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STUDY OF HISTAMINERGIC TRANSMISSION DURING SOCIAL INTERACTION IN MICE

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ABSTRACT

This study investigates the role of histaminergic transmission in social interaction and locomotor activity in mice. Histamine analogues, L-histidine, and histamine receptor antagonists were administered to male Swiss albino mice, and their effects on sociability and locomotion were examined using standardized behavioral assays. Histamine precursor L-histidine significantly increased sociability, while histamine receptor antagonists, cetirizine and ranitidine, reduced social behavior. Combination treatments of L-histidine with histamine receptor antagonists showed nuanced effects on sociability, suggesting complex interactions within the histaminergic system. Additionally, histaminergic analogues exerted diverse effects on locomotor activity, with implications for motor behavior regulation. These findings highlight the multifaceted role of histaminergic signaling in modulating social behavior and locomotion, providing insights into potential therapeutic interventions for conditions involving social dysfunction and motor impairments.

Key Words: Histaminergic transmission, social interaction, locomotor activity, mice, L-histidine, histamine receptor antagonists, sociability, histaminergic analogues, behavioral assays, neurotransmission, neurobiology

INTRODUCTION

Social interaction is a fundamental aspect of behavior in many species, including mice, where it plays a crucial role in various social behaviors such as mating, territoriality, and social bonding (Yoshikawa *et al.,* 2006). Understanding the neural mechanisms underlying social interaction is of great interest, not only for unraveling the complexities of social behavior but also for shedding light on potential therapeutic interventions for social deficits observed in neuropsychiatric disorders such as autism spectrum disorders (ASD) and schizophrenia.

Histamine, a neurotransmitter primarily known for its role in regulating allergic responses and sleep-wake cycles, has been implicated in modulating social behaviors (Passani & Blandina, 2011). The histaminergic system in the brain originates from neurons located in the tuberomammillary nucleus of the hypothalamus and projects widely throughout the central nervous system (Lee *et al.,* 2005). Mounting evidence suggests that histamine may play a pivotal role in regulating social behavior, yet the precise mechanisms by which histaminergic transmission influences social interaction remain to be fully elucidated. Several studies have highlighted the involvement of histamine in social behaviors.

For instance, pharmacological studies utilizing histamine receptor agonists and antagonists have demonstrated alterations in social behavior in rodents (Takahashi *et al.,* 2006). Additionally, genetic studies in mice with manipulations of histamine receptors or histamine synthesis enzymes have provided further evidence of the importance of histamine signaling in social interaction (Yoshikawa *et al.,* 2006).

Despite these advances, there is still a need for comprehensive investigations into the specific neural circuits and molecular mechanisms through which histaminergic transmission modulates social behavior (Passani & Blandina, 2011). Moreover, the majority of existing studies have focused on the role of histamine in either aggressive or affiliative aspects of social behavior, with less attention given to the dynamic changes in histaminergic activity during social interaction.

In this study, aim to investigate the role of histaminergic transmission during social interaction in mice. Firstly, we will assess the dose-dependent effects of histamine analogs and L-histidine on mice within a social interaction apparatus to understand their impact on social behavior. Additionally, we will explore the effects of histamine antagonists on social interaction in mice. To complement these behavioral analyses, we will utilize an actophotometer to examine the movement patterns of mice and study locomotor activity. Through these approaches, we seek to gain a comprehensive understanding of how histaminergic signaling influences social behavior in mice.

MATERIALS AND METHODS

In this study, male Swiss albino mice (25-30 g) of either sex were obtained from Jawaharlal Nehru Cancer Hospital and Research Centre in Bhopal. They were housed in acrylic cages (24 x 17 x 12 cm) under controlled environmental conditions (24 ± 1°C, 50 ± 2% RH), maintained on a 12:12 h light/dark cycle (lights on at 07:19:00h), and provided standard diet and water ad libitum. All experimental procedures were conducted following ethical guidelines approved by the Committee for the Control and Supervision of Experimental Animals (CCSEA) and the Institutional Animal Ethics Committee of Institute SIRT Pharmacy.

Drug and Solution

L-histidine was obtained from Bioven Ingredients, while histamine receptor antagonists, cetrizine (H1 receptor antagonist), and ranitidine hydrochloride (H2 receptor antagonist) were generously provided by Bioven Ingredients. All drugs were dissolved in 0.9% saline solution. The doses used were determined based on preliminary results and literature review. For intracerebroventricular (i.c.v.) administration, aCSF solution was employed, and drugs were administered at 5 µl/mice. For intraperitoneal (i.p.) administration, a dose of 10 ml/kg was used.

Intracerebroventricular (i.c.v.) Cannulation

Mice underwent i.c.v. cannulation under anesthesia induced by a combination of ketamine (100 mg/kg, s.c.) and xylazine (5 mg/kg, s.c.). A guide cannula (24 gauge) was stereotactically implanted according to coordinates referenced from Paxinos and

Franklin. After fixation with mounting screws and dental cement, a stainless steel dummy cannula was inserted to block the guide cannula when not in use. Post-surgery, mice received cefotaxime (50 mg/kg/day, subcutaneous) for 1 week for recovery and habituation to experimental protocols. Injections were performed using a Hamilton syringe connected to an internal cannula (41 gauge), with a volume of 1.0 µL injected into the right ventricle over 2 minutes (Passani *et al.,* 2011).

Sociability Test

The sociability test was conducted in a three-chambered device made of transparent plexiglass. Experimental mice were habituated for 5 minutes in a central room with closed doors to both side rooms, one containing an adult male stranger (Stranger 1) and the other an inanimate object. The doors were then opened, allowing mice to explore for 10 minutes. Time spent interacting with Stranger 1 was recorded. Subsequently, mice were subjected to a social novelty test, wherein they were acclimated for 10 minutes in the presence of two unfamiliar mice (one on each side) in a central chamber, with the second unfamiliar mouse acting as the novel stimulus (Stranger 2). Time spent interacting with Stranger 2 was recorded during a 10-minute exploration period (Yoshikawa *et al.,* 2006).

$$\%\ \text{time interaction with stranger 1} = \frac{\text{Time interacting with stranger 1}}{\left(\text{Time intracting with inanimate object}\right) + \left(\text{Time intracting with stranger 1}\right)}$$

$$\%\ \text{time interaction with stranger 2} = \frac{\text{Time interacting with stranger 2}}{\left(\text{Time intracting with stranger 1}\right) + \left(\text{Time intracting with stranger 2}\right)}$$

Equipment and room setup

The equipment setup for the sociability and social novelty preference tests, based on Crawley's method, consists of a three-chambered rectangular box, each chamber measuring 19 x 45 cm, with clear plexiglass septa allowing free access. Two wire containers, each housing an unfamiliar mouse, are placed vertically in the side chambers. Behavioral testing is conducted between 10:00 am and 5:00 pm, with general room lighting maintained at 650 lux. Chambers are cleaned with 70% ethanol between trials for proper disinfection. Observations are made from a distance of at least 2 meters from the apparatus to minimize interference (Lee *et al.,* 2005).

Habituation (Adaptation)

To acclimate the mice to the testing environment, a plexiglass partition wall is used to separate the left and right chambers. Empty wire cages are placed in the center of the left and right chambers, with one cage for each side. The experimental mice are placed in the center of the central chamber for a 5-minute habituation period (Takahashi *et al.,* 2006).

Social Affiliation Aspect of the Test (Session I)

During this session, a control mouse ("Stranger 1") is placed in a wire cup in one of the side compartments, with the placement alternating systematically between trials. The walls between the chambers are removed, allowing the experimental mice to freely explore all three chambers. The following parameters are monitored and recorded:

Number and duration of direct interactions between the experimental mice and the cup containing or without the Stranger 1 mouse, separately for each chamber. Direct contact or stretching of the experimental mice's body within a 1 cm radius around the cup is considered as active contact. Duration and frequency of other behaviors exhibited by the experimental mice in each compartment, including walking, grooming, freezing (not moving the body for more than 5 seconds), jumping, and repetitive actions. Duration and frequency of entries into each compartment, with a mouse considered inside a chamber when its head and four paws are within. Session I lasts for a duration of 10 minutes (Umathe *et al.,* 2008).

Social Novelty/Preference Session of the Test (Session II):

In this session, a second control mouse ("Stranger 2") is placed in an identical wire cup in the opposite compartment, which was empty during Session I. The same parameters as described in the first session are monitored to differentiate the behavior of the experimental mice in the presence of Stranger 1 compared to Stranger 2. Session II also lasts for 10 minutes.

Experimental Design

Effect of Histamine Precursor L-histidine on Sociability Test:

Separate groups of mice (n=6) received intraperitoneal (i.p.) injections of either vehicle (10 ml/kg) or L-histidine (500 or 1000 mg/kg). Since the maximum effect of 500 or 1000 mg/kg of L-histidine occurred 2 hours post-treatment, mice were treated with L-histidine 2 hours before the test. After 20 minutes, mice were subjected to the sociability test apparatus for 10 minutes to assess sociability and social novelty-seeking behavior (Moy *et al.,* 2004).

Effect of H1 and H2 Receptor Antagonists on Sociability Test:

In separate groups (n=6), mice were intracerebroventricularly (i.c.v.) treated with either artificial cerebrospinal fluid (aCSF) (5 µl/mice), H1 receptor antagonist cetirizine (20 µg/mice), or H2 receptor antagonist ranitidine (20 µg/mice). After 20 minutes, mice underwent the sociability test for 10 minutes to evaluate sociability and social novelty-seeking behavior.

Effect of H1 or H2 Receptor Antagonists on L-histidine-induced Effects on Sociability Test:

Separate groups of mice (n=6) received i.c.v. administration of aCSF (5 µl/mice), H1 receptor antagonist cetirizine (20 µg/mice), or H2 receptor antagonist ranitidine (20 µg/mice) after 1 hour and 45 minutes of i.p. injection of either vehicle (10 ml/kg) or L-histidine (1000 mg/kg). After 20 minutes, mice underwent the sociability test for 10 minutes to assess sociability and social novelty-seeking behavior.

Effect of Histaminergic Analogs on Locomotor Activity:

Different groups of mice received injections of vehicle (10 ml/kg, i.p.), L-histidine (10 µg/mice, i.c.v or 1000 mg/kg, i.p.), H1 receptor antagonist cetirizine (20 µg/mice, i.c.v.), H2 receptor antagonist ranitidine (20 µg/mice, i.c.v.), or a combination of both antagonists with L-histidine (1000 mg/kg, i.p.). After appropriate time periods, mice were subjected to the sociability test apparatus

for 10 minutes to evaluate sociability and social novelty-seeking behavior (Crawley; 2007).

Statistical Analysis

Data are presented as mean ± SEM. One-way analysis of variance (ANOVA) was used for most data analyses. Repeated-measures ANOVA was applied to analyze data from the sociability apparatus and locomotor activity trials. Newman-Keuls or Tukey's multiple comparison tests were used as follow-up tests. A significance level of $P < 0.05$ was considered statistically significant. Data analysis was performed using Prism Pad Chart.

RESULTS AND DISCUSSION

Effect of histamine precursor L-histidine in sociability test

In the sociability test, the effects of L-histidine treatment at doses of 500 and 1000 mg/kg (i.p.) were evaluated using one-way ANOVA, revealing a significant impact [$F(6, 35) = 39.93$, $^*P < 0.0001$]. Newman-Keuls test indicated a significant increase in sociability with L-histidine at 1000 mg/kg (i.p.) compared to saline-treated mice ($^*P < 0.001$). However, the 500 mg/kg dose showed no significant effect ($^*P < 0.05$). Histamine precursor L-histidine potentiates the Histamine H1 receptor subunit, influencing social behavior. Histamine synthesis can be enhanced by L-histidine administration, as seen in increased histamine levels in mice after 1000 mg/kg i.p. administration. Modulating endogenous histamine levels may offer a novel approach to controlling social behavior. Additionally, histamine H1 receptor antagonism in mice reduces activity, potentially influencing serotonin levels in the brain.

Effects of H1 receptor antagonist and H2 receptor antagonist on sociability test

The effects of H1 receptor antagonist cetirizine (20 µg/mice; i.c.v.) and H2 receptor antagonist ranitidine (20 µg/mice; i.c.v.) on the sociability test were evaluated through one-way ANOVA, revealing significant impacts [$F(2, 15) = 61.06$, $^*P < 0.0001$]. Newman-Keuls test indicated significant reductions in sociability with both cetirizine and ranitidine compared to aCSF-treated mice ($^*P < 0.001$). Furthermore, histamine H2 receptor antagonism and H1 receptor agonism enhance serotonin release, suggesting an enhancement of social interaction through H1 and H2 receptor-mediated serotonin enhancement.

To further investigate this hypothesis, the effects of histamine H1 and H2 receptor antagonists on L-histidine-induced social ability were explored. It was found that central administration of H2 receptor antagonist ranitidine blocked the increase in social interaction induced by L-histidine, while H1 receptor antagonist showed no effect. These findings support the hypothesis that enhancing central histaminergic transmission with L-histidine leads to increased serotonin levels, further promoting histaminergic transmission.

Additionally, blocking histamine H2 receptors reduces task performance in mice, indicating a potential role in exercise-related actions. The study also detected increased social interaction after administration of H1 and H2 receptor antagonists, suggesting their potential

utility in relieving negative symptoms such as social withdrawal in schizophrenia and psychotic disorders.

Effect of H1 and H2 receptor antagonist on L-histidine induced effect on sociability test

The effects of H1 and H2 receptor antagonists on L-histidine-induced sociability test were examined through one-way ANOVA, revealing significant impacts [$F(3, 20) = 51.16$, $^*P < 0.0001$]. Post hoc Newman-Keuls test showed that H2 receptor antagonist ranitidine (20 µg/mice; i.c.v.) reversed the decrease in social interaction induced by L-histidine ($^*P < 0.001$). However, H1 receptor antagonist cetirizine (20 µg/mice; i.c.v.) failed to affect L-histidine-induced effects on the sociability test ($^*P > 0.05$). The study further suggests an interaction between histamine and serotonin systems, as evidenced by an increase in histamine levels upon local serotonin infusion in the hypothalamus, attenuated by a 5-HT2C receptor antagonist. Anxiety disorders, believed to stem from serotonin 5-HT2C receptor activation, are influenced by serotonin agonists like mCPP, which modulate animal behavior via the 5-HT2C receptor subtype. The reduction in social behavior after enhancing central histaminergic transmission with histamine or L-histidine may be attributed to increased serotonin levels, further augmenting histaminergic transmission via 5-HT2C/2A receptor activation. These findings suggest potential interactions between histamine and serotonin in modulating social behavior, warranting further investigation with specific serotonin analogues. However, some researchers argue that the sociability test may not effectively differentiate between anxiolytic and anticompulsive agents.

Effect of histaminergic analogues on locomotor activity

Various histaminergic analogues were tested for their effect on locomotor activity at the doses as described previously using actophotometer. Table 1 illustrates the effects of various histaminergic analogues on locomotor activity in mice. Baseline locomotor activity readings were taken before treatment, and subsequent readings were recorded after treatment administration. Histamine administration via intracerebroventricular (i.c.v.) route at doses of 0.5 µg/mice and 10 µg/mice resulted in slight increases in locomotor activity compared to baseline. Conversely, L-histidine administration at 10 µg/mice via i.c.v. route led to a modest decrease in locomotor activity, while at a higher dose of 1000 mg/kg via intraperitoneal (i.p.) route, it caused a significant increase.

Treatment with the H1 receptor antagonist cetirizine (20 µg/mice; i.c.v.) slightly elevated locomotor activity, whereas treatment with the H2 receptor antagonist ranitidine (20 µg/mice; i.c.v.) led to a slight decrease. Interestingly, the combination of cetirizine or ranitidine with L-histidine (1000 mg/kg; i.p.) resulted in an increase in locomotor activity compared to baseline. The results suggest that histaminergic analogues can modulate locomotor activity in mice, with histamine and L-histidine exerting contrasting effects depending on the dose and route of administration. Moreover, the combination of histamine receptor antagonists with L-histidine appears to influence locomotor activity differently than either compound alone.

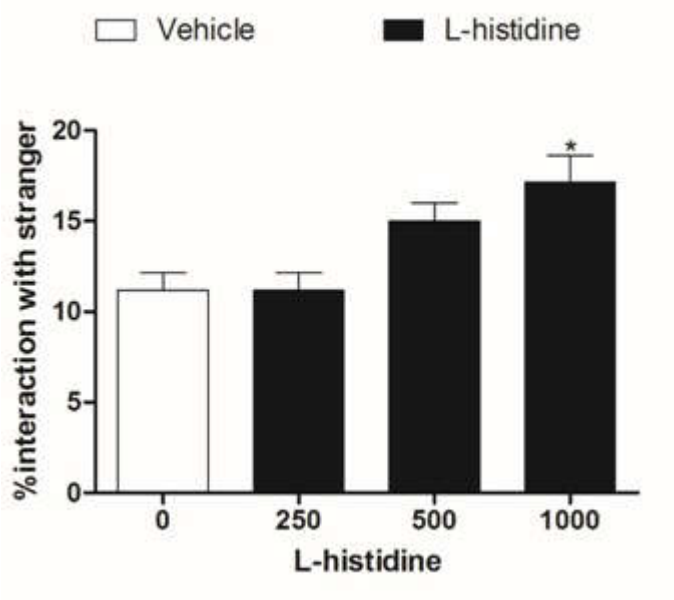

Figure 1: Effects of histamine, histamine precursor; L-histidine on sociability test

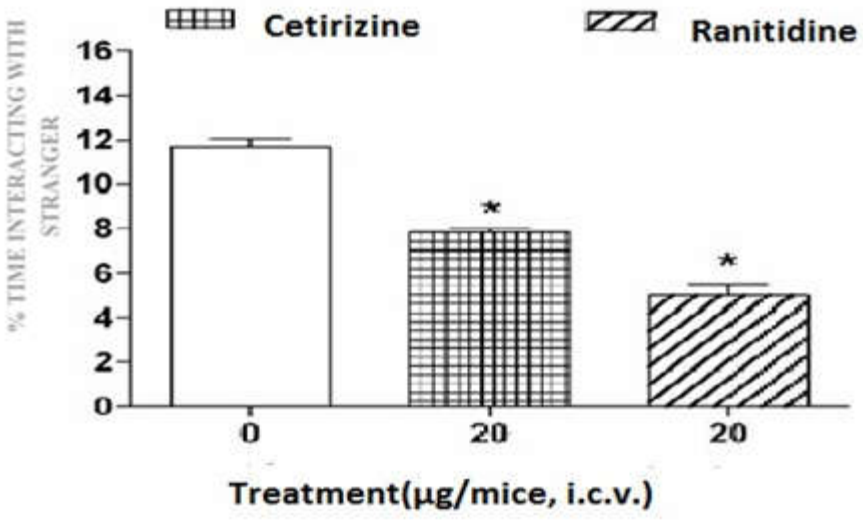

Figure 2: Effects of H1 receptor antagonist and H2 receptor antagonist on sociability test

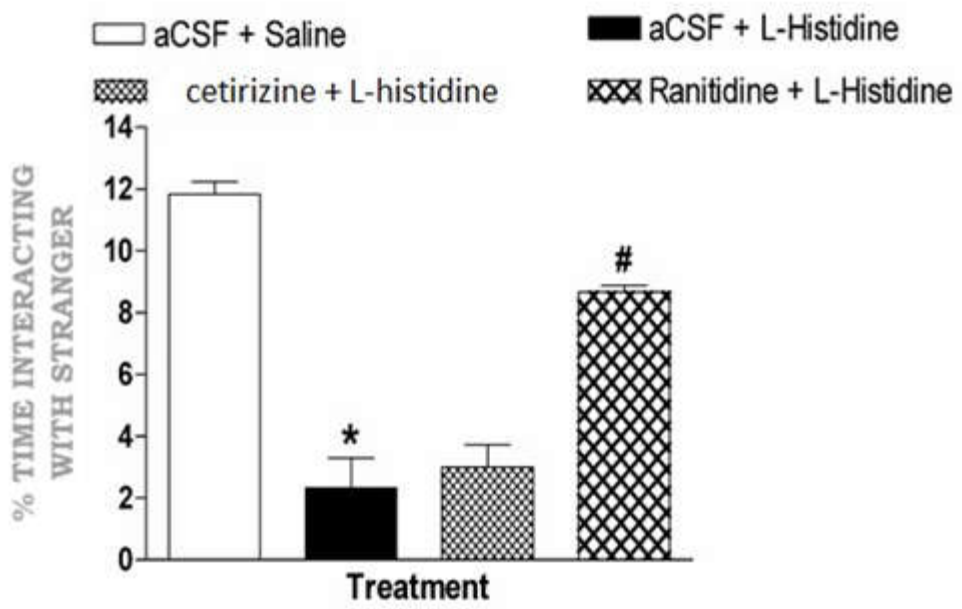

Figure 3: Effects of H1 or H2 receptor antagonist on L-histidine induced decrease in sociability test

Table 1: Effect of histaminergic analogues on locomotor activity

Sr. No.	Treatment	Locomotor activity (15 min)	
		Baseline activity	Reading after treatment
1.	Vehicle (10 mg/kg; i.p.)	285	298
2.	aCSF (5 µg/mice; i.c.v.)	250	267
3.	Histamine (0.5 µg/mice; i.c.v.)	239	257
4.	Histamine (10 µg/mice; i.c.v.)	246	249
5.	L-histidine (10 µg/mice; i.c.v.)	221	243
6.	L-histidine (1000 mg/kg; i.p.)	391	378
7.	Cetirizine (20 µg/mice; i.c.v.)	261	281
8.	Ranitidine (20 µg/mice; i.c.v.)	257	242
9.	Cetirizine (20 µg/mice; i.c.v.) + L-histidine (1000 mg/kg; i.p.)	261	294
10.	Ranitidine (20 µg/mice; i.c.v.) + L-histidine (1000 mg/kg; i.p.)	234	258

CONCLUSION

In conclusion, our study elucidates the significant role of histaminergic transmission in modulating social interaction and locomotor activity in mice. We found that histamine precursor L-histidine enhances sociability, while histamine receptor antagonists, particularly H1 and H2 receptor antagonists, reduce social behavior. Moreover, the combined administration of L-histidine with histamine receptor antagonists alters sociability differently, indicating complex interactions within the histaminergic system. Additionally, histaminergic analogues exert diverse effects on locomotor activity, with implications for understanding motor behavior regulation. Overall, these findings underscore the intricate involvement of histaminergic signaling in regulating both social behavior and locomotion, providing insights into potential therapeutic strategies for conditions involving social dysfunction and motor impairments.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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