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Effect of histamine H1 and H2 receptor antagonists, microinjected into cerebellar vermis, on emotional memory consolidation in mice

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Abstract

This study investigated the effects of histamine H1 or H2 receptor antagonists on emotional memory consolidation in mice submitted to the elevated plus maze (EPM). The cerebellar vermis of male mice (Swiss albino) was implanted using a cannula guide. Three days after recovery, behavioral tests were performed in the EPM on 2 consecutive days (T1 and T2). Immediately after exposure to the EPM (T1), animals received a microinjection of saline (SAL) or the H1 antagonist chlorpheniramine (CPA; 0.016, 0.052, or 0.16 nmol/0.1 μL) in Experiment 1, and SAL or the H2 antagonist ranitidine (RA; 0.57, 2.85, or 5.7 nmol/0.1 μL) in Experiment 2. Twenty-four hours later, mice were reexposed to the EPM (T2) under the same experimental conditions but they did not receive any injection. Data were analyzed using one-way ANOVA and the Duncan test. In Experiment 1, mice microinjected with SAL and with CPA entered the open arms less often (%OAE) and spent less time in the open arms (%OAT) in T2, and there was no difference among groups. The results of Experiment 2 demonstrated that the values of %OAE and %OAT in T2 were lower compared to T1 for the groups that were microinjected with SAL and 2.85 nmol/0.1 μL RA. However, when animals were microinjected with 5.7 nmol/0.1 μL RA, they did not show a reduction in %OAE and %OAT. These results demonstrate that CPA did not affect behavior at the doses used in this study, while 5.7 nmol/0.1 μL RA induced impairment of memory consolidation in the EPM.

Key words: Chlorpheniramine; Ranitidine; Cerebellar vermis; Emotional memory consolidation

Introduction

The central histaminergic nervous system originates from the tuberomammillary nucleus (TMN) of the hypothalamus, and, in many species, it widely innervates almost the whole brain including the cerebellum and other subcortical motor structures (1). The four histamine receptors identified as H1, H2, H3, and H4 subtypes are expressed in the brain (1,2). H1 and H2 receptors potentiate excitatory inputs while H3 receptors down regulate histamine synthesis and release as well as the release of other neurotransmitters (3). Because the H4 receptor has been recently discovered in the mammalian central nervous system, its role in the brain remains unclear.

The neural histaminergic system is involved in several behavioral and neurobiological functions, such as arousal, food intake, motor activity, nociception, learning, and memory (1,4). However, the part that histaminergic circuits play in mnemonic effects is complex. Histamine seems to have different effects in distinct brain regions and may have modulatory effects that differ according to memory type. The exact role of this neurotransmitter in learning processes and memory consolidation and the action of the receptor subtypes and how they affect key circuits related to a specific memory system are not well understood (5).

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Previous studies have shown that the histamine-containing fibers project from the tuberomammillary nucleus to the cerebellar cortex and the deep cerebellar nucleus, with a high density of histaminergic terminations in the vermis and flocculus (6,7). A moderately dense network of histamine fibers has been seen in the molecular and granular layers of the cerebellum in several species including humans (1). These fibers run parallel to the Purkinje cell layer after traversing it perpendicularly.

Autoradiographic mapping and *in situ* hybridization experiments demonstrated the presence of H1 and H2 receptors in the rat cerebellar cortex and deep in the cerebellar nuclei (8). These studies suggest that histamine may play an important role in modulating the excitability of cerebellar neurons. The Purkinje cells of the cerebellar cortex and the neurons in the nucleus interpositus all exhibit H2-receptor-mediated excitatory responses when exposed to a histamine bath perfusion (9). Granule cells are excited through the activation of H1 and H2 receptors (10,11).

The cerebellum has traditionally been considered an important motor structure, but several lines of evidence support the role of the cerebellum as more complex than previously thought and include more than just the regulation of motor responses (12). An increasing number of studies have demonstrated its involvement in cognitive and emotional functions. Functional neuroimaging studies and studies of patients with cerebellar lesions have been conducted to elucidate the role of the cerebellum in the processing of emotion (13-15). Moreover, Ruediger et al. (16) demonstrated that fear conditioning learning is specifically correlated with the growth of feedforward inhibition connectivity in hippocampal and cerebellar circuits.

Experimental evidence indicates that the cerebellum plays a role in emotional learning. The capacity to learn and retain fear-conditioned responses was investigated in *hotfoot* mutant mice. These animals are characterized by a primary deficiency in the synapses made by parallel fibers onto the Purkinje cells. In these mutant mice, the cerebellar dysfunction impairs learning, which suggests that these synapses are involved in fear memory consolidation (17). Studies have related the cerebellar vermis to emotional memory consolidation, since vermis inactivation caused amnesic effects after a fear conditioning task (18). Thus, the participation of the vermis in emotional memory is independent of its role in sensory or motor processes, and the vermis may represent an interface between sensory stimuli, emotional state, and motor responses (12,18).

Histaminergic modulation of learning and memory was studied using lesions and pharmacological interventions in the tuberomammilary nucleus and other decisive brain regions. In our first study (19), microinjection of histamine into the cerebellar vermis demonstrated that the cerebellar histaminergic system is involved in the process of consolidation of emotional memory. These results indicated that there was a dose-dependent inhibition of memory consolidation when histamine was injected into the cerebellar vermis in mice reexposed to the elevated plus maze (EPM). Therefore, in the present study, we investigated the effects of H1 and/or H2 receptor antagonists on emotional memory consolidation.

Material and Methods

Animals

Male Swiss mice (Universidade Federal de São Carlos, Brazil) weighing 25-35 g at the beginning of the experiments were housed in polypropylene cages ($31 \times 20 \times 13$ cm) in groups of five and were maintained under a 12:12-h light-dark cycle (lights on at 7:00 am) in a controlled environment at a temperature of $23 \pm 1^{\circ}C$ and a humidity level of $50 \pm 5\%$. Food and drinking water were provided *ad libitum*, except during the brief test periods. All mice were experimentally naive, and the experimental sessions were conducted during the light period of the cycle (9:00 am to 1:00 pm).

Drugs

The H1 receptor antagonist chlorpheniramine (CPA) maleate salt and the H2 receptor antagonist ranitidine hydrochloride (RA; Sigma Chemical Co., USA) were prepared using saline as vehicle. Saline (SAL) was used as an experimental control. The doses were based on previous research (20) and on pilot work in our laboratory. The substances were coded, and the experimenter was blinded to the codes when the tests and behavioral analysis were performed.

EPM apparatus

The EPM used was similar to the one originally described by Lister (21). The EPM consisted of two open arms ($30 \times 5 \times 0.25$ cm) and two enclosed arms ($30 \times 5 \times 15$ cm) that were connected to a common central platform (5×5 cm). The apparatus was made of crystal acrylic and was raised 38.5 cm above floor level.

Stereotaxic surgery and drug infusion

Mice were intraperitoneally anesthetized using 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine solution in association with local anesthesia (3% lidocaine with norepinephrine 1:50,000) and were placed in a Stoelting stereotaxic instrument. A single, 7-mm stainless steel guide cannula (25 gauge) was implanted in the cerebellar vermis according to the following coordinates from the mouse brain atlas (22): 6.5 mm posterior to the bregma, 0 mm lateral to the midline, and 2.0 mm ventral to the skull surface. The guide cannula was fixed to the skull using dental acrylic and jeweler's screws. A dummy cannula (33-gauge stainless steel wire) was inserted into the guide cannula at the time of surgery to reduce the

incidence of occlusion. Postoperative analgesia was provided for 3 days by adding acetaminophen (200 mg/mL) to the drinking water in a ratio of 0.2 mL acetaminophen to 250 mL water (i.e., the final concentration was 0.16 mg/mL).

Saline and drug solutions were infused into the cerebellar vermis using a microinjection unit (33-gauge cannula; Insight Equipamentos Científicos Ltda., Brazil), which extended 2.0 mm beyond the tip of the guide cannula. The microinjection unit was attached to a 5-μL Hamilton microsyringe via polyethylene tubing (PE-10), and the administration was controlled by an infusion pump (Insight Equipamentos Científicos Ltda.) that was programmed to deliver a volume of 0.1 μL over a period of 60 s. The microinjection procedure consisted of gently restraining the animal, inserting the injection unit, infusing the solution, and keeping the injection needle *in situ* for a further 60 s to avoid reflux. Confirmation of successful infusion was obtained by monitoring the movement of a small air bubble inside the PE-10 tubing.

General conditions and data collection

Three days after surgery, the animals were transported to the behavioral space and left undisturbed for at least 1 h before testing, to facilitate adaptation. The test was performed on 2 consecutive days, and the trials in the EPM were denoted Trial 1 and Trial 2. Mice were individually placed on the central platform of the maze facing the open arm and were able to explore the maze for 5 min.

In Trial 1, immediately after exposure to the EPM, the animals received a microinjection of the drugs as follows: in Experiment 1, SAL or 0.016, 0.052, or 0.16 nmol/0.1 μL CPA, and in Experiment 2, SAL or 0.57, 2.85, or 5.7 nmol/0.1 μL RA. Twenty-four hours later (Trial 2), the mice were reexposed to the EPM under the same experimental conditions as in Trial 1, with the exception that they did not receive an injection. Between subjects, the maze was thoroughly cleaned with 5% ethanol and a dry cloth. All tests were conducted under moderate illumination (140 lux) as measured on the central platform of the EPM and in an environment isolated from the rest of the room by a black protective curtain.

All sessions were video recorded with a digital camera that was linked to a computer in an adjacent room. Images were analyzed by a highly trained observer using X-PLO-RAT, which is an ethological analysis software package developed at the Laboratory of Exploratory Behavior, USP, Ribeirão Preto (23). Behavioral parameters were defined in a way that was consistent with previous studies (21,24) and included the following observations: the frequency of open- and enclosed-arm entries (OAE and EAE; where an entry was defined as the entry of all four of an animal's paws into an arm) and the total amount of time spent in the open arms (OAT), enclosed arms (EAT), and central area (CT). These data were used to calculate the percentage of OAE [%OAE = (open entries/open + enclosed entries) × 100], the percentage of OAT [%OAT = (open time/300) × 100], and the percentage of EAT [%EAT = (enclosed time/300) × 100]. The number of stretched-attend postures (SAP; an exploratory posture in which the body stretches forward and then retracts to its original position without any forward locomotion), immobility time (stillness but some movement of the chest), and the frequency of head dipping (HD; exploratory movement of head or shoulders over the sides of the maze) were also scored. Total SAP was considered a primary index of risk assessment, and HD was considered an index of exploratory behavior.

Histology

At the end of testing, all animals received a 0.1-μL infusion of 1% methylene blue according to the microinjection procedure described earlier. The animals received an anesthetic overdose, their brains were removed, and the injection sites were verified histologically according to the atlas of Franklin and Paxinos (22). Data from animals with injection sites outside the cerebellar vermis were excluded from the study. The final sample size of each cohort ranged between 9 and 12. Histology confirmed that a total of 78 mice had accurate cannula placement in the cerebellar dorsal vermis (Figure 1).

Statistical analysis

All results were initially analyzed using the Levene test for homogeneity of variance. Data were analyzed using one-way ANOVA. When differences were indicated by significant *F* values, they were further analyzed using the Duncan multiple range test. A P value of less than 0.05 was considered to be significant.

Ethics

The experiments performed as part of this study were approved by the Animal Ethics Commission of the Universidade Federal de São Carlos (CEEA #049/09) and were in compliance with the norms of the Brazilian Neuroscience and Behavior Society, which are based on the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results

Experiment 1: effects of the H1 receptor antagonist CPA on memory consolidation

One-way ANOVA showed no significant difference between the groups in Trial 1 for all the measures analyzed (Table 1). Therefore, the data were pooled because the animals had received no pharmacological treatment at that point. Data are summarized in Figure 2A and B, and Table 2. ANOVA showed differences in %OAE between sessions ($F_{4,65} = 6.12$, $P = 0.0003$). The *post hoc* Duncan test indicated that all groups entered the open arms less often in Trial 2 in comparison with Trial 1.

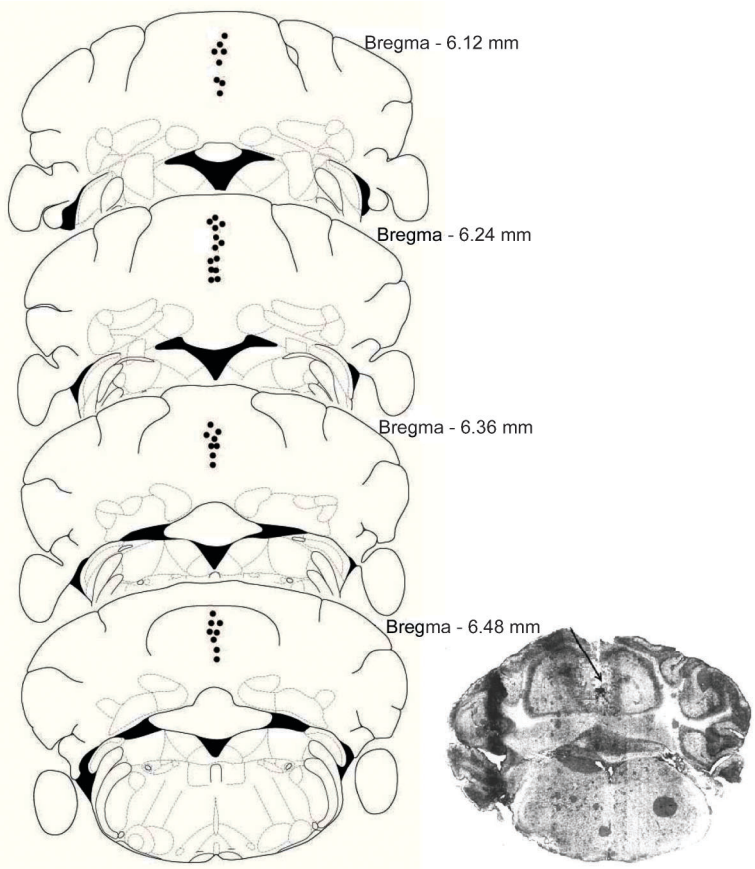

Figure 1. *A*, Schematic representation (adapted from Ref. 22) of sites of microinfusion (filled circles) into the cerebellum of mice. *B*, Photomicrograph showing a typical injection site (indicated by an arrow) in the cerebellar vermis.

Figure 2B shows %OAT for the first and second sessions. ANOVA detected differences in %OAT between sessions ($F_{4,65} = 6.94$, $P = 0.0001$), and *post hoc* analysis determined that animals explored the open arms for a shorter time in the second trial when they had been microinjected with SAL or CPA (0.016, 0.052, and

Table 1. One-way ANOVA statistical results for the behavior of mice with no pharmacological treatment in Trial 1.

Behavioral measures	Experiment 1		Experiment 2	
	F	P	*F*	P
OAE	0.93	0.45	1.39	0.26
%OAE	1.55	0.20	0.58	0.63
OAT	0.94	0.44	1.22	0.31
%OAT	1.25	0.30	1.22	0.31
EAE	0.81	0.52	1.20	0.32
EAT	0.33	0.85	0.73	0.54
%EAT	0.33	0.85	0.73	0.54
CT	0.57	0.68	1.03	0.38
SAP	1.19	0.32	0.87	0.46
Head dipping	1.37	0.25	0.58	0.63
Immobility time	0.64	0.60	2.06	0.12

OAE: number of open-arm entries; %OAE: percentage of OAE; OAT: time spent in the open arms; %OAT: percentage of OAT; EAE: number of enclosed-arm entries; EAT: time spent in the enclosed arms; %EAT: percentage of EAT; CT: central platform time; SAP: frequency of stretched-attend postures.

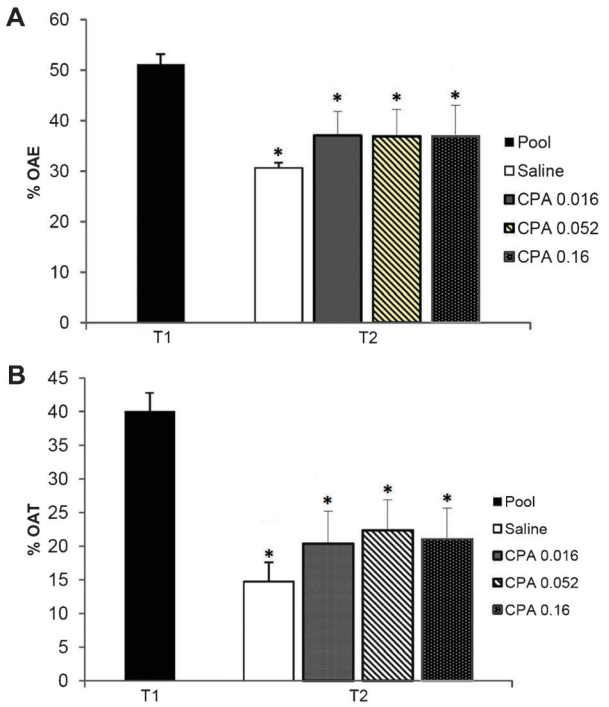

Figure 2. Effects of H1 antagonist chlorpheniramine (CPA; 0.016, 0.052, or 0.16 nmol/0.1 μL) microinjected into the cerebellar vermis on *A*, the percentage of open-arm entries (%OAE) and *B*, the percentage of time spent in the open arms (%OAT) in Trials 1 and 2 in the elevated plus maze. Pool: animals exposed to the elevated plus maze with no pharmacological treatment. Data are reported as means ± SE (n = 9). *P<0.05 Trial 2 *vs* Trial 1 (Duncan test).

0.16 nmol/0.1 μL). These results demonstrate that CPA did not alter behavioral parameters at the doses used.

Table 2 shows the results for all other behaviors. ANOVA did not indicate differences in EAE ($F_{4,65} = 1.08$, $P = 0.37$), which is an EPM index of general exploratory activity. ANOVA revealed significant differences between trials in OAT ($F_{4,65} = 6.94$, $P = 0.0001$) and OAE ($F_{4,65} = 3.96$, $P = 0.006$). Furthermore, there were differences between sessions for EAT ($F_{4,65} = 10.20$, $P<0.0001$), %EAT ($F_{4,65} = 10.20$, $P<0.0001$), and frequency of HD ($F_{4,65} = 6.33$, $P = 0.0002$). ANOVA did not detect any significant differences in immobility time ($F_{4,65} = 0.45$, $P = 0.77$), CT ($F_{4,65} = 0.33$, $P = 0.85$), and total SAP ($F_{4,65} = 1.13$, $P = 0.34$) between trials.

Experiment 2: effects of the H2 receptor antagonist RA on memory consolidation

One-way ANOVA did not determine that there were significant differences between the groups in Trial 1 for any of the measures analyzed (Table 1). ANOVA indicated that there were differences in %OAE ($F_{4,87} = 3.76$, $P = 0.007$) between trials. The *post hoc* analysis indicated that differences in %OAE existed in groups that were microinjected with SAL ($P = 0.03$), and 0.57 and 2.85 nmol/0.1 μL RA ($P<0.05$). The animals that received treatment with 5.7 nmol/0.1 μL RA did not have a reduced %OAE (Figure 3A).

Figure 3B shows %OAT for the first and second sessions. ANOVA determined that there were differences in %OAT between sessions ($F_{4,87} = 3.38$, $P = 0.013$). The Duncan test indicated that the animals that were microinjected with SAL ($P = 0.03$) and 2.85 nmol/0.1 μL RA ($P = 0.009$) exhibited a decreased %OAT in Trial 2 relative to Trial 1, while the groups microinjected with 0.57 and

Table 2. Effects of H1 antagonist chlorpheniramine (CPA) microinjected into the cerebellar vermis on the behavior of mice in Trials 1 and 2 in the elevated plus maze (EPM).

	Trial 1	Trial 2			
	Pool	SAL	CPA 0.016	CPA 0.052	CPA 0.16
OAE	8.9 ± 0.6	4.0 ± 0.6*	6.6 ± 1.4	6.3 ± 1.2	6.3 ± 1.4
OAT	112.6 ± 8.3	44.2 ± 8.7*	61.1 ± 14.5*	67.2 ± 13.9*	63.3 ± 13.6*
EAE	8.3 ± 0.5	9.7 ± 1.3	10.1 ± 1.3	9.9 ± 0.8	10.2 ± 1.2
EAT	94.4 ± 5.7	173.4 ± 12.0*	150.5 ± 17.5*	152.9 ± 16.6*	150.0 ± 19.5*
%EAT	31.5 ± 1.9	57.8 ± 4.0*	50.2 ± 5.8*	51.0 ± 5.5*	50.0 ± 6.5*
CT	93.0 ± 6.4	82.4 ± 10.5	88.4 ± 12.0	80.0 ± 7.3	86.7 ± 11.7
SAP	9.1 ± 0.8	8.2 ± 1.3	4.9 ± 1.1	6.8 ± 1.3	8.6 ± 1.5
Head dipping	10.0 ± 1.3	2.2 ± 1.0*	2.4 ± 0.8*	2.1 ± 1.6*	4.4 ± 1.3*
Immobility time	0.3 ± 0.2	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0

Immediately after exposure to the EPM, animals received a microinjection of CPA (0.016, 0.052, or 0.16 nmol/0.1 μL). Pool: animals exposed to EPM with no pharmacological treatment; SAL: saline; OAE: number of open-arm entries; OAT: time spent in the open arms; EAE: number of enclosed-arm entries; EAT: time spent in the enclosed arms; %EAT: percentage of time spent in EAT; CT: central platform time; SAP: frequency of stretched-attend postures. Data are reported as means ± SE in seconds. *P<0.05 Trial 2 *vs* Trial 1 (Duncan test).

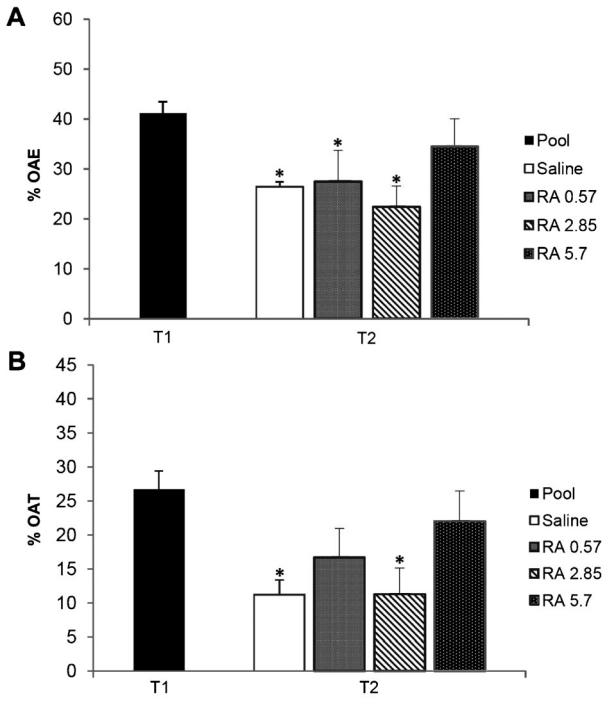

Figure 3. Effects of H2 antagonist ranitidine (RA; 0.57, 2.85, or 5.7 nmol/0.1 μL) microinjected into the cerebellar vermis on *A*, the percentage of open-arm entries (%OAE) and *B*, the percentage of time spent in the open arms (%OAT) in Trials 1 and 2 in the elevated plus maze. Pool: animals exposed to the elevated plus maze with no pharmacological treatment. Data are reported as means ± SE (n = 9-12). *P < 0.05 Trial 2 *vs* Trial 1 (Duncan test).

5.7 nmol/0.1 μL RA did not reduce %OAT. These results demonstrated that the H2 antagonist RA, at the dose of 5.7 nmol/0.1 μL, impairs memory consolidation in mice reexposed to the EPM.

Table 3 shows the results for all other behaviors. ANOVA revealed significant differences in the OAT between trials ($F_{4,87} = 3.38$, $P = 0.01$). *Post hoc* comparisons indicated that differences existed for the groups microinjected with SAL ($P = 0.03$) and 2.85 nmol/0.1 μL RA ($P = 0.03$). Additionally, differences between sessions for OAE ($F_{4,87} = 2.99$, $P = 0.02$), EAT ($F_{4,87} = 7.59$, $P < 0.001$), %EAT ($F_{4,87} = 7.59$, $P < 0.001$), CT ($F_{4,87} = 4.60$, $P = 0.002$), and EAE ($F_{4,87} = 3.22$, $P = 0.01$) and the frequency of HD ($F_{4,87} = 5.14$, $P = 0.0009$) were detected. ANOVA did not detect any significant differences between trials in immobility time ($F_{4,87} = 1.30$, $P = 0.28$) or total SAP ($F_{4,87} = 1.41$, $P = 0.23$).

Discussion

The primary findings of the present study are that infusion into the cerebellar vermis with the H1 antagonist CPA (0.016, 0.052, and 0.16 nmol/0.1 μL) did not show any behavioral effects, whereas microinjection with the H2 antagonist 5.7 nmol/0.1 μL RA impaired emotional memory consolidation in mice reexposed to the EPM.

In the EPM, the behavior expressed during the test are due to a conflict between motivation to explore the maze and the natural tendency to avoid open spaces (24,25). According to File (26), after the initial exploration of the apparatus, rodents acquire, consolidate, and retrieve some memory related to exploration of potentially dangerous areas of the maze. Several studies show that EPM-experienced animals exhibit a significant decrease

Table 3. Effects of H2 antagonist ranitidine (RA) microinjected into the cerebellar vermis on the behavior of mice in Trials 1 and 2 in the elevated plus maze (EPM).

	Trial 1	Trial 2			
	Pool	SAL	RA 0.57	RA 2.85	RA 5.7
OAE	6.7 ± 0.3	4.5 ± 0.5	4.1 ± 0.6	2.6 ± 0.4*	6.6 ± 0.6
OAT	78.4 ± 1.1	33.7 ± 1.4*	50.1 ± 2.2	33.9 ± 1.9*	66.0 ± 2.0
EAE	9.0 ± 0.3	11.5 ± 0.5*	7.7 ± 0.6	8.4 ± 0.5	10.9 ± 0.5
EAT	118.6 ± 1.0	161.1 ± 1.7*	192.4 ± 2.8*	193.0 ± 2.0*	149.5 ± 2.3
%EAT	39.5 ± 0.6	53.7 ± 1.0*	64.1 ± 1.6*	64.3 ± 1.1*	49.8 ± 1.3
CT	103.1 ± 0.8	105.2 ± 1.6	57.5 ± 1.9*#	73.2 ± 1.7*#	84.5 ± 1.6
SAP	7.9 ± 0.3	5.4 ± 0.5	6.3 ± 0.6	6.0 ± 0.5	9.0 ± 0.7
Head dipping	7.3 ± 0.4	1.6 ± 0.4*	2.1 ± 0.5*	2.0 ± 0.5*	2.6 ± 0.6*
Immobility time	0.1 ± 0.1	0.1 ± 0.2	0.4 ± 0.3	0.0 ± 0.0	0.7 ± 0.4

Immediately after exposure to the EPM, animals received a microinjection of RA (0.57, 2.85 or 5.7 nmol/0.1 μL). Pool: animals exposed to EPM with no pharmacological treatment; SAL: saline; OAE: number of open-arm entries; OAT: time spent in the open arms; EAE: number of enclosed arm entries; EAT: time spent in the enclosed arms; %EAT: percentage of EAT; CT: central platform time; SAP: frequency of stretched-attend postures. Data are reported as means ± SE in seconds. * P < 0.05 Trial 2 *vs* Trial 1; # P < 0.05 *vs* control (SAL; Duncan test).

in %OAE and %OAT during retesting (19,20,25). Therefore, the test/retest protocol in the EPM has demonstrated usefulness to investigate both short- and long-term memory components (27).

Histaminergic projections to the cerebellar vermis, amygdala, and hippocampus can be involved in the modulation of emotional memory consolidation. It has been previously proposed that the amygdala and cerebellum are functionally interconnected during aversive learning (12,15). According to Sacchetti et al. (28), the vermis and amygdala may interact, and the vermal electrical stimulation modulates amygdala activity. These effects are mediated by both direct and indirect anatomical connections between the cerebellum and the limbic areas.

Evidence demonstrates the existence of the histaminergic receptors H1, H2, and H3 in the cerebellum of rodents (8). Generally, H1 and H2 receptors excite or potentiate excitatory impulses, while H3 activation mediates autoinhibition of TMN neurons (6,8).

Evidence regarding the role of the histaminergic system in the learning and memory process is controversial, and the function of the histaminergic receptors and how they affect the memory system is still unclear (5). H1 receptors mediate actions on brain activity, and classic antihistamines such as CPA act as H1 antagonists (1). Several studies have demonstrated the anxiolytic effects of CPA in behavioral tests (1,29). Furthermore, CPA involvement has been proposed in spatial learning and emotional memory processes; however, results in this area are indefinite. For instance, the pharmacological blockade of the H1 receptor with CPA improved spatial learning in the Morris water maze (30), but it conversely impaired spatial learning in the eight-arm radial maze (31). In a recent study, Serafim et al. (20) showed that microinjection of CPA in the amygdala impaired emotional memory performances at a dose of 0.16 nmol/0.1 μL, and suggested that the H1 receptors in the amygdala are not implicated in anxiety-like behaviors but are involved in emotional states induced by the T1/T2 EPM protocol in mice. However, in the present study, CPA microinjected into the cerebellar vermis did not affect behavioral measures in mice reexposed to the EPM.

Some studies have examined the role of H2 receptors on cognitive performance. In the study by Benetti et al. (32), the H2 receptor agonist ampthamine improved fear memory expression, and the authors suggested that activation of postsynaptic H2 receptors within the nucleus basalis magnocellularis by endogenous histamine is involved in the expression of fear responses. Another H2 receptor agonist, dimaprit, improved extinction of aversive memory, while the H2 receptor antagonist, RA, impaired this memory (33,34). Da Silva et al. (35) showed that knockout mice lacking the H1 and H2 receptor subtypes enhanced learning and memory in auditory and contextual fear conditioning tests, but impaired learning of the Barnes maze and short-term memory of an object recognition test. In addition, electrophysiological examination indicated that both knockout mice H1 and H2 showed impaired long-term potentiation in CA1 areas of the hippocampus (35). Recently, Da Silveira et al. (36) showed that the H1 receptor antagonist, pyrilamine, the H2 receptor antagonist, RA, and the H3 receptor agonist, imetit, injected in the CA1 region immediately, 30, 120, or 360 min posttraining, blocked long-term memory retention in a time-dependent manner (30-120 min) without affecting general exploratory behavior, anxiety state, or hippocampal function.

In the present study, animals that received 5.7 nmol/0.1 μL RA did not reduce open-arm exploration during retesting on the apparatus, which demonstrates that RA microinjected into the cerebellar vermis impairs emotional memory consolidation in mice reexposed to the EPM. Using a similar protocol, we recently demonstrated that animals microinjected with histamine into the cerebellar vermis did not avoid the open arms less on retesting, and pretreatment with CPA abolished the inhibitory effect of histamine on memory consolidation, while pretreatment with RA did not show any memory effect, which indicated that the H1 receptor is probably involved in histamine-induced emotional memory impairment (37). The present results did not reveal memory effects for the two lower doses of RA and further indicate that H1 mediation seems to be the main mechanism involved in EPM memory impairment. Therefore, the results of the higher dose of RA may be due to a massive blockade of H2 receptors by RA, which induced endogenous histamine to bind only to the H1 receptors reproducing the same kind of effect found with microinfusion of exogenous histamine.

Because emotional memory has an important role in controlling behavior and it is critical for survival, a memory deficit can expose the animal to a needlessly dangerous situation while persistence of an aversive memory is a considerable factor associated with the development of anxiety and fear disorders, including phobias and post-traumatic stress disorder. Therefore, in view of our results, these histamine-modulated effects leading to memory erasure are potentially important and may give clues to new studies regarding the histaminergic system and trauma-related disorders (37).

In conclusion, the results of the present study demonstrated that H2 receptor antagonist ranitidine in the cerebellar vermis impaired emotional memory consolidation in mice reexposed to the EPM.

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