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Research report

The activation of histamine-sensitive sites of the ventral hippocampus modulates the consolidation of a learned active avoidance response in rats

Edgardo O. Alvarez *, Arturo M. Banzan

Área de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, and IMBECU, CONICET, Mendoza, Argentina

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Abstract

Previous evidence from our laboratory has shown that histamine receptors located into the ventral hippocampus modulate learning and memory processes. Stimulation of histamine hippocampal sensitive receptors during the acquisition phase of a conditioned avoidance response to an ultrasonic tone was able to increase latency to escape and impair memory in the rat. Histamine application into the same hippocampal region also impaired the evocation of the response. The purpose of the present work was to evaluate if histaminergic neuron circuits have participation on the consolidation processes of the conditioned avoiding response. Male adult rats were implanted into the ventral hippocampus with microinjection cannulae and subjected consecutively to 2 sessions of 8 trials to learn an avoidance response after an ultrasonic tone of 40 kHz was on, as it was previously described. Immediately after the training period was over, or 15 min after, different groups of rats were microinjected with saline, histamine or a combination of histamine H_1- or H_2-receptor antagonists. Twenty four hours later, animals were tested in a new session for the retention of the avoiding response. Results showed that histamine treatment interfered with the consolidation of the avoiding response, affecting latency and the memory efficiency. This interference was mediated by histamine H_1- and H_2-receptors, since pretreatment with pyrilamine or ranitidine blocked the inhibitory effect of histamine. Results support the concept that histaminergic neurotransmission modulates learning and memory by affecting selectively the three stages of learning.

Keywords: Learning; Avoidance response; Hippocampus; Memory consolidation; Histamine receptors

1. Introduction

Learning is the property giving living systems the capacity to adapt to a changing environment by behaviorally responding with appropriate actions based on previous experience. This capacity represented an important evolutionary advantage to living systems because it gave the brain the mechanisms to compare previous stimuli with new ones on a time scale. In this way, the organisms acquired the means to visualize changes of the environment in terms of time. Many complex events are necessary in the brain in order to keep a memory. Environmental stimuli, which in essence represent information, have to be processed and stored as coded information into specific neuron circuits. Once coded information is stored, it is necessary to retrieve it in order to compare it with incoming information to discern if it is familiar or not. It is thought that after some unknown process, comparison of stored information with the new one, gives a resultant action originating some specific behavioral output [10,37,5]. Evidence has shown that learning is a complex process that apparently occurs into three stages: acquisition, consolidation and retrieval [37,34,17,9]. In spite that the three stages of learning appear as a continuum, there is evidence that they are sequential and discrete [18,40,15]. Thus, it is quite possible to think that intrinsic brain mechanisms might control the efficacy of any of these processes. For example, it is known that stress, drastic emotional contingencies or the emotionally content of the task to be learned facilitate the process of acquisition [42,8,14]. Administration of drugs or bioactive compounds into some brain

* Corresponding author at: Área de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza 5500, Argentina.
Tel.: +54 261 4494116.
E-mail addresses: ealvarez@fcm.uncu.edu.ar, edgardo1144@yahoo.com (E.O. Alvarez).

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structures thought to be involved in memory processes, are able to disrupt consolidation of an auditory Pavlovian fear conditioning response, or the evocation of a conditioned avoiding response to an ultrasonic tone in rats [35,2]. Procedures such as exposure of rats to novelty before a memory retention test, facilitates the retrieval of a one-trial inhibitory avoidance response [23]. This evidence highly suggests that the three processes involved in learning have separate regulations.

Previous evidence from our laboratory has shown that the histaminergic neurons of the hippocampus are involved in acquisition and retrieval of a conditioned active avoidance response induced by an ultrasonic tone [1–4]. These data gave support to the idea that histamine is at least one of many neurotransmitters that regulates the process of learning. In the present report it was studied if histamine into the ventral hippocampal neurons is able to modify the consolidation phase of the learning of an active avoidance response to an ultrasonic tone in rats.

2. Materials and methods

2.1. Animals

Male rats of the Holzman-derived colony, weighting 250–300 g, 90 days old and maintained in thermoregulated (22–24 °C) and light controlled conditions (06.00–20.00 h) were used. Standard rat chow and water were available *ad libitum*.

2.2. Implantation procedures

Animals were anesthetized with ether and unilaterally implanted with guide steel cannulae (23 gauge, 15 mm length into the caudal ventral hippocampus, as it was described elsewhere) [1–3]. After implantation rats were caged individually and remained at rest for at least 72 h.

2.3. Drugs

Histamine dihydrochloride (HA, Sigma Chemical Co., U.S.A.), as target drug; pyrilamine maleate (PYR, Sigma Chemical Co, U.S.A.) and ranitidine (RAN, R.B.I., U.S.A.), as specific antagonists of histamine receptors, freshly prepared in saline solution were used.

2.4. Experimental schedule

The conditioned active avoiding response to an ultrasonic 40 kHz tone was used as experimental model of learning and memory. Animals were trained in a two-compartment wooden cage painted black with a wall separating both compartments. Animals may pass from compartment 2 (the punishment box) to compartment 1 (the safe box) through a swinging door that can be locked in its place. Rats were conditioned to escape through by opening the door after the ultrasonic sine-wave tone was on, as it was described in detail elsewhere [1]. Training sessions included 8 trials with a maximum duration of 4.5 min each. Following suggestions of the Institution Board for experimental animal care, rats were minimally exposed to the current shock, and if animals did not pass after a second current pulse (25 μA maximum), trial was terminated, and rats returned to the resting compartment. Experiments were performed in two stages:

Stage 1 *Learning the avoiding response*: implanted animals were put in groups of five into compartment 2 with the communicating door unlocked so rats could pass freely from one side to the other one. No electric shock neither ultrasonic tone was given at this time. The purpose of this stage was familiarization of the testing environment in order to diminish the novelty at later time. The period of adaptation to the cage lasted about 15 min. Twenty four hours later; rats were individually subjected to the learning schedule (training session 1). At the following day they were subjected to the 8 trials schedule again (training session 2). At the end of this session, rats usually got a conditioned avoiding response (CAR) of at least 70%. Animals with poor performance (less than 70% CAR) were discarded and not included in the experiments. At the end of the eighth training trial of session 2 rats were microinjected into the ventral hippocampus with saline (Sal), HA or RAN in a total volume of 1 μl of saline solution, as previously described [1–3]. Microinjection pulse lasted about 15–20 s.

A diagram describing the experimental schedule is shown in Fig. 1.

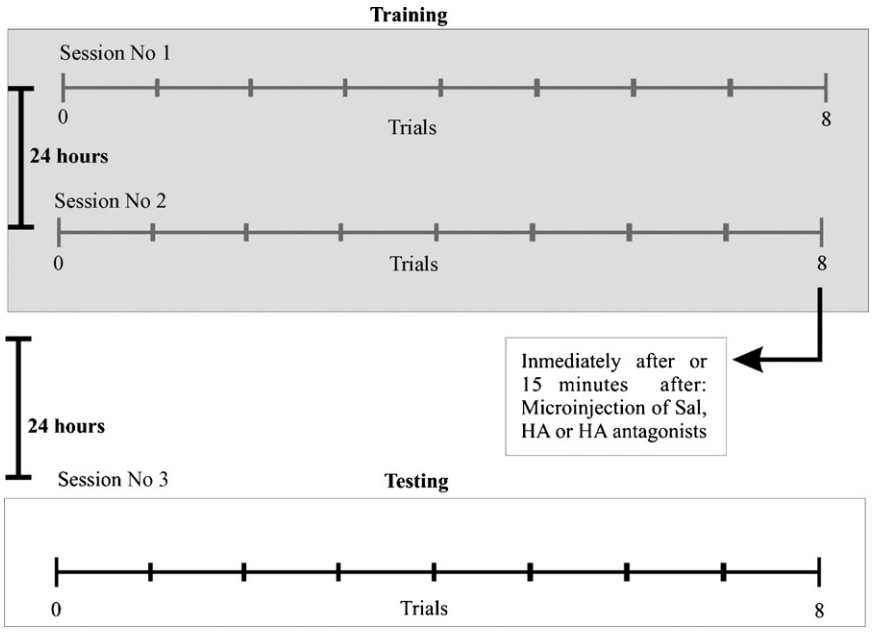

Fig. 1. Schematic diagram of the experimental setup of the experiments performed in the present study. A total of 3 sessions were done in all experiments, two for training and one for testing.

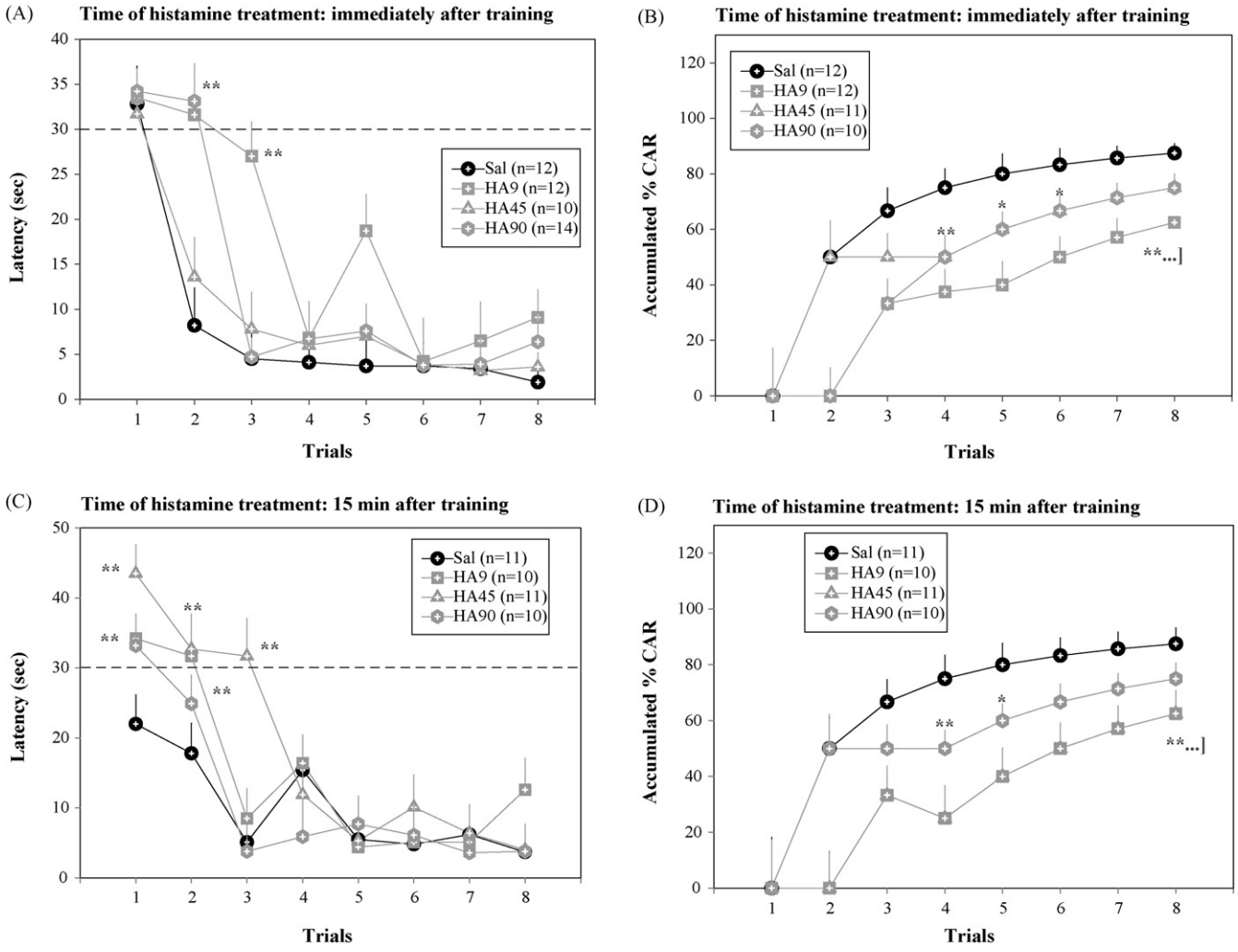

Fig. 2. Consolidation of an avoiding response to an ultrasonic tone in rats bearing hippocampus microinjection cannulae implants and treated with various doses of histamine. (A) Latency curves. Histamine or saline treatments immediately after session 2 were over. (B) % of correct avoiding responses curves of animals shown in (A). $^{**}p < 0.01$ vs. the respective control point. $^{*}p < 0.05$ vs. the respective control point. **. . .], all trials with exception of trial 1 significantly different from control curve, $p < 0.01$. (C) Latency curves. Histamine or saline treatments 15 min after session 2 was ended. (D) % of correct avoiding responses curves of animals shown in (C). $^{**}p < 0.01$ vs. the respective control point. $^{*}p < 0.05$ vs. the respective control point. **. . .], all trials with exception of trial 1 significantly different from control curve, $p < 0.01$.

Stage 2 *Testing retrieval of the conditioned response*: twenty-four hours after finalizing the 2 days training period, animals were tested once more in a new session of 8 trials. Neither experimental procedure, nor injections to the ventral hippocampus were performed at this stage. Animals were simply tested for the avoiding response. After the testing was concluded, rats were sacrificed by ether excess and their brains dissected out for histological verification of sites of implants as described earlier [1–3].

2.4.1. Experiments

The following experiments were performed:

1. Effect of histamine into the ventral hippocampus on the consolidation of the avoiding response to an ultrasonic tone immediately after training. In this experiment the possible effect of histamine administration on the consolidation of the avoiding response to an ultrasonic tone was investigated. A total of 48 implanted rats were used. The following groups were formed: (1) control animals (Sal, $n = 12$), receiving only 1 μl of saline solutions in the microinjection schedule; (2) histamine-treated animals, dose 9 nmol/μl (HA9, $n = 12$); (3) histamine-treated animals, dose 45 nmol/μl (HA45, $n = 10$); histamine-treated animals, dose 90 nmol/μl (HA90, $n = 14$). At the end of the training period (Fig. 1), all rats were immediately microinjected into the ventral hippocampus with saline or the different doses of histamine, according to the experimental groups. No further treatment was performed and animals remained at rest up to the following day. Doses of histamine and its antagonists were found previously to affect specifically learning [1,2]. Thus, in the present study it was decided to use the same doses.
2. Effect of histamine into the ventral hippocampus on the consolidation of the avoiding response to an ultrasonic tone 15 min after training. This experiment was the same that experiment 1, except that at the end of the second day of the training period, saline and histamine treatment were performed 15 min after ending of the eighth trial. A total number of 42 rats were used. Groups were: Sal ($n = 11$); HA9 ($n = 10$); HA45 ($n = 11$) and HA90 ($n = 10$).
3. Effect of histamine and histamine receptors antagonists on the consolidation of the avoiding response to an ultrasonic tone immediately after training. In this experiment, the possible participation of H_1- or H_2-histamine receptors on the consolidation of the avoiding response to the ultrasonic tone after a chemical stimulation with histamine of the ventral hippocampus was investigated. Antagonists were microinjected into the hippocampus 15 min after the finalization of the eighth trial of the second training session (time 15 min). At the next 5 min (20 min after the ending of the training period), rats were microinjected once again with saline or 9 nmol of histamine (time 20 min). In this schedule, animals had to be injected into the hippocampus

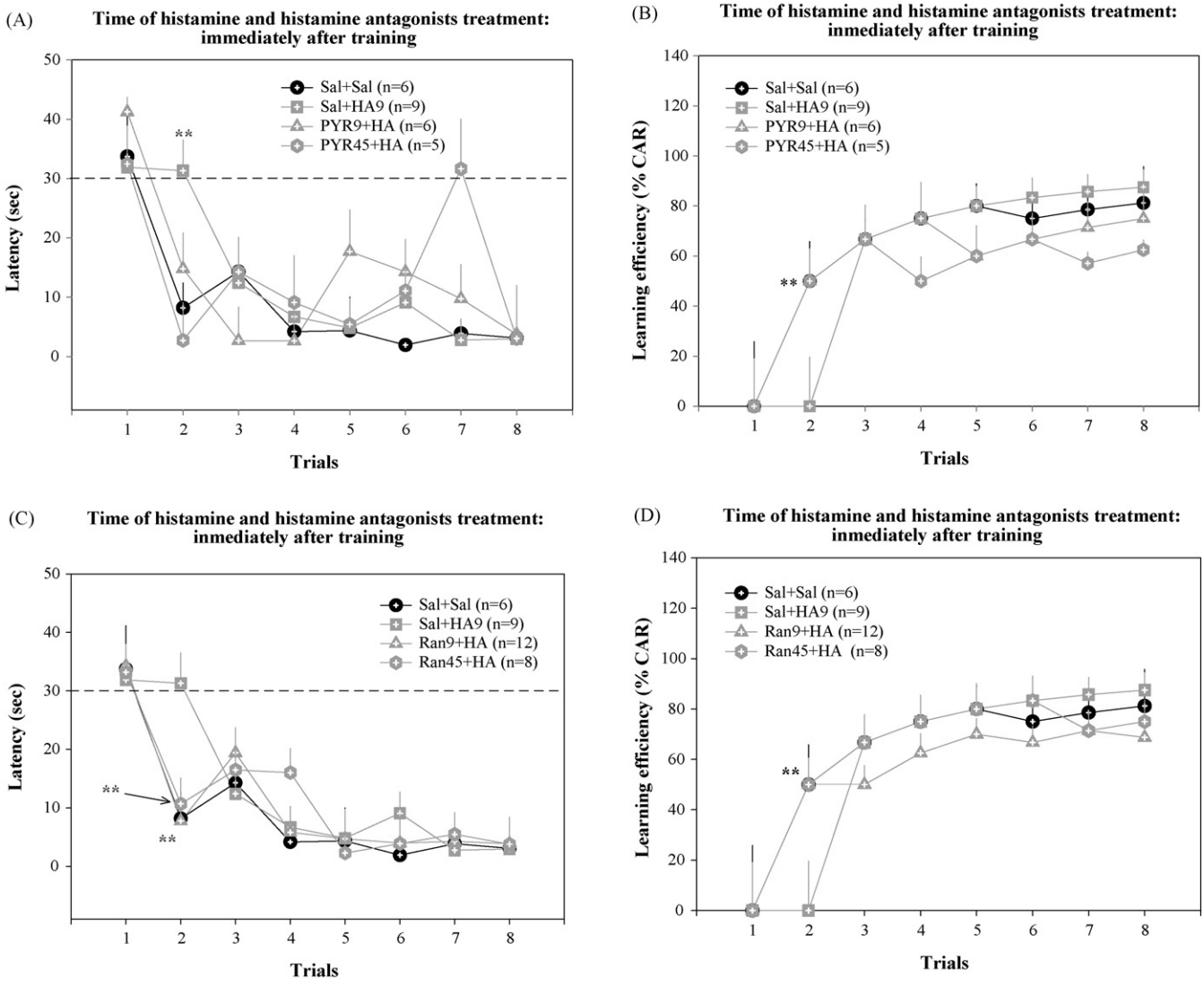

Fig. 3. Consolidation of an avoiding response to an ultrasonic tone in rats bearing hippocampus microinjection cannulae implants, and treated with various doses of histamine antagonists and histamine, immediately after training. (A) Latency curves of animals treated with pyrilamine and histamine. (B) % of correct avoiding responses curves of animals shown in (A). $^{**}p < 0.01$ vs. the respective point of group Sal + HA9. (C) Latency curves of animals treated with ranitidine and histamine. (D) % of correct avoiding responses curves of animals shown in (C). $^{**}p < 0.01$ vs. the respective point of group Sal + HA9. Note that HA45 curve coincides with HA90 curve.

twice. A total number of 46 rats were used. Groups were: Sal + Sal, rats microinjected with 1 μl of saline at times 15 and 20 ($n = 6$); Sal + HA9, animals microinjected with 1 μl of saline at times 15 and 9 nmol HA/μl at time 20 ($n = 9$); PYR9 + HA, animals microinjected with 9 nmol pyrilamine/μl at time 15, and with 9 nmol of histamine at time 20 ($n = 6$); PYR45 + HA, animals microinjected with 45 nmol of pyrilamine/μl at time 15, and 9 nmol of histamine/μl at time 20 ($n = 5$); RAN9 + HA, animals microinjected with 9 nmol of ranitidine/μl at time 15, and 9 nmol of histamine/μl at time 20 ($n = 12$) and RAN45 + HA, rats microinjected with 45 nmol of ranitidine/μl at time 15, and with 9 nmol of histamine/μl at time 20 ($n = 8$). Preliminary groups with histamine antagonists alone were also tested. However, their performances were not different from control Sal + Sal. Thus, these results were not included.

2.5. Statistics

Medians of latency to escape or CAR for the different groups were analyzed by the Non-parametric Multiple Comparisons Test of Dunn [12], since variables were found non-parametric. A probability value of less than 0.05 was considered as statistical significant.

3. Results

Histological inspection of coronal 10%-formaldehyde fixed sections revealed that the localized microinjections were found into the medial ventral portion of the hippocampus. According to this, the area of the chemical stimulation included part of the dentate area and subiculum complex, covering the CA_1–CA_4 cells zone (results not shown). Therefore, the stimulated zone was about the same of that of previous experiments [1–3].

3.1. Experiment 1 (Fig. 2A and B)

Fig. 2(A) shows the latency to escape from the electric shock after the ultrasonic tone was on, in rats microinjected into the ventral hippocampus with saline or increasing doses of histamine. As shown, control animals (Sal group) displayed a median latency of about 33 s at the first trial. From there

Table 1
Experiment no. 1

Group	Session	Trials							
		1	2	3	4	5	6	7	8
(A) Latency to escape (s)									
Sal	1	51.80 ± 10.0	56.3 ± 8.1	40.1 ± 7.0	34.1 ± 3.7	32.2 ± 3.5	32.6 ± 4.2	32.0 ± 5.7	31.9 ± 4.3
	2	37.7 ± 1.8	28.0 ± 4.4	11.7 ± 3.9	6.30 ± 3.2	3.80 ± 0.7	8.10 ± 3.5	3.30 ± 1.7	3.20 ± 2.7
HA9	1	55.5 ± 11.0	56.4 ± 11.9	48.5 ± 10.2	36.4 ± 11.2	36.0 ± 5.2	35.0 ± 4.7	8.20 ± 6.0	24.8 ± 4.9
	2	34.9 ± 1.4	32.5 ± 3.8	31.9 ± 4.8	19.0 ± 4.0	8.80 ± 3.5	6.60 ± 2.3	5.80 ± 3.4	5.20 ± 1.4
HA45	1	57.5 ± 12.8	43.5 ± 7.8	35.70 ± 3.9	34.30 ± 2.8	34.20 ± 4.8	33.00 ± 5.5	7.80 ± 5.9	21.90 ± 5.1
	2	36.8 ± 3.7	24.80 ± 4.2	21.30 ± 4.6	9.10 ± 4.6	7.30 ± 5.4	5.40 ± 3.4	7.20 ± 2.7	6.50 ± 1.6
HA90	1	120 ± 14.5	47.80 ± 10.4	40.00 ± 11.9	34.00 ± 10.9	34.00 ± 4.7	36.90 ± 4.5	27.60 ± 4.5	31.40 ± 4.3
	2	35.80 ± 3.3	16.10 ± 3.6	9.20 ± 4.1	9.60 ± 3.6	4.60 ± 2.8	6.80 ± 3.6	5.20 ± 3.6	4.60 ± 2.6
(B) Memory efficiency (% CAR)									
Sal	1	0 ± 0	0 ± 0	0 ± 2.8	0 ± 3.6	0 ± 4.4	0 ± 6	7.1 ± 6.4	12.5 ± 6.1
	2	0 ± 0	25 ± 7.2	33.3 ± 7.3	50 ± 6.2	60 ± 5	66.7 ± 4.6	71.4 ± 4	75 ± 3.4
HA9	1	0 ± 0	0 ± 0	0 ± 3.9	0 ± 5.9	10 ± 5.5	16.7 ± 4.4	28.6 ± 3.9	37.5 ± 5
	2	0 ± 0	0 ± 7.2	16.6 ± 7.3	25 ± 5.1	40 ± 4.1	50 ± 3.4	57.1 ± 3.4	75 ± 3.3
HA45	1	0 ± 0	0 ± 0	0 ± 5.8	0 ± 6.6	0 ± 7.2	16.7 ± 6	21.4 ± 6.8	25 ± 6.8
	2	0 ± 10	50 ± 11.2	50 ± 9.7	50 ± 5.6	60 ± 6	66.7 ± 6.2	71.4 ± 6.5	75 ± 5.7
HA90	1	0 ± 7.1	0 ± 3.6	0 ± 4.7	0 ± 5.9	20 ± 3.8	16.7 ± 7.1	14.3 ± 5.1	25 ± 4.7
	2	0 ± 10.1	50 ± 7.1	66.7 ± 9.2	62.5 ± 7	70 ± 6.6	66.7 ± 5.9	71.4 ± 5.5	75 ± 5

on latency was around 5 s for all subsequent trials. Animals microinjected into the hippocampus at the end of their training period 24 h earlier with 9 nmol or 90 nmol of histamine, displayed a higher latency significantly different from control values at trials 2 and 3 (Fig. 2A, $p < 0.01$). Rats that received 45 nmol of histamine, however showed a latency curve pattern not significantly different from control (Fig. 2A). When learning efficiency was examined (Fig. 2B), control animals showed an steady increasing curve reaching about 87% at trial 8. All groups treated with histamine showed a significantly lower efficiency curve than control. For rats microinjected with 45 nmol of histamine, CAR curve was statistically lower than control at trials 3–6 (Fig. 2B). For rats microinjected with 9 nmol of histamine significant lower CAR scores were found at trials 2–6, and for rats treated with 90 nmol of histamine, with exception of trial 1, the entire curve was significantly lower than control.

3.2. Experiment 2 (Fig. 2C and D)

When treatment began 15 min after the termination of the training session at day 2, control animals at day 3 showed a similar latency curve than the one found in experiment 1 (Fig. 2C). Histamine treatment on day 2 in the same conditions was able to interfere with the animal's latency to escape.

Table 2
Experiment no. 2

Group	Session	Trials							
		1	2	3	4	5	6	7	8
(A) Latency to escape (s)									
Sal	1	53.5 ± 12.3	44.8 ± 7.6	38.1 ± 14.9	38.1 ± 4.8	34.8 ± 3.7	32.6 ± 5.6	7.6 ± 4.7	32.4 ± 4.3
	2	36.4 ± 7.9	31.9 ± 5.8	13.7 ± 5.1	10.3 ± 3.7	5.11 ± 1.4	6.1 ± 2.7	4.5 ± 2.8	6.3 ± 3.8
HA9	1	50.8 ± 13.4	55.7 ± 6.9	47.2 ± 10.1	34.8 ± 3.6	36.8 ± 4.5	9.8 ± 5.4	12.8 ± 5.9	22.7 ± 4.3
	2	35.3 ± 4.2	32.2 ± 5.2	20.1 ± 5.1	32.3 ± 5.1	3.7 ± 2.9	4.8 ± 4.1	4.4 ± 3.1	4.8 ± 2.9
HA45	1	120 ± 8.9	47.9 ± 11.5	38.6 ± 1.9	33.3 ± 8.7	34 ± 4.7	31.7 ± 4.2	34.05 ± 5.5	32.5 ± 4.9
	2	35.7 ± 2.8	32.5 ± 4	33.1 ± 0.5	31.4 ± 5.5	4.5 ± 3.7	13.5 ± 3.6	5.5 ± 1.6	3.46 ± 1.7
HA90	1	120 ± 15.9	50.6 ± 10.1	35.4 ± 14.9	36.5 ± 8.6	33.5 ± 3.1	34.7 ± 5.2	21.9 ± 5	35.4 ± 3
	2	36.0 ± 3.1	32.5 ± 3.7	15.3 ± 4.4	15.9 ± 3.5	3.6 ± 3	5 ± 2.4	2.90 ± 1.6	4.5 ± 2.2
(B) Memory efficiency (% CAR)									
Sal	1	0 ± 0	0 ± 5.4	0 ± 4.4	0 ± 5.8	20 ± 4.9	16.7 ± 4.4	28.6 ± 5.1	25 ± 5.3
	2	0 ± 21.1	50 ± 11.8	66.7 ± 11.1	50 ± 8.8	60 ± 7.4	66.7 ± 6.2	71.4 ± 5.3	75 ± 5.3
HA9	1	0 ± 0	0 ± 0	0 ± 4.1	0 ± 7	0 ± 9.3	16.7 ± 7.2	21.4 ± 7.6	25 ± 7.8
	2	0 ± 17.7	50 ± 14	66.7 ± 8.3	75 ± 8.3	80 ± 6.6	83.3 ± 5.5	85.7 ± 4.7	87.5 ± 4.1
HA45	1	0 ± 0	0 ± 7.8	0 ± 6.4	0 ± 8.8	20 ± 5	16.7 ± 5.2	28.6 ± 5.5	25 ± 6.4
	2	100 ± 31.6	50 ± 15.8	66.7 ± 10.5	75 ± 7.9	80 ± 6.3	83.3 ± 5.2	85.7 ± 4.5	87.5 ± 3.9
HA90	1	0 ± 0	50 ± 16	33.3 ± 7.3	50 ± 5.4	60 ± 4.6	66.7 ± 4	71.4 ± 3.5	75 ± 3.9
	2	0 ± 0	50 ± 8.6	33.3 ± 7.3	50 ± 5.4	60 ± 4.6	66.7 ± 4	71.4 ± 3.5	75 ± 3

Table 3
Experiment no. 3

Group	Session	Trials							
		1	2	3	4	5	6	7	8
(A) Latency to escape (s)									
Sal + Sal	1	55.8 ± 12.5	40.8 ± 9.6	37.0 ± 2.7	34.0 ± 4.5	32.3 ± 4.0	32.5 ± 4.1	22.4 ± 3.5	33.4 ± 4.5
	2	32.9 ± 4.5	31.4 ± 3.9	31.5 ± 4.2	16.0 ± 4.0	4.9 ± 2.9	2.3 ± 0.9	6.5 ± 2.1	2.7 ± 3.4
PYR9 + HA9	1	49.8 ± 15.5	44.8 ± 13.4	35.0 ± 3.8	23.6 ± 4.4	33.1 ± 6.4	21.2 ± 6.3	34.6 ± 6.1	32.7 ± 3.9
	2	33.5 ± 11.9	8.6 ± 5.2	6.5 ± 3.9	4.0 ± 3.7	2.5 ± 0.5	1.9 ± 2.6	2.4 ± 0.9	4.3 ± 1.1
PYR45 + HA9	1	47.1 ± 8.4	39.3 ± 10.3	38.4 ± 3.9	34.9 ± 5.9	34.4 ± 5.1	15.6 ± 4.7	31.6 ± 4.5	9.9 ± 6.9
	2	32.2 ± 10.4	16.4 ± 5.3	32.2 ± 4.3	8.9 ± 5.8	3.4 ± 1.2	5.3 ± 1.0	4.1 ± 3.2	3.9 ± 2.8
RAN9 + HA9	1	54.5 ± 10.3	42.6 ± 10.4	37.8 ± 6.7	38.01 ± 4.2	19.80 ± 4.0	33.90 ± 5.1	34.5 ± 4.7	19.7 ± 2.5
	2	36.0 ± 1.1	19.5 ± 4.1	9.1 ± 4.3	4.3 ± 3.7	3.6 ± 2.7	2.80 ± 2.3	3.60 ± 1.5	4.4 ± 1.6
RAN45 + HA9	1	49.2 ± 11.2	40.9 ± 3.5	31.7 ± 5.3	26.9 ± 9.5	31.9 ± 4.9	31.5 ± 5.3	31.7 ± 3.9	32.16 ± 5.3
	2	34.5 ± 4.2	21.2 ± 3.8	12.1 ± 4.1	5.2 ± 5.2	11.1 ± 3.3	4.80 ± 2.7	6.80 ± 2.1	6.4 ± 2.5
(B) Memory efficiency (% CAR)									
Sal + Sal	1	0 ± 0	0 ± 5.4	0 ± 4.4	0 ± 5.8	20 ± 4.9	16.7 ± 4.4	28.6 ± 5.1	25 ± 5.3
	2	0 ± 21.1	50 ± 11.8	66.7 ± 11.1	50 ± 8.8	60 ± 7.4	66.7 ± 6.2	71.4 ± 5.3	75 ± 5.3
PYR9 + HA9	1	0 ± 0	0 ± 0	0 ± 4.1	0 ± 7	0 ± 9.3	16.7 ± 7.2	21.4 ± 7.6	25 ± 7.8
	2	0 ± 17.7	50 ± 14	66.7 ± 8.3	75 ± 8.3	80 ± 6.6	83.3 ± 5.5	85.7 ± 4.7	87.5 ± 4.1
PYR45 + HA9	1	0 ± 0	0 ± 7.8	0 ± 6.4	0 ± 8.8	20 ± 5	16.7 ± 5.2	28.6 ± 5.5	25 ± 6.4
	2	100 ± 31.6	50 ± 15.8	66.7 ± 10.5	75 ± 7.9	80 ± 6.3	83.3 ± 5.2	85.7 ± 4.5	87.5 ± 3.9
RAN9 + HA9	1	0 ± 0	50 ± 16	33.3 ± 7.3	50 ± 5.4	60 ± 4.6	66.7 ± 4	71.4 ± 3.5	75 ± 3.9
	2	0 ± 0	50 ± 8.6	33.3 ± 7.3	50 ± 5.4	60 ± 4.6	66.7 ± 4	71.4 ± 3.5	75 ± 3
RAN45 + HA9	1	0 ± 0	0 ± 4.5	0 ± 8.6	25 ± 6.8	20 ± 6.3	33.3 ± 5.7	42.8 ± 6.7	37.5 ± 6.5
	2	0 ± 15.7	50 ± 11.1	66.7 ± 12.2	62.5 ± 10.7	70 ± 8.6	75 ± 7.1	78.5 ± 6.1	81.2 ± 5.3

For 9 nmol histamine dose, latency was significantly higher than control at trials 1–2. For 45 nmol histamine dose, latency was different from control at trials 1–3, and for 90 nmol histamine dose, latency was higher than control at trial 1 (Fig. 2C). When learning efficiency was examined (Fig. 2D), CAR responses were significantly lower than control at trials 2–8 for 9 and 45 histamine doses, and trials 4–5 for 90 nmol histamine dose.

3.3. Experiment 3 (Fig. 3)

Pre-treatment with the H_1-histamine antagonist pyrilamine in doses of 9 nmol or 45 nmol and 9 nmol of histamine on day 2, was able to block the inhibitory effect of histamine on latency at trial 2 on day 3 (Fig. 3A). Regarding the learning efficiency, displacement of the learning curve to the right at trial 2 was completely blocked with the pretreatment of pyrilamine as shown in Fig. 3(B). Both doses of pyrilamine were effective. Pretreatment with ranitidine in 9 nmol or 45 nmol doses with 9 nmol of histamine at day 2 was effective also to block the inhibitory effect of histamine on latency at day 3 (Fig. 3C). Regarding learning efficiency, pre-treatment with 9 nmol of ranitidine with 9 nmol of histamine at day 2, partially blocked the inhibitory effect of histamine on CAR responses at day 3 (Fig. 3D). Dose of 45 nmol ranitidine, was completely effective to block the histamine effects.

As explained in Section 2 sessions on consecutive days were performed in order to achieve learning criteria in rats. Performance of sessions 1 and 2 for latency and CAR responses for the three experiments is shown in Tables 1–3. As shown, all animals reached a median latency of about 5 s for latency and about 70% CAR at the end of session 2 where treatment with histamine or histamine antagonists was applied.

4. Discussion

Consolidation is considered the final step whereby the memory trace is converted into a long-term memory in the brain [22,20,28,29]. It is followed that the learning neural circuits need to actively invest into an additional process in order to codify the new information into a permanent register of memory. Although it is not known exactly the molecular mechanisms whereby consolidation occurs in neurons, there is agreement that this process takes some finite time after the "training" period has been terminated [30]. During this period, several mechanisms have been proposed to explain finally the generation of a long-term memory [26,9,38,32,43]. Nevertheless, whatever the intrinsic mechanism may be, it is known that it is susceptible to be interfered, impairing the permanency of memory. Previous evidence from our laboratory has shown that hippocampal histamine modulates the acquisition and recall of an avoiding response to an ultrasonic tone [1–4].

The first point to be determined in the present work was to know if the neural region of the ventral hippocampus was the same that those in previous studies. As described, microinjections of saline, histamine or histamine antagonists into the ventral hippocampus were made in the central region of the hippocampal formation. Thus, area of diffusion reached the CA_4–CA_1 neuronal population, the same region where the histamine effects were found previously [1–3]. Inspection of Tables 1–3 shows that animals at the end of session 2 in the present experimental conditions were able to learn the appro-

priate avoiding response to the ultrasonic tone. Overall learning efficiency was about 75% and latency was around 4 s. These two variables, although related to learning and memory, are showing however different things. Latency measures the time input information takes to reach the higher brain centers, the integration of data and the delay of the efferent command signals to stimulate the appropriate muscle response. Learning efficiency on the other hand is an expression of the capacity of the neural circuits to store new information (memory). Regarding latency, integration requires linking the ultrasonic signal to the events that come after the ultrasonic tone is over, i.e. the electric shock. Thus, in order to avoid it, rats must show an escape response to the next compartment through the unlocked door. If latency (L) is represented in simplified mathematical terms, then $L = t_{INPUT} + t_{INT} + t_{OUTPUT}$; where t_{INPUT} = time the afferent signal takes to reach the higher brain centers, t_{INT} = time the neural circuits (including the hippocampus) take to integrate the context events and t_{OUTPUT} = time that the efferent command from the motor cortex takes to reach the muscles of the animal to give the behavioral response. It is thought that t_{INPUT} and t_{OUTPUT} remain about the same during the training period, since they depend mainly on the neurophysiologic characteristics of the sensory receptors and the nerves of the animal, and it is assumed to be constant. Thus, variation of latency during repeating trials, must reflects mainly the time needed to associate the stimulus (the ultrasonic tone) to punishment (the electric shock) in the rat's brain. After the task has been acquired (sessions 1 and 2) in the present experimental design t_{INT} decreased to less than 5 s, since latency was around 5 s in control animals (Fig. 2A). The application of histamine immediately after trials were over at session 2, significantly affected latency 24 h later during the recall session (Fig. 2A). This evidence suggests that the activation of histamine-sensitive neurons of the ventral hippocampus during the onset of consolidation of the avoiding response interfered with the process. It is interesting to note that the inhibitory effect of histamine at this stage is dissipated eventually by repetition of trials, thus after trial 3, the latency curve of those animals previously treated with histamine behaved as control (Fig. 2A). It can be speculated that the interference by histamine into the ventral hippocampus neurons *delayed* or *interrupted* the normal course of the consolidation mechanism in such a way that 24 h later, the system needed to reinforce or restart the learning phase in order to the avoiding response is acquired. This interpretation is supported by the displacement of the latency curve to the right and the lower learning efficiency scores observed in the histamine-treated animals (Fig. 2A and B). It is not known how the type of task influences the timing of the consolidation process, but since it is thought that consolidation may take at least 2 h for some determined tasks [30,21,33], it was not surprising that histamine applied 15 min after the training period in session 2, still interfered latency and CAR responses 24 h later. Since longer times for microinjections of histamine on session 2 were not applied, it was not possible in the present work to have an idea of the exact duration of the consolidation of the avoidance response in our experimental conditions. Examining results from experiment 3 (Fig. 3), it is evident that blocking the hippocampal histamine H_1- or H_2-receptors, the histamine treatment was not able to impair the consolidation of the avoiding response 24 h later. Data suggest that hippocampal histamine H_1- and H_2-receptors participate in the modulation of the histamine effect. However, it is necessary the activation of both types of receptors in order to the inhibitory effect of histamine is present. Present results complete the study in our laboratory about the participation of the hippocampal histamine neurons on the learning of an active conditioned avoiding response. In our experimental conditions, it was found that histamine inhibits the three stages of learning of the avoiding response; evocation [1,2]; acquisition [3]; consolidation (present results). Regarding the role that the histaminergic systems have on cognitive mechanisms, opposing actions for histamine on learning and memory processes have been described, and facilitatory or inhibitory effects have been found [24,44,31,11,19,39,7,25,13]. This apparent contradictory evidence for the same neural function is not surprising, since histamine has complex actions on neurons. Stimulation of H_1-histamine receptors can activate the $G_{q/11}$ GTP-hydrolyzing protein which activates phospholipase C, which hydrolyzes phosphatidyl-4,5-bisphosphate, generating two second messengers DAG and IP_3 [27,6,16]. Final responses to these intracellular mediators produce varying cell effects including inhibitory actions, such as blocking of the leak K^+ current [16], opening of the small conductance K^+ channels, resulting in hyperpolarization and inhibition of the pyramidal cells in the hippocampus [41,36]; or stimulatory actions, such as cation-channel depolarizations [6,16]. Activation of H_2-histamine receptors follows a similarly complex activation of intracellular mediators [16]. It is difficult to predict a determined action for histamine starting from the simple binding to an H_1- or H_2-receptor in some determined neuron in a brain circuit, since neurons interactions such as an stimulation of an inhibitory interneuron, a positive effect is transformed into an inhibitory effect. Thus, it is possible to find inhibitory or facilitatory behavioral effects in experiments using whole animals where histamine pharmacology has been studied after peripherical, intracerebroventricular or localized brain injections in different experimental designs. Present results support the inhibitory actions of histamine found in previous studies of this laboratory [1–4], and in addition to the evidence reported from some other authors [19,11,25], the concept that histamine neuronal systems exert important modulatory actions on the learning and memory functions in the brain has gained strong experimental support.

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