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# L-histidine induces state-dependent memory deficit in mice mediated by $H_1$ receptor

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ABSTRACT

This study investigated the role of $H_1$ receptor in the state-dependent memory deficit induced by L-histidine (LH) in mice using Trial 1/2 protocol in the elevated plus-maze (EPM). The test was performed for two consecutive days: Trial 1 (T1) and Trial 2 (T2). Before both trials, mice received a combined injection i.p. of saline + saline (SAL/SAL), 500 mg/kg L-histidine + saline (LH/SAL), 500 mg/kg L-histidine + 16 mg/kg chlorpheniramine (LH/CPA) or saline + 16 mg/kg chlorpheniramine (SAL/CPA). The trials were performed in the EPM 10 min after the last injection. Each animal was placed in the center of the maze facing the open arm and had five minutes to explore it. On both days, test sessions were videotaped. The behavioral measures were scored from videotape. Data were analyzed based on Analysis of Variance (ANOVA) and the Fisher's LSD test. The data showed no effects on anxiety since there was no difference between the SAL/SAL and the other groups in Trial 1, respectively, open arm entries (OAE), open arm time (OAT) and their percentages (%OAE and %OAT). During Trial 2, OAE, OAT, %OAE and %OAT were reduced in mice treated with SAL/SAL, LH/CPA and SAL/CPA, while the group LH/SAL did not show any difference in these measures. No significant changes were observed in enclosed arm entries (EAE), an EPM index of general exploratory activity. Thus, it can be suggested that LH induces emotional memory deficit and the treatment with chlorpheniramine was able to revert this effect, suggesting this action of LH was mediated by the $H_1$ receptor.



## 1. Introduction

The biogenic amine histamine is an important neurotransmitter-neuromodulator in the central nervous system (CNS). Neurons that synthesize and release histamine are exclusively located in the nucleus tuberomamillaris at the posterior hypothalamus and project fibers to practically all the brain areas. (Hass and Panula, 2003; Prell and Green, 1986).

Neuronal histamine synthesis is carried out by histidine descarboxylase which converts L-histidine to histamine via oxidative descarboxilation (Dere et al., 2010). The action of histamine on CNS is mediated by four types of receptors, $H_1$, $H_2$, $H_3$ and $H_4$ which differ in pharmacology, localization and the intracellular response they mediate (Strakhova et al., 2009; Leurs et al., 1995).

The neural histaminergic system (NHS) has been implicated in several of biological functions including control of the waking state, in motivated behaviors and behavioral disorders (Onodera et al., 1994), as well as in neuroplastic changes associated with functional recovery from brain damage (Piratello and Mattioli, 2004), anxiety (Faganello and Mattioli, 2007; Privou et al., 1998; Imaizumi and Onodera, 1993) and learning and memory (Medalha et al., 2000; Mattioli et al., 1998; De Almeida and Izquierdo, 1986). Besides, according to Dere et al. (2010) in recent review, clinical investigations demonstrate the involvement of neural histaminergic system in neurodegenerative diseases such as Alzheimer and Parkinson.

The role of this system during learning and memory has been studied, and contradictory results have also been observed. For example, the depletion of neuronal histamine induced by the inhibition of the histamine-synthesizing enzyme L-histidine descarboxylase was reported to both impair (Kamei et al., 1993) and promote (Cacabelos and Alvarez, 1991) learning in active avoidance tests. Pre-trial and pre-retention intracerebroventricular (i.c.v.) injection of histamine was reported to facilitate mnemonic functioning (Prast et al., 1996) and injections of histamine caused facilitation of memory retrieval in aged rats (Kamei and Tasaka, 1993).

The elevated plus-maze (EPM) is one of the most widely used animal models in research on anxiety (Carobrez and Bertoglio, 2005; Rodgers and Cole, 1994). Nowadays, its usefulness has spread towards the understanding of the biological basis of emotionality related to learning and memory (Stern et al., 2008; Bannerman et al., 2004; Lamprea et al., 2000). According to Bertoglio and Carobrez (2004), in the EPM test, after the initial (Trial 1) exploration of the whole

*Abbreviations:* LH, L-histidine; EPM, elevated plus maze; T1, trial 1; T2, trial 2; SAL, saline; CPA, chlorpheniramine; ANOVA, analysis of variance; i.c.v., intracerebroventricular; OAE, open arm entries; OAT, open arm time; %OAE, percentage of open arm entries; %OAT, percentage of open arm time; EAE, enclosed arm entries; %EAT, percentage of enclosed arm time; SAP, stretched-attend postures; %CT, percentage of central area time; CNS, central nervous system; NHS, Neural histaminergic system.

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apparatus, rodents express increased inhibitory avoidance response during retesting (Trial 2). This finding is thought to reflect the acquisition of information through the exploration of potentially dangerous areas of the maze — the open arms (Bertoglio et al., 2006; Bertoglio and Carobrez, 2004; Carobrez and Bertoglio, 2005).

Based on the fact that neural histaminergic system is related with anxiety, learning and memory, the EPM test has been employed to investigate its involvement on these processes (Serafim et al., 2010; Moghaddam et al., 2008; Frisch et al., 1998). Recent results in our laboratory demonstrated that systemic injections of L-histidine induced a state-dependent memory retrieval deficit in mice re-exposed to the EPM (Serafim et al., 2010). In view of these findings, the present study was designed to investigate whether LH state-dependent memory deficit could be mediated by $H_1$ receptor blocker in mice using Trial 1/2 protocol in the elevated plus-maze (EPM).

## 2. Methods

### 2.1. Animals

Subjects were male Swiss mice (Federal University of São Carlos, UFSCar, SP, Brasil) weighing 25–35 g at testing. They were housed in groups of 10 per cage (41 × 34 × 16 cm) and maintained under a 12 h light cycle (lights on at 7:00 a.m.) in a controlled environment — temperature (23 ± 1 °C) and humidity (50 ± 5%). Food and drinking water were freely available except during the brief test periods. All mice were experimentally naïve, and experimental sessions were carried out during the light phase of the cycle (9:00 – 13:00 h).

### 2.2. Drugs

L-histidine hydrochloride (precursor of Histamine) (LH — 500 mg/kg) (RBI, USA) and the $H_1$ receptor antagonist, chlorpheniramine maleat salt (CPA — 16 mg/kg) (Sigma, MO, USA) were dissolved in 0.9% saline solution (SAL). Saline solution was used as an experimental control. All drugs were administered intraperitoneally (i.p.) in a volume of 2 ml/kg of body weight. The doses were selected on the basis of previous studies (Serafim et al., 2010; Faganello and Mattioli, 2007; Medalha et al., 2000). The substances were coded and the coding was unknown by the experimenter at the moment of the tests and behavioral analysis.

### 2.3. Apparatus and general procedure

The elevated plus maze used was similar to that originally described by Lister (1987). The EPM consisted of two open arms (30 × 5 × 0.25 cm) and two enclosed arms (30 × 5 × 0.25 cm) connected to a common central platform (5 × 5). The apparatus was made of wood – the floor – and transparent glass – clear walls – and was raised to a height of 38.5 cm above floor level. All tests were conducted under moderated illumination (77 lx) measured on the central platform of the EPM.

### 2.4. Experimental procedure

To facilitate adaptation, the animals were transported to the experimental room and left there undisturbed for at least 1 h prior to the test sessions. The test was performed for two consecutive days: Trial 1 (T1) and Trial 2 (T2). In T1, they were injected with a combined injection, which consisted by one first injection, followed by a second injection 30 min later. The combined injections employed were: SAL + SAL (n = 12), LH + SAL (n = 13), LH + CPA (12) and SAL + CPA (n = 11). Ten minutes after the second injection, animals were exposed to the EPM for 5 min. For the test on the EPM, mice were individually placed on the central platform of the maze facing the open arm. Twenty four hours later (T2), mice were injected with the same combined injection that the previous day and they were re-exposed to the EPM under the same experimental conditions. Between subjects, the maze was thoroughly cleaned with ethanol 20% and a clean dry cloth. All sessions were video recorded by a camera linked to a monitor and VCR in the adjacent room.

### 2.5. Behavioral analysis

Videotapes were scored by a highly trained observer using an ethological analysis packing X-PLO-RAT developed at Laboratory of Exploratory Behavior USP/ Ribeirão Preto (Becerra-Garcia et al., 2005). Behavioral parameters were defined according to previous studies (Rodgers and Johnson, 1995; Lister, 1987): the frequency of open and enclosed-arm entries (OAE and EAE) defined as all four paws placed inside an arm, and total time spent in the open (OAT) and enclosed arms (EAT) and in the central area (CT). These data were used to calculate the percentage of open-arm entries {%OAE; [(open entries/open + enclosed entries) × 100]}, the percentage of time spent in the open {%OAT; [(open time/300) × 100]} and enclosed {%EAT; [(enclosed time/300) × 100]} arms, as well as on the central platform {%CT; [(central platform time/300) × 100]}. The number of stretched-attend postures (SAP; exploratory posture in which the body stretches forward and then retracts to its original position without any forward locomotion) and the frequency of the head dipping (exploratory movement of head/shoulders over sides of the maze) were also scored. Total SAP was considered a primary index of risk assessment and head dipping evaluated the exploratory behavior (Rodgers et al., 1997).

The conventional measure of anxiety consisted of %OAE and %OAT (Rodgers et al., 1997). In the EPM, emotional memory can be evaluated by the Trial 1/Trial 2 protocol. The decreased open-arm activity (OAE, OAT, %OAE and %OAT) in T2 was defined as learning and memory index (Serafim et al., 2010; Stern et al., 2008; Bertoglio et al., 2006; Lamprea et al., 2000). Total enclosed arm entries were measured as an index of locomotor activity (Cruz et al., 1994).

### 2.6. Statistical analysis

All results were initially submitted to Levene's test for homogeneity of variance. When appropriate, the data were transformed to square root and then analyzed by two-way analysis of variance (ANOVA; factor 1: treatment, factor 2: day). When differences were indicated by significant *F* values, they were identified by Fisher's LSD test (protected *t*-tests). A P value of $<0.05$ was required for significance.

### 2.7. Ethics

The experiments carried out in this study are in compliance with the norms of the Brazilian Neuroscience and Behavior Society (SBNeC), based on the US National Institutes of Health Guide for Care and use of Laboratory Animals.

## 3. Results

### 3.1. Effects of combined injection on anxiety in mice on EPM

As summarized in Fig. 1A–B, on the first exposure to the EPM combined injection (SAL/SAL, LH/SAL, LH/CPA and SAL/CPA) did not significantly affect conventional measures of anxiety in the EPM (percentage of open arm entries (%OAE): $F_{(3,141)} = 1.07$, $p > 0.05$; and percentage of open arm time (%OAT): $F_{(3,141)} = 1.49$, $p > 0.05$). The ANOVA did not reveal a significant treatment effect for open arm entries (OAE): $F_{(3,141)} = 2.50$, $p > 0.05$; open arm time (OAT): $F_{(3,141)} = 2.26$; $p > 0.05$; percentage of enclosed time (%EAT) and percentage of central platform time (%CT): ($F_{(3,141)} = 0.99$, $p > 0.05$; $F_{(3,141)} = 2,13$, $p > 0.05$) (Table 1). Treatment effect did not change the locomotor activity, once there was no statistically significant effect on enclosed arm entries (EAE): $F_{(3,141)} = 0.22$, $p > 0.05$ (Fig. 1C). Regarding SAPs and head