2. Effect of histamine infusion into the BLA, vmPFC, or CA1 on a-FMHis– or anisomycin-induced amnesia. The schematic drawings show the sequence of behavior procedures and treatments. Rats were implanted with infusion cannula in the LV to administer a-FMHis or saline (SAL) and a second cannula bilaterally in the (*A*) BLA, (*B*) vmPFC, or (*C* and *D*) CA1. Data are expressed as means ± SEMs of 10–12 animals for each group. ****$P < 0.0001$ vs. respective controls (one-way ANOVA and Bonferroni's MCT). ANI, anisomycin; HA, histamine.

with H_1 and H_2 receptors (25–27). To characterize the histamine receptor type involved in the reversal of a-FMHis–induced amnesia (Fig. 2*C*), rats were infused with a-FMHis into the LV 24 h after IA training and 2-(2-pyridyl)ethylamine (PEA; 1.2 μg/μL) or dimaprit (DIM; 2.3 μg/μL) into the CA1 10 min before the retention test (Fig. 3*A*). PEA selectively activates H_1 receptors, whereas DIM is an H_2 agonist with no H_1 activity (28). Controls received equivalent infusions of saline. One-way ANOVA performed at the retention test displayed a significant difference in latency across groups ($F_{2,42} = 118.1$; $P < 0.0001$), and Bonferroni's MCT analysis indicated that rats infused with DIM had significantly shorter latencies than those infused with saline or PEA. Hence, DIM did not reverse a-FMHis–induced amnesia. Controls and PEA-treated rats displayed similar levels of latency during the retention test, thus suggesting that the H_1 receptor contributed to the full reinstatement of IA-LTM expression in animals rendered amnesic by a-FMHis. To further investigate the role of H_1 receptors, we examined the effects of pyrilamine (20 μg/μL), an H_1 antagonist (29) infused in the CA1 10 min before the retention test. During IA training, there were no significant differences among the groups in their latencies (Fig. 3*B*). However, on the retention test, rats infused with pyrilamine displayed shorter latencies than those infused with saline (unpaired *t* test; $P < 0.05$) (Fig. 3*B*), thus indicating that blockade of H_1 receptors in the CA1 impaired IA-LTM expression. This finding extends the observations with PEA, strongly suggesting an active involvement of endogenous histamine in IA-LTM retrieval.

Effect of Histamine Depletion on c-Fos Expression After IA-LTM Retrieval in Rat BLA, vmPFC, and CA1. In an attempt to clarify how brain histamine deficit may influence IA-LTM retrieval, we measured c-Fos protein expression in three brain regions of rats given saline or a-FMHis into the LV 24 h after IA training and subjected to a retention test 48 h after training. Rats were euthanized 90 min after the retention test. No differences in c-Fos expression were found in the BLA or the vmPFC of rats treated with either saline or a-FMHis (Fig. 4 *A* and *B*). Conversely, neurons immunopositive for c-Fos were significantly fewer in the CA1 of a-FMHis–treated rats compared with in the control group (unpaired *t* test; $P < 0.0001$) (Fig. 4*C*).

Effect of Histamine Depletion on Levels of pCREB After IA-LTM Retrieval in Rat BLA, vmPFC, and CA1. CREB is a crucial mediator in the formation of IA-LTM (17, 30), and an increase in

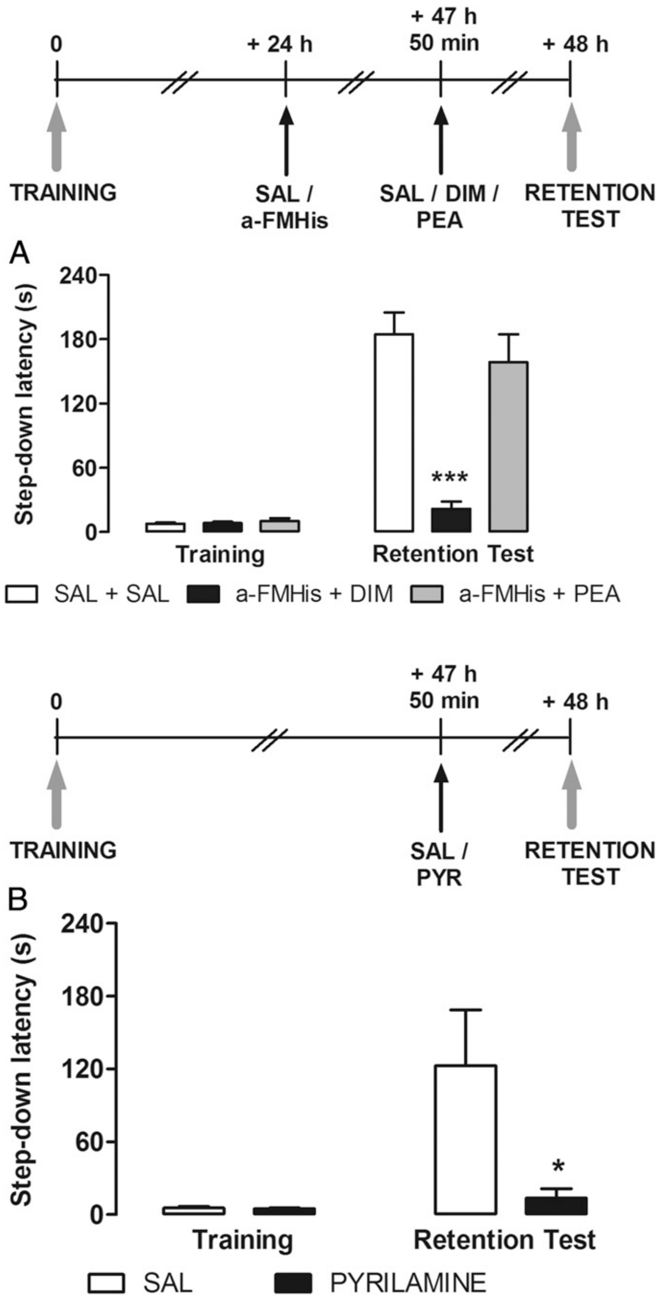

Fig. 3. Effect of histamine receptor ligands infusion into CA1 on a-FMHis–induced amnesia. The schematic drawings show the sequence of procedures and treatments. Rats were implanted with infusion cannulas in the (*A*) LV and (*B*) CA1 bilaterally. Data are expressed as means ± SEMs of 8–12 animals for each group. PYR, pyrilamine; SAL, saline. **P* < 0.05 vs. controls (unpaired *t* test); ****P* < 0.001 vs. controls (one-way ANOVA and Bonferroni's MCT).

CREB phosphorylation at Ser-133 in the hippocampus is specifically associated with the consolidation of IA memory (31, 32). To investigate the influences of histamine depletion on phosphorylated cAMP-responsive element binding protein (pCREB) after IA-LTM retrieval, rats were euthanized immediately after the retention test, and pCREB levels were assessed in the BLA, vmPFC, and CA1 of rats given saline or a-FMHis infusions in the LV 24 h after IA training (Fig. 5). No difference of pCREB density was found in the BLA or the vmPFC of rats given saline or a-FMHis. Conversely, a significant decrease of pCREB was detected in the CA1 of rats treated with a-FMHis compared with saline-treated animals (one-way ANOVA and Bonferroni's MCT; $F_{5,31} = 5.199$; $P < 0.001$).

Discussion

This study shows that intact histamine neurotransmission is required to enable not only IA consolidation but also, retrieval. Furthermore, it shows that H_1 receptor activation in the CA1 is necessary for IA-LTM retrieval. This finding adds strong support to the view that retrieval is a dynamic process that requires neuromodulatory signaling, just like acquisition, consolidation, reconsolidation, and memory maintenance (6, 33, 34), and identifies a crucial role for the neurotransmitter histamine in this process. Indeed, rats temporarily depleted of histamine through infusion of a-FMHis, an irreversible inhibitor of histidine decarboxylase, into the LV 24 h either before or after IA training displayed amnesia of IA training 48 h posttraining. We reported earlier that pretraining administration of a-FMHis disrupted the consolidation of IA memory, while leaving IA short-term memory intact (17), and suppressed brain histamine release for ∼48 h (17). Because molecular changes specific for IA-LTM in rat hippocampus begin immediately after the training session and progress for less than 20 h (35, 36), the amnesia resulting from a-FMHis infusion 24 h posttraining is likely caused by an impairment of retrieval rather than consolidation. This view is supported by the observation that intra-CA1 infusion of anisomycin 12 h after training failed to disrupt IA-LTM tested 2 d posttraining (35, 37). Indeed, the requirement of de novo protein synthesis to consolidate IA memory is crucial only during the immediate phase after training (35, 37). Delayed processes, such as gene expression-dependent phases, are necessary specifically for long-term maintenance of the memory but not for its formation (36, 38).

We found that intra-CA1 injections of histamine 10 min before retrieval (therefore 48 h after training) restored IA-LTM in rats given a-FMHis after training but failed to rescue memory loss of animals given a-FMHis before training. In keeping with these results, we reported earlier that application of exogenous histamine in the CA1 of rats depleted of brain histamine pretraining (hence, amnesic) restored IA-LTM only when given within 6 h after training (17). A plausible interpretation of the results presented here is that histamine injections reestablished retrieval mechanisms impaired by its depletion but only in rats that received a-FMHis posttraining and thus, had formed a memory trace. This explanation fits well with the finding that intra-CA1 infusion of exogenous histamine 10 min before the retention test did not reverse the amnesia induced by anisomycin injected into the CA1 immediately after training at a dose that blocked protein synthesis for about 6 h (39). De novo protein synthesis in the hippocampus at training is critical for the consolidation of IA memory (8, 35, 40), and local infusion of anisomycin around that time thwarts memory trace formation (35, 41). Recently, it has been reported that anisomycin also impaired memory retrieval when administered 10 min before the retention test (10). In this study, anisomycin is no longer active during testing, thus ruling out the possibility that memory loss was caused by retrieval inhibition.

The observation that exogenous histamine restored memory in histamine-depleted rats only when given into the CA1, whereas its administration into the BLA or the vmPFC was ineffective, is intriguing, because the IA task can be learned when the hippocampus is inactivated (42, 43). We recently showed that IA-LTM normally involves the hippocampus but can be mediated by other structures if this region is compromised (17). Activation of the glutamate AMPA receptor simultaneously in the hippocampus, amygdala, entorhinal cortex, and parietal cortex is a requirement for IA retrieval 1 d after training (33). This simultaneous activation is not the case of histamine receptors, with operational presence in the CA1 that is sufficient to complete retrieval, at least for the timeframe

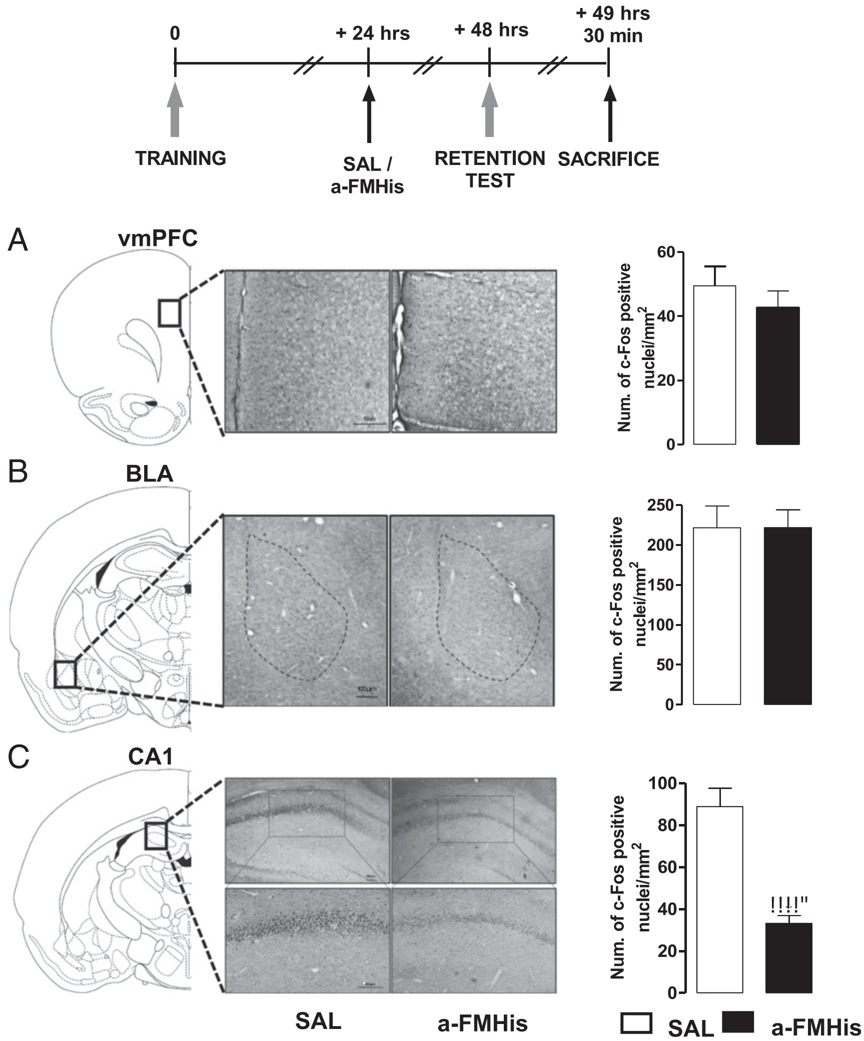

Fig. 4. c-Fos expression in the CA1 of rats trained and tested for IA memory is blunted in a-FMHis–treated rats. The schematic drawing shows the sequence of procedures and treatment. Brain coronal sections show the effect of exposure to retention test on c-Fos protein expression in the (*A*) BLA, (*B*) vmPFC, and (*C*) CA1 of rats given intra-LV infusions of saline (SAL) or a-FMHis 24 h after IA training. Data are expressed as means ± SEM of three to four rats for each group. (Scale bars: *A*, 100 μm; *B*, 500 μm; *C*, *Upper*, 100 μm; *C*, *Lower*, 500 μm.) ****$P < 0.0001$ (unpaired *t* test).

investigated in this study. There is much evidence that activation of H_2 receptors potentiates consolidation in aversive tasks (14, 27, 44), including IA (26, 45). The hippocampus expresses H_1 and H_2 receptors (46, 47), and both facilitate cAMP accumulation (48). Hippocampal infusion with the H_2 receptor agonist DIM 10 min before the retention test failed to restore a-FMHis–induced amnesia, whereas rats injected with the H_1 receptor agonist PEA showed latencies similar to those of saline-injected controls. Furthermore, administering the H_1 antagonist pyrilamine 10 min before retention test in rats with normal levels of histamine completely disrupted IA retention. This result strongly supports the notion that histamine signaling through H_1 receptors is essential for IA-LTM retrieval. Our results are in agreement with previous reports showing that the H_1 receptor is implicated in memory processes, because H_1 receptor KO mice showed impaired memory performance in the Barnes maze (49) and the radial maze task (50). Accordingly, long-term potentiation in the CA1 was significantly reduced in H_1-KO compared with WT mice (49). Studies using c-fos-tTA reporter mice have shown that the same neurons in the amygdala, hippocampus, and cortex that are active during contextual fear learning are reactivated when memory is retrieved (51, 52). Recently, it has been reported that CA1 neurons that were engaged during contextual fear learning reduced levels of pCREB and were responsible for reinstating memory representations that occurred during learning in the cortex at the time of retrieval (53). When those specific CA1 neurons were silenced, specific cortical reactivation was reduced, and mice became unable to retrieve a previously formed contextual fear memory (53). We may speculate that IA-LTM shares these features with contextual fear memory and that H_1 receptor activation is necessary for reactivating the CA1 neurons engaged during learning, thus being responsible for reinstatement of cortical-specific activity necessary for retrieval. Consistent with this idea, given that gene expression in c-fos-tTA mice largely recapitulated endogenous c-Fos expression (52), we found that, 90 min after exposure to the retention test, c-Fos–positive neurons were significantly fewer in the CA1 of a-FMHis–treated rats that also displayed amnesia compared with those in the same region of animals given saline and displaying intact IA-LTM retention. Previous studies supported this contention (53, 54). Immediately after the retention test, we found reduced levels of pCREB in the CA1 of a-FMHis–treated animals compared with in controls. CREB phosphorylation represents a crucial step for the consolidation of LTM (30), and increases in pCREB levels associated with retrieval of fear conditioning as well as spatial memory in different brain regions,

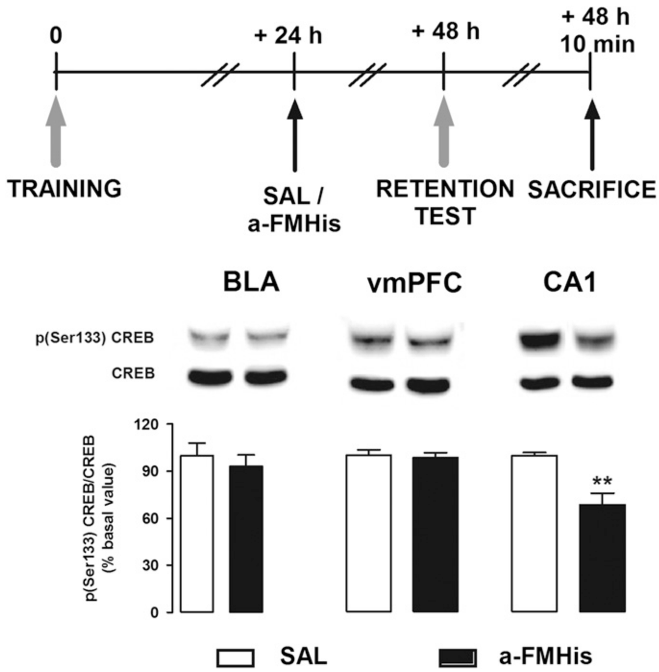

Fig. 5. Effect of histamine depletion on levels of CREB phosphorylation in the BLA, vmPFC, and CA1 of rats trained and tested for IA memory. The schematic drawing displays the sequence of procedures and treatments. Rats were implanted with an infusion cannula in the LV. Representative immunoblots and densitometric quantification are shown. Data are expressed as means ± SEMs of four rats. $^{**}P < 0.01$ vs. respective saline (SAL; one-way ANOVA and Bonferroni's MCT).

including the hippocampus, have been reported (55, 56). We suggest that increased levels of pCREB are required for the retrieval of IA-LTM through activation of H_1 receptors. Consistently, earlier reports indicate that histamine signaling is necessary to trigger CREB phosphorylation in the hippocampus (17, 57). The hippocampus is involved in both the aversive component of the IA and its contextual aspect as reviewed in the work by Izquierdo et al. (58).

Taken together, this study suggests (*i*) that the integrity of the histaminergic system in the CA1 region of the hippocampus is crucial for IA-LTM retrieval, (*ii*) that histamine-depleted rats do not retrieve IA-LTM and display a reduced number of c-Fos–positive cells as well as a lower level of pCREB in CA1 after retrieval compared with controls, and (*iii*) that blockade of H_1 receptors in the CA1 of normal rats impairs the retrieval of IA-LTM 2 d after training. Advances in the understanding of mechanisms underlying IA memory may help in the search for treatments of psychiatric diseases, such as posttraumatic stress disorder or obsessive–compulsive disorder. Here, we provide evidence that targeting the histaminergic system may modify the encoding, consolidation, and retrieval of emotional memory.

Materials and Methods

Animals. Male Wistar rats (3 mo old; 300–330 g) purchased from Centro de Modelos Biologicos Experimentais of the Pontifical Catholic University of Rio Grande do Sul (our regular provider) were used. Animals were housed four to a cage with water and food ad libitum under a 12-h light/dark cycle (lights on at 7:00 AM). The temperature of the animals' room was maintained at 23 °C ± 1 °C. All procedures were in accordance with the NIH's *Guide for the Care and Use of Laboratory Animals* (59) and approved by Animal Committee on Ethics in the Care and Use of Laboratory Animals of the Pontifical Catholic University of Rio Grande do Sul.

Surgery. At least 1 wk after their arrival, animals were anesthetized (75 mg/kg ketamine plus 10 mg/kg xylazine i.p.) and placed on a stereotaxic frame (Kopf). A stainless steel cannula (22 gauge) was implanted in the LV (anterior, −0.9 mm; lateral, −1.5 mm; ventral, −2.6 mm from Bregma) (60) and fixed to the skull by using dental cement. Animals were also implanted bilaterally with 22-gauge guide cannulas 1 mm above the CA1 region of the dorsal hippocampus (anterior, −4.2 mm; lateral, ±3.0 mm; ventral, −1.8 mm from Bregma), the BLA (anterior, −2.4 mm; lateral, ±5.1 mm; ventral, −7.5 mm from Bregma), or the vmPFC (anterior, +3.2 mm; lateral, ±0.8 mm; ventral, −2.0 mm from Bregma) (60). Cannula placements were verified postmortem as described in detail in *SI Materials and Methods*. Animals were allowed 7 d to recover from surgery before behavioral procedures. All rats were handled once daily for 3 consecutive d, and all behavioral procedures was conducted between 8:00 and 11:00 AM.

IA Task. The apparatus consisted of a Plexiglas box (50 × 25 × 25 cm) with a floor made of parallel 1-mm-caliber bronze bars spaced 0.8 cm apart and a wood platform (5-cm high, 8-cm wide, and 25-cm long) on the left extreme of the box. For the IA training session, animals were gently placed on the platform facing the left rear corner. When stepping down and placing their four paws on the grid, animals received a 2-s 0.5-mA scrambled foot shock and then, were immediately withdrawn from the training box. After 48 h, animals were placed again on the platform as described for a retention test without the foot shock. In the retention test, the stepdown latency was 300 s. Latency to stepdown was measured with an automated stopwatch.

Drugs and Infusion Procedures. At the time of drug microinfusions, animals were gently restrained by hand, and an injection needle (30 gauge) was fitted tightly into the guide, extending 1 mm from the tip of the guide cannulas. The injection needle was connected to a 10-μL Hamilton microsyringe, and the infusions were performed at a rate of 0.5 μL/30 s. The injection needle was left in place for an additional 60 s to minimize backflow. It was then carefully withdrawn and placed on the other side, where the procedure was repeated. Infusions into the BLA, vmPFC, or CA1 were performed 10 min before the retention test. Infusions into the LV were performed 24 h either before or after IA training. The drugs used were histamine (1 μg/μL), DIM (2.3 μg/μL), PEA (1.2 μg/μL), pyrilamine (20 μg/μL), and anisomycin (80 μg/μL) purchased from Sigma-Aldrich. The a-FMHis (5 μg/μL) was synthesized at Abbott Laboratories. The doses were chosen among those found to be effective in previous papers and had no effects on locomotion or exploration activity (26, 61). The volume of the drugs infused was 0.5 μL per side in the BLA and 1 μL per side into the CA1, vmPFC, and LV. Control groups received equal volumes of sterile saline (0.9%).

Immunohistochemistry. Ninety minutes after the retention test, animals were deeply anesthetized with chloral hydrate (400 mg/kg i.p.) and perfused transcardially with saline followed by 4% (vol/vol) paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were postfixed in the same solution overnight (4 °C) and cryoprotected in 30% (wt/vol) sucrose in phosphate buffer. Forty-micrometer-thick sections were then processed for standard immunostaining (details in *SI Materials and Methods*).

Western Blotting Analysis. Animals were killed immediately after the IA retention test, the brain was dissected out on ice, and the amygdala, the vmPFC, and the CA1 were immediately isolated. The pooled structures (left and right) were individually homogenized in 200 μL ice-cold lysis buffer containing protease and phosphatase inhibitors [50 mM Tris·HCl (pH 7.5), 50 mM NaCl, 10 mM EGTA, 5 mM EDTA, 2 mM sodium pyrophosphate, 4 mM *p*-nitrophenyl phosphate, 1 mM Na_3VO_4, 1.1 mM PMSF, 20 μg/μL leupeptin, 50 μg/μL aprotinin, 0.1% SDS] and centrifuged at 13.8 × *g* at 4 °C for 15 min. The following procedure is described in detail in *SI Materials and Methods*. For each sample, a ratio of p^{Ser133}-CREB/CREB densities was calculated, and then, all of the individual rates were expressed as a percentage of the average of ratios obtained from the control group.

Data and Statistical Analysis. Statistical analysis was performed by using Prism Software (GraphPad). Data are expressed as means ± SEMs. IA latencies and number of c-Fos–positive nuclei as well as the pCREB/CREB ratio were analyzed with unpaired *t* test or one-way ANOVA. The source of the detected significances was determined by Bonferroni's multiple comparison posthoc test. *P* values less than 0.05 were considered statistically significant. The number of rats per group is indicated in the figures.

ACKNOWLEDGMENTS. We thank Dr. Alessia Costa for technical support and Dr. M. Cowart for the synthesis of a-FMHis. Work was supported by research grants from the National Council of Research of Brazil, the CNPq, the Compagnia di San Paolo (Italy), and the Ente Cassa di Risparmio di Firenze (Italy).

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Supporting Information

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SI Materials and Methods

Correct Cannula Placements. Correct cannula placement was verified by infusing a 4% (wt/vol) methylene blue solution over 30 s into the CA1 region of the dorsal hippocampus, the vmPFC (both 1 μL per side), or the BLA (0.5 μL per side) 2 d after the behavioral procedures. Rats were euthanized 30 min later with an overdose of anesthetic. Brains were removed and stored in formalin. The spread of the dye was taken as an estimate of drug infusions in the same animal. Cannula placements were considered correct when the spread was 1 mm^3 (62–64) or less from the intended infusion sites, which occurred in 98% of the rats. Only behavioral data from animals with cannulas placed correctly were analyzed.

Immunohistochemistry Procedure. Ninety minutes after IA retention test, to fully assess *c-Fos* expression, rats were deeply anesthetized with chloral hydrate (400 mg/kg i.p.) and perfused transcardially with cold physiological saline followed by 4% (vol/vol) paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). Brains were postfixed in the same solution overnight (4 °C) and cryoprotected in 30% (wt/vol) sucrose in PB; 40-μm-thick sections were cut on a cryostat and collected in PB. Sections were preincubated in 0.75% H_2O_2 in PB for 30 min and 0.2% BSA for 30 min and then incubated overnight in rabbit *c-Fos* primary antibody (1:5,000; Sigma-Aldrich) at 4 °C. The immunoreactive product was detected with the avidin-biotin peroxidase system (Vectastain Kit; Vector Laboratories). After washing, sections were mounted on gelatin-coated slides, dehydrated, coverslipped, and observed using an Olympus BX40 Microscope equipped with a Nikon DS-F1 Camera. *c-Fos*–immunopositive nuclei were counted bilaterally using the ImageJ software (NIH) on four to five sections per region per rat and normalized to a 1-mm^2 area according to the work by Provensi et al. (65). Atlas coordinates relative to Bregma (60) for the sections analyzed were from −3.8 to −4.4 mm for hippocampal CA1, from −2.12 to −2.75 mm for the BLA, and from 2.7 to 1.95 mm for the vmPFC. All regions analyzed receive histaminergic fibers. Statistics were calculated on the average values from four to five sections of individual regions for each animal.

pCREB Experiments and Western Blotting Analysis. For the experiments aimed to evaluate pCREB levels, male Wistar rats were infused with saline or a-FMHis through a cannula into the LV 24 h after the IA training session. Immediately after the IA retention test, animals were killed, rat brains were dissected out on ice, and the BLA, the CA1 region of the hippocampus, and the vmPFC were isolated immediately. Pools of structures (left and right) were individually homogenized in 200 μL ice-cold lysis buffer containing protease and phosphatase inhibitors [50 mM Tris·HCl (pH 7.5), 50 mM NaCl, 10 mM EGTA, 5 mM EDTA 2 mM sodium pyrophosphate, 4 mM *p*-nitrophenyl phosphate, 1 mM Na_3VO_4, 1.1 mM PMSF, 20 μg/μL leupeptin, 50 μg/μL aprotinin, 0.1% SDS] and centrifuged at 13.8 × *g* at 4 °C for 15 min. Supernatants were collected, and levels of total protein were quantified using Pierce BCA Protein Assay (Thermo Scientific). Aliquots of protein homogenates were diluted with a mix of lysis buffer and loading buffer two times [50 mM Tris (pH 6.8), 100 mM DTT, 10% (vol/vol) glycerol, 1% bromophenol blue, 2% (vol/vol) SDS] and boiled for 10 min. Aliquots containing 50 μg total proteins were resolved by electrophoresis on a 10% SDS/PAGE and transferred on PVDF membranes (Immobilon Transfer Membranes; Millipore). Membranes were then blocked in Tris-buffered saline (pH 7.6) containing 0.1% Tween 20 (TBS-T) and 5% (wt/vol) nonfat dry milk (Bio-Rad Laboratories) for 2 h at room temperature and incubated overnight at 4 °C with mAbs against pCREB (Ser133; Cell Signaling Technology) or CREB (Cell Signaling Technology) diluted 1:1,000 in TBS-T containing 5% (wt/vol) BSA or 5% (wt/vol) nonfat dry milk, respectively. Immunodetection was performed with secondary antibodies (anti-rabbit IgG conjugated to HRP; Cell Signaling Technology) diluted 1:5,000 in TBS-T containing 1% nonfat dry milk. Blots were washed in TBS-T, and then, reactive bands were detected by using ECL (Luminata Crescendo; Millipore). Quantitative densitometry was assessed using QuantityOne software (Bio-Rad Laboratories). A ratio of pSer133-CREB/CREB densities was calculated of all samples, and all of the individual rates were expressed as percentages of the average of ratios obtained from the control group.