Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

Pitolisant disrupts memory consolidation and hippocampal synaptic plasticity: Impaired excitability and long-term potentiation in the CA_1 area

Siamak Beheshti [1]*, Adeleh Badiyifard [1], Zahra Badiei Darani [2], Mansour Azimzadeh [3], Alireza Halabian [1]

[1] Department of Plant and Animal Biology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran
[2] Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
[3] Department of Psychology, Faculty of Arts and Science, University of Toronto, St. George Street, Toronto, Ontario, M5S3G3, Canada

ARTICLE INFO

Article type:
Original

Article history:
Received: Oct 3, 2025
Accepted: Nov 15, 2025

Keywords:
Hippocampus
Long-term potentiation
Memory consolidation
Pitolisant
Synaptic plasticity

ABSTRACT

Objective(s): The histaminergic system plays a vital role in memory function, but the underlying cellular and circuit mechanisms remain poorly understood. Pitolisant, an inverse agonist of the histamine H3 receptor (H3R), is widely used in the treatment of narcolepsy and has shown potential anti-seizure effects. However, its impact on memory and synaptic plasticity is unclear. This study investigated the effects of intracerebroventricular administration of pitolisant on passive avoidance memory consolidation. Meanwhile, the impact of pitolisant on synaptic excitability, short-term plasticity, and long-term potentiation (LTP) in the hippocampus was investigated.

Materials and Methods: Adult male Wistar rats received pitolisant (10 or 100 µg/rat; ICV) or saline immediately after training in the passive avoidance apparatus to test memory consolidation. Meanwhile, pitolisant was microinjected into the CA_1 region to assess its targeted effects. Subsequently, field potentials were recorded to measure fundamental synaptic properties: input-output (I/O) function, paired-pulse facilitation, and LTP function.

Results: Pitolisant significantly impaired memory consolidation. It also reduced the fEPSP slope in I/O function and paired-pulse facilitation, suggesting impaired synaptic excitability and increased presynaptic inhibition. Additionally, LTP was reduced in pitolisant-treated groups, indicating disrupted long-term plasticity.

Conclusion: These findings highlight a potential inhibitory effect of pitolisant on passive-avoidance memory consolidation in healthy rats and on hippocampal synaptic function, raising concerns about its use in neurological disorders.

▶Please cite this article as:
Beheshti S, Badiyifard A, Badiei Darani Z, Azimzadeh M, Halabian AR. Pitolisant disrupts memory consolidation and hippocampal synaptic plasticity: Impaired excitability and long-term potentiation in the CA_1 area. Iran J Basic Med Sci 2026; 29: 378-386. doi: https://dx.doi.org/10.22038/ijbms.2026.90953.19621

Introduction

The central nervous system relies on histamine as a principal neuromodulator for coordinating several physiological functions, including wakefulness, cognition, and synaptic plasticity. The four known histamine receptors (H1R-H4R), all G protein-coupled, mediate histaminergic signaling (1). H1Rs and H2Rs are widely expressed in the brain, including in glial cells and neurons in regions like the amygdala, cortex, and hippocampus. These receptors, located postsynaptically, play a role in functions like alertness and wakefulness. H3R, a pre- and post-synaptic receptor, regulates histamine release (2) and influences other neurotransmitters like GABA, serotonin, and glutamate (3). H4R is found in microglia, but its expression in neurons is uncertain.

Research has shown that histamine plays a role in memory formation. Histamine signaling enhances memory consolidation and retrieval (4). Agonists of the H3R hinder memory consolidation, while antagonists improve it. When H3R agonists were administered before training, passive avoidance and object recognition responses were impaired (5). In the CA_1 region, post-training injection of an H3R agonist disrupted long-term memory consolidation (6). On the other hand, H3R antagonists administered after training improved memory retention in a two-trial delayed comparison model using a Y-maze, a finding blocked by an H2R antagonist (7). However, when H3R agonists and antagonists were injected locally into the basolateral amygdala (BLA), the results were different. An H3R antagonist reduced the conditioned fear response in the BLA when infused post-training (8), while an H3R agonist enhanced the fear response when injected into the BLA (9).

Although the role of histamine receptor agonists and antagonists in memory modulation has been widely studied, the results remain controversial, and the underlying cellular and circuit mechanisms remain poorly understood. Histamine H3Rs exhibit constitutive activity, allowing them to signal spontaneously in the absence of a ligand. Inverse agonists block this basal activity, thereby altering

***Corresponding author:** Siamak Beheshti. Department of Plant and Animal Biology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran. Tel: +98-31-37932458, +98- 31-37936127, Email: s.beheshti@sci.ui.ac.ir, siamak.beheshti@yahoo.com

histaminergic signaling. Pitolisant, an inverse agonist of H3Rs, is currently used to treat narcolepsy (10) and has also demonstrated potential anti-seizure properties (11). Given the histaminergic system's influence on memory performance, this study aimed to investigate the effects of intracerebroventricular (ICV) administration of pitolisant on passive avoidance memory consolidation. Meanwhile, to assess the underlying circuit mechanisms, the impact of intra-CA_1 pitolisant injection on synaptic excitability, short-term plasticity, and long-term potentiation (LTP) induction were examined in the hippocampus. By evaluating input-output (I/O) function, paired-pulse facilitation, and LTP, we aimed to clarify the role of H3R inverse agonism in hippocampal function and its potential implications for cognitive processes.

Materials and Methods

Drugs

Anesthesia was induced with ketamine (100 mg/kg) and xylazine (10 mg/kg) (Alfasan, Netherlands) in behavioral experiments. Urethane (1.5 g/kg) was used as the anesthetic (Exir; Austria) in electrophysiological studies. Pitolisant (Sigma, United States) was prepared in a saline solution.

Animals

Thirty-six male Wistar rats, weighing 301 ± 20 g and 12 weeks old, were used in the study (18 for behavioral and 18 for electrophysiological studies). The rats were procured from the Animal Breeding Center of Isfahan University of Medical Sciences (Isfahan, Iran) and maintained under standard laboratory conditions in compliance with ethical guidelines established by the Iran National Committee for Ethics in Biomedical Research. All experimental procedures received ethical approval from the Institutional Animal Care and Use Committee at the University of Isfahan (IR.UI.REC.1399.078). Animals were maintained in standard polycarbonate cages (42 cm x 26.5 cm x 15 cm) with free access to rodent chow and water throughout the study period, in a facility with a stable temperature of 22 ± 2 °C and a 12-hr light/dark cycle starting at 7 AM.

Experimental procedure

The rats were randomly assigned to two experimental groups. Each group was divided into three subgroups to receive either saline (n=6) or pitolisant (10 µg/rat, 100 µg/rat; n=6 per subgroup) into the right lateral ventricles (for behavioral studies) or hippocampal CA_1 area (for electrophysiological studies). The dose of pitolisant was chosen based on a previous study [9]. Intracerebroventricular injections were administered using a 27-gauge injection needle inserted through an implanted guide cannula. The needle was attached via polyethylene tubing (PE-20, Stoelting) to a 5 µl Hamilton microsyringe. A total volume of 5 µl was infused slowly at 0.5 µl/min.

In electrophysiological studies, the injections were administered using the stereotaxic setup (Stoelting Co., USA). Intra-CA_1 injections were delivered in a volume of 1 µl at a rate of 0.5 µl per minute. After completing the injections, the needles remained in place for an additional minute before being carefully withdrawn.

Assessment of passive avoidance memory consolidation

One week before behavioral testing, rats underwent stereotaxic surgery under anesthesia induced by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). A 22-gauge guide cannula was implanted 1 mm above the right lateral ventricle using the following coordinates relative to bregma: anterior-posterior -0.9 mm, medio-lateral ±1.4 mm, and dorso-ventral -2.5 mm (Paxinos & Watson, 2007). Two skull screws were placed for stabilization, and doxycycline powder was applied topically to prevent infection before securing the cannula with dental cement. To maintain patency, the cannula was occluded with a mineral-oil-coated 27-gauge stainless steel stylet to avoid blood clot obstruction.

The step-through passive avoidance test was conducted by the method outlined previously (12). Initially, each rat was positioned in the white starting room of the apparatus, facing the sliding door. After a five-second delay, the door was lifted, allowing the rat to move into the adjacent black room. Once all four paws entered the dark room, the door closed, and the rat remained there for 20 sec before being transferred to a holding cage. Thirty minutes later, the procedure was repeated: the rat was returned to the white room for five seconds, and upon entering the black room, it received a 1 mA foot shock for two seconds. After another 20-second interval, the rat was returned to the holding cage. Two minutes later, the test was repeated, with a shock administered each time the rat fully entered the dark room. Training ended when the rat stayed in the white compartment for two minutes without crossing. All animals learned the task within a maximum of two trials. Drug injections were done immediately after training.

The following day, a retrieval test was administered to assess long-term memory. Each rat was placed in the white room for 20 sec before the door opened. Step-through latency (STL) and time spent in the dark compartment (TDC) were recorded over 600 sec.

In vivo electrophysiological field potential recordings

Electrophysiological studies were conducted after the drug injections. Extracellular field potential recordings were performed following established protocols adapted from our prior research (13, 14). The rats were anesthetized with urethane (1.5 g/kg, IP). A bipolar stainless steel electrode (Teflon insulated: Model 791500, A-M Systems Inc., USA) was implanted in the right hippocampal Schaffer collateral pathway for stimulation using the Paxinos and Watson atlas (15) coordinates as follows: anteroposterior (AP) = -4.2 mm, mediolateral (ML) = 3.8 mm, and dorsoventral (DV) = -2.7 to -3.8 mm. Extracellular field potentials from CA_1 pyramidal neurons were recorded using a Teflon-coated stainless-steel monopolar electrode inserted at a 52.5° angle to target the right CA_1 region from the upper left. The recording coordinates were AP = -3.4 mm, ML = 1.5 mm, and DV = -4.4 to -5.1 mm. These placements were optimized to maximize field excitatory postsynaptic potential (fEPSP) responses.

The fEPSP slope and amplitude were selected as primary measures of synaptic plasticity. Field potentials were evoked at 0.1 Hz in the CA_1 region, amplified 1,000×, and filtered with a 1–3 kHz band-pass filter. Data were acquired using eProbe software (Science Beam, Tehran, Iran) and analyzed with eTrace analysis software (Science Beam, Tehran, Iran).

Before inducing LTP, input-output (I/O) curves (stimulus-response functions) were generated by varying the stimulus current between 100 and 1000 µA. Once the I/O curve was established, the stimulus intensity was adjusted to evoke ~ 50% of the maximum fEPSP slope. A 30-min stable baseline was recorded.

In the paired-pulse facilitation (PPF) protocol, the stimulus intensity was set to 50% of the maximum intensity. Pairs of stimuli were delivered with a 50 ms inter-stimulus interval.

LTP was induced using a high-frequency stimulation (HFS) protocol consisting of four bursts of 25 pulses (100 Hz, 0.15 ms pulse duration) separated by 10-second intervals. LTP magnitude was quantified by analyzing changes in fEPSP slope and amplitude, expressed as a percentage of the pre-stimulation baseline, and monitored for 90 min post-tetanus to assess synaptic changes in CA_1 pyramidal neurons.

Statistical analysis

Normality of data distribution was evaluated using both the Shapiro-Wilk and Kolmogorov-Smirnov goodness-of-fit tests. Behavioral parameters were statistically evaluated using one-way ANOVA with Tukey's honest significant difference (HSD) *post hoc* test for multiple comparisons between groups. Electrophysiological data were analyzed using repeated-measures two-way ANOVA, followed by Tukey's *post hoc* test for pairwise comparisons. Statistical analyses were conducted using SPSS (version 21), while GraphPad Prism (version 9.0.1) was used for data visualization. Results are presented as mean ± SEM. A p-value of less than 0.05 was considered statistically significant. When the assumption of sphericity was violated, the Greenhouse-Geisser correction was applied, as indicated in the text.

Results

The effect of pitolisant on memory consolidation

The analysis revealed significant group differences in step-through latency (STL) following post-training treatments [F(2, 15) = 8.71, *P*= 0.004]. *Post-hoc* tests demonstrated that pitolisant (10 and 100 µg) significantly reduced STL compared to the control group (Figure1 (A); *P*<0.05).

Significant treatment effects were also observed for time in dark compartment (TDC) [F(2, 15) = 4.81, *P*= 0.02]. Specifically, pitolisant (100 µg) significantly increased TDC versus the control group (Figure 1 (B); *P*<0.05).

Input-output (I/O) functions

A two-way ANOVA with repeated measures revealed a significant increase in the I/O curve for fEPSP amplitude in the saline group (F(1.3, 6.54) = 9.69, *P*= 0.01) and the pitolisant 10 µg group (F(1.33, 6.67) = 12.43, *P*=0.008), while no significant change was observed in the pitolisant 100 µg group (*P*>0.05).

For fEPSP slope, significant differences were found in all groups: saline (F(1.37, 6.88) = 5.69, *P*= 0.04), pitolisant 10 µg (F(1.26, 6.34) = 18.92, *P*= 0.003), and pitolisant 100 µg (F(1.27, 6.35) = 5.71, *P*= 0.04). These results were analyzed using a two-way ANOVA with repeated measures and Greenhouse-Geisser correction.

Analysis of within-subject effects for fEPSP amplitude showed a significant impact of intensity (F(9, 135) = 18.87, *P*<0.001) and intensity × treatment interaction (F(18, **135)** = 3.07, *P*<0.001). Similarly, for fEPSP slope, intensity (F(9, 135) = 15.13, *P*<0.001) and intensity × treatment interaction (F(18, 135) = 1.86, *P*= 0.02) were significant. These analyses were performed using a two-way ANOVA with repeated measures, assuming sphericity.

Between-subject effects were also significant for both fEPSP amplitude (F(2, 15) = 13.12, *P*= 0.001) and fEPSP slope (F(2, 15) = 12.59, P = 0.001).

Post hoc comparisons for fEPSP amplitude revealed significant differences between the saline and pitolisant 10 µg groups (*P*<0.003) and between the saline and pitolisant 100 µg groups (*P*<0.001). Similarly, *post hoc* analysis for fEPSP slope indicated significant differences between the saline and pitolisant 10 µg groups (*P*<0.006) and between the saline and pitolisant 100 µg groups (*P*<0.001) (Tukey's multiple comparison test).

The significant decrease in I/O function in both 10 µg and 100 µg pitolisant groups suggests a reduction in synaptic excitability within CA_1 circuits. Since I/O curves measure the relationship between stimulation intensity and evoked synaptic responses, this result indicates that pitolisant reduces the overall responsiveness of CA_1 pyramidal neurons to Schaffer collateral stimulation. One explanation is that pitolisant enhances inhibitory control, dampening excitatory transmission (Figure 2).

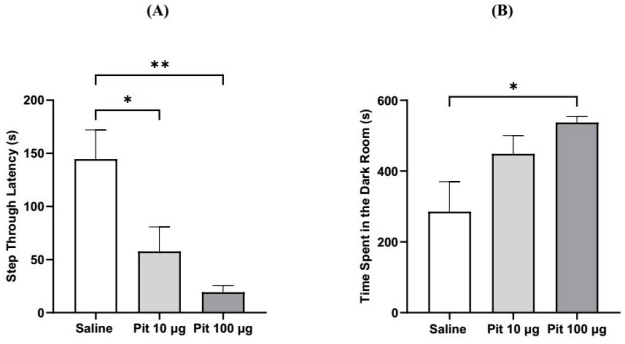

Figure 1. The effect of pitolisant on fear memory consolidation in rats
Pitolisant was administered post-training to assess memory consolidation in the passive avoidance task. Pitolisant decreased step-through latency (A) and increased time spent in the dark room (B), which indicated memory impairment. Data are presented as mean ± SEM and analyzed using ANOVA with Tukey's *post hoc* test. **P*<0.05; ***P*<0.01. Pit: Pitolisant.

Paired-pulse facilitation

In field potential recordings, paired-pulse facilitation (PPF) refers to an increase in the amplitude of the second evoked response when two stimuli are delivered in close succession. This effect is primarily attributed to residual calcium in the presynaptic terminal after the first pulse, which enhances neurotransmitter release during the second pulse. PPF is commonly used as an indicator of short-term synaptic plasticity. To assess PPF, paired-pulse responses were recorded at a 50 ms interstimulus interval (PP50). The facilitation effect was quantified by expressing the amplitude difference between the second and first responses as a percentage of the first pulse amplitude.

Pitolisant significantly reduced the fEPSP slope index compared to the saline group (F(2, 12) = 6.77, *P*= 0.01) (one-way ANOVA, Tukey's multiple comparison test).

The reduction in PPF at 50 ms suggests a shift toward paired-pulse inhibition, which is typically associated with increased presynaptic GABAergic inhibition or reduced excitatory neurotransmitter release. Since PPF at 50 ms usually reflects short-term facilitation due to residual calcium accumulation, the observed decrease suggests that pitolisant might have enhanced presynaptic inhibition, thereby reducing glutamate release from Schaffer collateral terminals (Figure 3).

Long-term potentiation (LTP)

LTP induction was observed in all groups when measured by the slope of the fEPSP: saline: F(2.11, 10.57) = 9.18, *P*= 0.005; pitolisant 10 µg: F(1.73, 8.66) = 5.96, *P*= 0.02; pitolisant 100 µg: F(2.9, 14.51) = 3.79, *P*=0.03. However, when assessing fEPSP amplitude, significant potentiation was only observed in the saline group: F(1.61, 8.06) = 23.03, *P*= 0.001. This suggests that although synaptic efficacy was enhanced across conditions, the magnitude of amplitude potentiation was more variable and may reflect differences

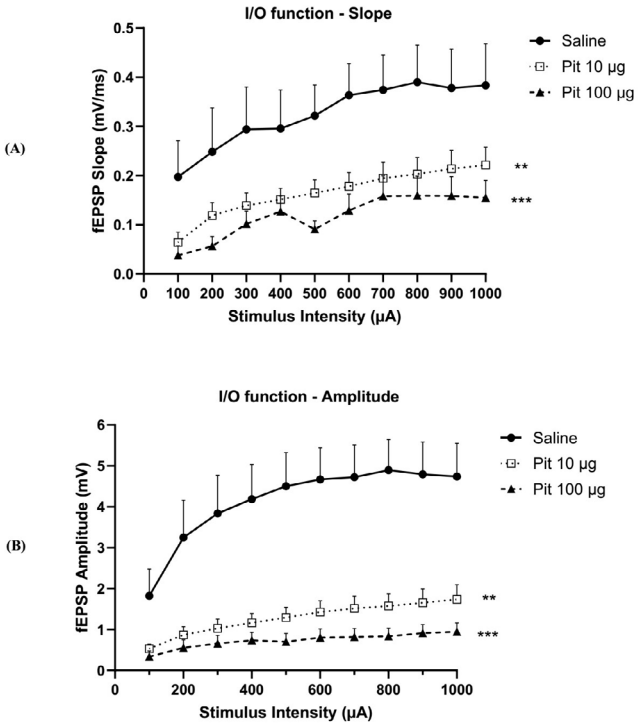

Figure 2. Input-output (I/O) curves of fEPSP slope (A) and amplitude (B) in the hippocampal CA_1 region of rat saline and pitolisant-treated groups (10 and 100 µg/rat, n=6 per group).
I/O curves assess synaptic excitability by measuring the relationship between stimulation intensity and evoked synaptic responses. The fEPSP slope reflects synaptic strength at the apical dendritic layer, while amplitude represents overall signal magnitude. Pitolisant administration significantly reduced fEPSP slope and amplitude, suggesting decreased synaptic excitability. Data are presented as mean ± SEM and analyzed using ANOVA with Tukey's *post hoc* test. $^{**}P<0.01$; $^{***}P<0.001$. CA1: Cornu ammonis; fEPSP: Field excitatory post synaptic potential; Pit: Pitolisant

in underlying mechanisms or recording conditions. All analyses were conducted using two-way ANOVA with repeated measures and Greenhouse-Geisser correction.

Within-subjects analysis revealed no significant effects of time or the time × treatment interaction on LTP maintenance, as measured by fEPSP slope ($P>0.05$, two-way ANOVA with repeated measures; sphericity assumed; correction for multiple comparisons). However, between-subjects analysis showed a significant effect of treatment ($F(2, 15) = 5.55$, $P = 0.01$). In contrast, fEPSP amplitude exhibited a significant effect of time ($F(8, 120) = 11.69$, $P<0.001$), but no significant between-subjects effect ($P>0.05$, two-way ANOVA, repeated measures, Sphericity Assumed correction).

Post hoc analysis using Tukey's multiple comparisons test revealed that the mean potentiation of fEPSP slope was significantly higher in the saline group compared to both the pitolisant 10 µg group (145.7% vs. 116%, $P= 0.04$) and the pitolisant 100 µg group (145.7% vs. 111.9%, $P= 0.02$). In contrast, the mean potentiation of fEPSP amplitude did not differ significantly between groups ($P>0.05$) (Figure 4).

The decrease in LTP strength after pitolisant administration indicates a disruption in excitatory synaptic plasticity. This could be due to several factors, such as reduced release of excitatory neurotransmitters, heightened activity of inhibitory interneurons hindering sustained depolarization needed for LTP, and changes in dopamine and acetylcholine signaling influenced by histaminergic modulation, all of which are crucial for hippocampal

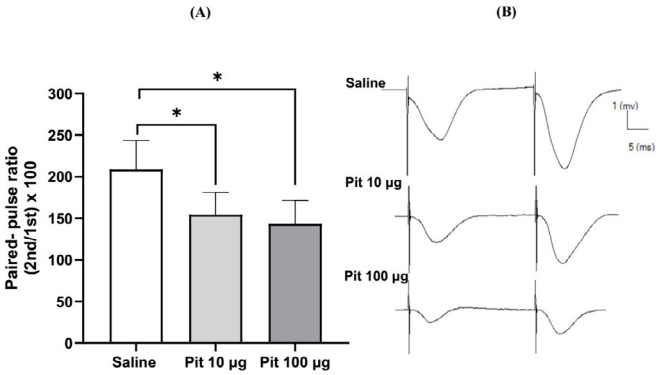

Figure 3. The effect of pitolisant on paired-pulse facilitation (PPF) in the rat hippocampal CA_1 region
(A) PPF ratios for saline and pitolisant-treated groups (10 and 100 µg/rat, n=6 per group), showing a reduction in facilitation at a 50 ms inter-stimulus interval. (B) Representative traces of fEPSPs under the three experimental conditions. Data are presented as mean ± SEM and analyzed using ANOVA with Tukey's *post hoc* test ($P<0.05$ considered significant). Pit: Pitolisant. CA1; Cornu ammonis; fEPSP: Field excitatory post synaptic potential; PPF: Paired-pulse facilitation

synaptic plasticity.

Discussion

Intracerebroventricular administration of pitolisant significantly disrupted memory consolidation, as evidenced by impaired performance in the passive avoidance paradigm. Pitolisant is a selective histamine H3R inverse agonist/antagonist, which enhances histaminergic and, to some extent, noradrenergic, dopaminergic, glutamatergic, serotonergic, GABAergic, and cholinergic neurotransmission by blocking presynaptic H3 autoreceptors and heteroreceptors (3, 16). Multiple preclinical studies have shown that pitolisant, when administered to healthy rodents before or immediately after a learning task, can impair the consolidation of hippocampal-dependent memories. A classic example is contextual fear conditioning, where an animal learns to associate an environment with a foot shock (17, 18). However, other H3R antagonists, like ABT-239 (19) or ciproxifan (20), often enhance memory consolidation in the same tasks.

While histamine may be procognitive at moderate levels, excessive histamine induced by pitolisant can activate postsynaptic H1 and H2 receptors to a degree that disrupts the delicate electrophysiological processes required for memory consolidation. Meanwhile, over-activation of H1/H2 receptors can disrupt hippocampal theta rhythms and long-term potentiation (LTP) (21), which is critical for memory consolidation. Pitolisant's impairment in contextual fear conditioning (a hippocampal task) but not in cued fear conditioning (an amygdala-dependent task) supports the region-specific effect (22). Pitolisant also exhibits appreciable affinity for the 5-HT2A serotonin receptor and the dopamine transporter (DAT) (23). In a healthy brain with optimal baseline monoamine levels, perturbing these systems during the critical consolidation window could introduce "noise" that interferes with the precise synaptic strengthening required. Pitolisant's interactions with 5-HT2A and DAT may contribute to its unique effects relative to more selective compounds such as ABT-239 or GSK-189254 (24). In contrast to the impairment of memory consolidation by pitolisant observed in our study, post-training injection of an H3R agonist into the CA_1 region disrupted long-term memory consolidation

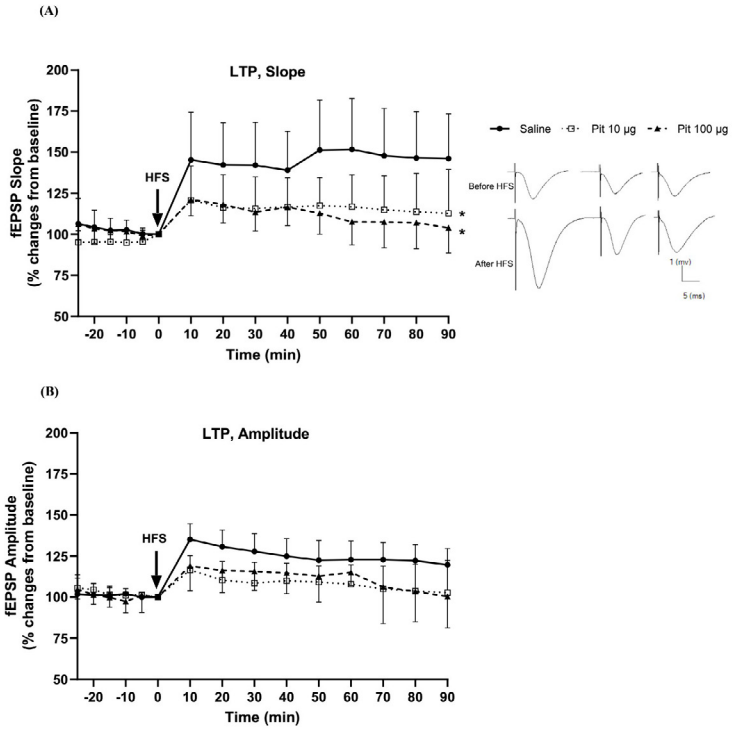

Figure 4. The effects of pitolisant (10 and 100 µg/rat) on LTP induction in the rat CA_1 area
(A) Changes in the fEPSP slope, expressed as percentages of the baseline response, and representative traces of typically recorded fEPSPs in hippocampal CA1 neurons before and after high-frequency stimulation (HFS) induction for LTP in all experimental groups. (B) Changes in the fEPSP amplitude, expressed as percentages of the baseline response. Data are presented as means ± SEM (ANOVA, Tukey's *post hoc* test). *P<0.05. fEPSP: Field excitatory postsynaptic potential; HFS: High-frequency stimulation; LTP: Long-term potentiation; Pit: Pitolisant; CA1: Cornu ammonis 1

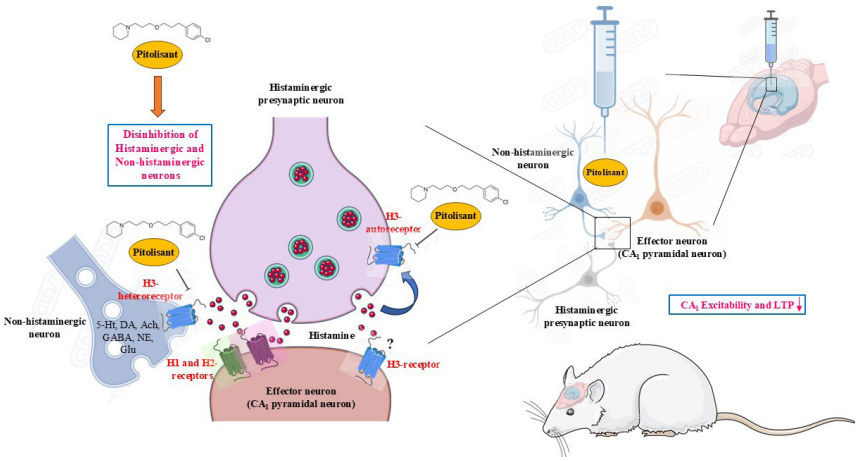

Figure 5. A graphical diagram illustrating the proposed effects of pitolisant for shifting the excitation-inhibition balance in rat CA_1
Excessive histamine resulting from pitolisant's effect on H3 Autoreceptors can disrupt CA1 synaptic excitability and long-term potentiation (LTP). Pitolisant acting on H3-heteroreceptors may also exacerbate the enhancement of GABAergic tone by urethane in the hippocampus, preventing LTP induction. CA1: Cornu ammonis; GABA: Gamma amino butyric acid; LTP: Long-term potentiation.

(6). The fact that both manipulations cause the same impairment suggests that the histaminergic system's role in memory consolidation is not a simple "on/off" switch but a tightly regulated, balanced process. The H3R, acting as a heteroreceptor on non-histaminergic neurons, inhibits the release of other critical neurotransmitters. An H3R agonist would inhibit the release of all these neurotransmitters. The memory impairment could be due to a combined deficit in ACh, NE, and DA, rather than solely to a lack of histamine. Pitolisant, as an H3R inverse agonist/antagonist, would disinhibit the release of all these neurotransmitters simultaneously. The resulting flood of ACh, NE, DA, and 5-HT, in addition to histamine, could create a chaotic neurochemical environment. For example, excessive ACh can lead to receptor desensitization, while high NE can induce stress-like states that impair hippocampal function. The impairment caused by pitolisant may thus be due to an imbalance in these other systems, thereby overriding the beneficial effect of increased histamine. Understanding why pitolisant can impair memory, while others do not, can guide the development of next-generation H3R ligands. The goal is to design compounds that provide a more balanced disinhibition, thereby avoiding the potential for hippocampal overactivation observed with pitolisant.

To assess the probable mechanisms by which pitolisant impairs memory consolidation, we examined its effects

on synaptic plasticity in the hippocampus. Pitolisant was injected directly into the hippocampal CA_1 region to evaluate its targeted action. Our results showed that intra-CA_1 administration of pitolisant significantly reduced I/O function, indicating decreased synaptic excitability. Since I/O curves measure the relationship between stimulus intensity and the resulting fEPSP, a downward shift suggests that CA_1 pyramidal neurons exhibited weaker responses to Schaffer collateral stimulation. This finding aligns with previous studies demonstrating that H3R antihistamines, including inverse agonists, modulate hippocampal excitability by influencing presynaptic neurotransmitter release and postsynaptic responsiveness. Specifically, blockade of H3 receptors enhances the release of histamine, dopamine, serotonin, norepinephrine, and GABA, thereby altering synaptic dynamics (25). The observed reduction in I/O function suggests that pitolisant might have enhanced inhibitory tone, thereby limiting excitatory transmission within CA_1 circuits.

Histamine affects neuronal excitability and seizure susceptibility in both animals and humans (26). It induces a long-term increase in the excitability of hippocampal CA_1 pyramidal cells and the dentate gyrus (27, 28). These outcomes suggest that the histaminergic system contributes to hippocampal synaptic excitation via H3R. However, one study showed that selective activation of the H3R by R-α-methylhistamine did not change excitatory or inhibitory postsynaptic currents, or cellular excitability (29). Conversely, intracerebroventricular administration of the H3R agonist R-α-methylhistamine significantly attenuated both population excitatory postsynaptic potentials (pEPSPs) and population spikes, while H3R antagonists (clobenpropit and thioperamide) enhanced both synaptic responses. Pitolisant negatively impacted CA_1 neuronal excitability, suggesting that H3R's histamine-independent signaling is important for modulating neuronal excitability (30).

In addition to decreased I/O function, we observed a reduction in paired-pulse facilitation (PPF) at 50 ms, which reflects paired-pulse inhibition. PPF is often used to assess short-term synaptic plasticity and the functional state of presynaptic mechanisms, such as neurotransmitter release probability. Normally, PPF at this interval is attributed to residual calcium accumulation, facilitating neurotransmitter release during the second stimulus. It can also provide insights into the health of synaptic connections and how they are modulated by factors like neuromodulators, brain states, or pathological conditions (31). The fact that pitolisant decreased the likelihood of glutamate release in the CA_1 area indicates that the presynaptic H3Rs might have been affected. The observed reduction also suggests that pitolisant might have enhanced presynaptic inhibition, potentially through increased GABAergic interneuron activity. Given that H3Rs are expressed on both excitatory and inhibitory terminals, pitolisant's inverse agonist properties might have preferentially enhanced inhibitory signaling, thereby dampening excitatory synaptic responses.

Previous studies have shown that histaminergic modulation affects both glutamate and GABA release in the hippocampus (32). The shift toward paired-pulse inhibition in our study supports the hypothesis that pitolisant increases inhibitory control over excitatory circuits, which could explain the accompanying reductions in I/O function and synaptic plasticity.

We also indicated that injecting pitolisant into the CA_1 area dose-dependently impaired LTP induction. LTP is a lasting increase in the efficacy of excitatory synaptic transmission, commonly observed in the hippocampus as a form of synaptic plasticity. Histamine is thought to enhance memory consolidation through altering synaptic plasticity, a key mechanism in learning and memory (33). One study showed that isoflurane, a general anesthetic, reduced memory performance in object recognition and passive avoidance tests and hippocampal LTP, whereas ciproxifan, a histamine H3R antagonist, reversed this effect (34). The observed reduction in LTP magnitude following pitolisant treatment suggests that histaminergic modulation influences synaptic plasticity in CA_1. LTP induction requires a balance between excitatory and inhibitory inputs, with excessive inhibition preventing the sustained depolarization necessary for NMDA receptor activation and long-term synaptic strengthening. The paired-pulse inhibition observed in our study suggests that increased GABAergic activity may be a contributing factor to the observed LTP deficits.

Clobenpropit, an H3R antagonist, reduced NMDA-induced neuronal toxicity in cultured cortical neurons by promoting GABA release via the cAMP/PKA signaling cascade (35). Thioperamide, a noncompetitive antagonist of 5-HT3 receptors, increased serotonin (5-HT) levels in the prefrontal cortex (36). The administration of H3R agonists and antagonists in the basolateral amygdala (BLA) has been shown to enhance or reduce acetylcholine release in the BLA, respectively (8, 9). Studies have demonstrated the essential role of muscarinic receptor activation in the amygdala in declarative memory consolidation (37, 38), which suggests that the interaction between histamine and acetylcholine in the BLA may regulate memory consolidation. Additionally, H3R blockade has been shown to affect neuromodulatory systems, including dopamine and acetylcholine, both of which play essential roles in hippocampal plasticity (39, 40). Thus, pitolisant's effects on LTP may be mediated by its influence on other neurotransmitter systems and broader network interactions rather than by direct effects on CA_1 synapses alone.

While H3Rs are primarily located presynaptically, they also occur postsynaptically. However, research on postsynaptic H3Rs is less extensive than that on presynaptic H3Rs (41), and the role of postsynaptic H3Rs in memory formation remains unclear.

It is essential to recognize that the results in the present study were obtained from healthy animals. Studies conducted in animals with histaminergic system deficiencies might yield different results. For example, administration of ciproxifan, an H3R antagonist, potently prevented the deleterious effects of chronic restraint stress when administered before learning, immediately after learning, or before retrieval, on both recognition and passive avoidance behavior (42). Meanwhile, H3R inverse agonism can enhance LTP in affected animals but disrupt it in healthy controls. For example, one study observed that H3R inverse agonism reduced I/O curve responses and numerically reduced LTP in health control animals (31), whereas another study observed improved LTP in ethanol-exposed animals that displayed LTP deficiencies (43). In other pathological models like Alzheimer's disease, schizophrenia, and attention deficit hyperactivity disorder (ADHD), where the brain is

neurochemically deficient, pitolisant consistently restores cognitive function and enhances memory consolidation (44). These conditions are characterized by reductions in the very neurotransmitters (histamine, acetylcholine, and dopamine) that H3R antagonists aim to boost. The same dose that impairs a healthy brain improves a diseased one. This occurs because the overactivation observed in a healthy brain is not possible in a pathological brain, as the system is already underactive. Pitolisant's disinhibition normalizes the system, returning it from a state of deficiency toward homeostasis. Therefore, it is worth noting that the results of the present study do not mean that H3R inverse agonism broadly leads to general impairments in synaptic plasticity and synaptic transmission.

The discussion around pitolisant's impairing effects on memory consolidation and synaptic plasticity is a powerful reminder that neuropharmacology is not one-size-fits-all. The impact of pitolisant on cognition is state-dependent. Its ability to potently increase histamine can disrupt the finely tuned hippocampal processes in a healthy brain, leading to consolidation impairments that are not always observed with other, more selective H3R ligands. However, in the context of a pathological brain with inherent neuromodulatory deficits, this same mechanism becomes therapeutically beneficial, restoring cognitive function by bringing a hypoactive system back into balance. This dichotomy underscores the importance of evaluating cognitive drugs not only in disease models but also in healthy systems to understand their potential risks and mechanisms of action fully.

There might be another explanation for the impairing effect of pitolisant on LTP. Urethane is known to potentiate the action of the inhibitory neurotransmitter GABA at $GABA_A$ receptors (45). The tuberomammillary nucleus (TMN) histaminergic neurons are under potent GABAergic inhibition (46). By enhancing this inhibitory tone, urethane effectively suppresses the activity of the brain's histaminergic system. This suppression is a primary reason why urethane anesthesia mimics natural sleep states (particularly slow-wave sleep) so effectively. Reduced firing of histaminergic neurons results in decreased histamine release in projection areas such as the cortex, hippocampus, and thalamus. The suppression of this key "wake-on" system is central to the state of unconsciousness induced by urethane.

In a normal, awake brain, pitolisant increases histamine release (by blocking autoreceptors) and also increases ACh, NE, DA, and glutamate in certain areas (by blocking heteroreceptors) (16). This global boost in pro-wakefulness neuromodulators may enhance cognitive function and facilitate plasticity. However, under urethane anesthesia, the histaminergic neurons themselves are silenced by urethane's enhancement of GABAergic inhibition. Therefore, there is little to no histamine for pitolisant to release from the somata. The effect of pitolisant is no longer a net increase in neurotransmitter release, but rather the removal of a critical inhibitory brake (the H3R) on a system that is already being powerfully driven into a slow-wave state by urethane. Pitolisant could exacerbate the enhancement of GABAergic tone by urethane in the hippocampus, leading to such powerful inhibition that the postsynaptic neuron cannot depolarize sufficiently to unblock the NMDA receptors, thus completely preventing LTP induction.

Although histamine release is minimal, pitolisant may still cause a slight, unregulated increase in ACh or NE due to residual tonic firing in their neurons. In an awake brain, this is beneficial. In a deeply anesthetized brain, a sudden, unphased "burst" of ACh or NE without the coordinated activity of other systems (like histamine) could be disruptive. It may trigger aberrant signaling pathways that interfere with, rather than facilitate, LTP induction. Therefore, the effects of a drug targeting a neuromodulatory system can be inverted or altered by the brain's background state (e.g., awake vs. anesthetized). A drug like pitolisant that might be cognitive-enhancing in a normal subject can become disruptive when an anesthetic like urethane has changed the fundamental operating mode of the brain. Therefore, data on plasticity and neuromodulation obtained under anesthesia must be interpreted with extreme caution and should ideally be validated in awake, behaving models.

The observed suppression of synaptic plasticity by pitolisant raises important considerations for its clinical use. While the drug is primarily used for treating narcolepsy, its broader neuromodulatory effects warrant further investigation, particularly in conditions where cognitive function is a concern. Future studies should examine the dose-dependent effects of pitolisant on hippocampal plasticity and its impact on other brain regions involved in learning and memory. The H3R knockout models could also provide valuable insights into the role of histamine receptors in regulating synaptic plasticity.

Finally, the generalizability of our findings is constrained by the limited sample size. Additionally, the open-label design, without blinding of outcome assessors, introduces the potential for bias, which must be acknowledged when interpreting the results.

Conclusion

This study demonstrates that H3R blockade by pitolisant, an H3R inverse agonist, impairs passive avoidance memory consolidation, reduces synaptic excitability, impairs short-term plasticity, and disrupts LTP in the hippocampal CA_1 area of healthy rats. These findings suggest that H3R modulation plays a crucial role in memory consolidation by maintaining a balance among excitation, inhibition, and synaptic strength. Understanding the broader impact of pitolisant on neural plasticity is important for evaluating its cognitive and neurological effects, considering its clinical use.

Acknowledgment

The results presented in this paper were taken from master's thesis of Adeleh Badiyifard and Zahra Badiei Darani, which were funded by the University of Isfahan, Iran, and Zahra Badiei Darani.

Authors' Contributions

S B was responsible for study design, conceptualization, supervision, formal analysis, methodology, writing the original draft, review, editing, and final approval of the version to be published. A B performed electrophysiological experiments and collected data. Z BD performed behavioral experiments. M A analyzed the electrophysiological data, reviewed and edited the manuscript, and contributed to methodology. A H contributed to the methodology.

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

Declaration

We have not used any AI tools or technologies to prepare this manuscript. Figure 5 was prepared using the BioGDP website (BioGDP.com) (47).

References

1. Panula P. Histamine receptors, agonists, and antagonists in health and disease. Handb Clin Neurol 2021; 180:377-387.
2. Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. Nature 1983; 302:832-837.
3. Passani MB, Blandina P. Histamine receptors in the CNS as targets for therapeutic intervention. Trends Pharmacol Sci 2011; 32:242-249.
4. Nomura H, Shimizume R, Ikegaya Y. Histamine: A key neuromodulator of memory consolidation and retrieval. Curr Top Behav Neurosci 2022; 59:329-353.
5. Blandina P, Giorgetti M, Bartolini L, Cecchi M, Timmerman H, Leurs R, *et al*. Inhibition of cortical acetylcholine release and cognitive performance by histamine H3 receptor activation in rats. Br J Pharmacol 1996; 119:1656-1664.
6. da Silveira CK, Furini CR, Benetti F, Monteiro Sda C, Izquierdo I. The role of histamine receptors in the consolidation of object recognition memory. Neurobiol Learn Mem 2013; 103:64-71.
7. Orsetti M, Ghi P, Di Carlo G. Histamine H(3)-receptor antagonism improves memory retention and reverses the cognitive deficit induced by scopolamine in a two-trial place recognition task. Behav Brain Res 2001; 124:235-242.
8. Passani MB, Cangioli I, Baldi E, Bucherelli C, Mannaioni PF, Blandina P. Histamine H3 receptor-mediated impairment of contextual fear conditioning and *in-vivo* inhibition of cholinergic transmission in the rat basolateral amygdala. Eur J Neurosci 2001; 14:1522-1532.
9. Cangioli I, Baldi E, Mannaioni PF, Bucherelli C, Blandina P, Passani MB. Activation of histaminergic H3 receptors in the rat basolateral amygdala improves expression of fear memory and enhances acetylcholine release. Eur J Neurosci 2002; 16:521-528.
10. Lamb YN. Pitolisant: A review in narcolepsy with or without cataplexy. CNS Drugs 2020; 34:207-218.
11. Beheshti S, Wesal MW. Anticonvulsant activity of the histamine H3 receptor inverse agonist pitolisant in an electrical kindling model of epilepsy. Neurosci Lett 2022; 782:136685.
12. Beheshti S, Shahrokhi S. Blocking the ghrelin receptor type 1a in the rat brain impairs memory encoding. Neuropeptides 2015; 52:97-102.
13. Azimzadeh M, Mohd Azmi MAN, Reisi P, Cheah PS, Ling KH. Step-by-step approach: Stereotaxic surgery for *in vivo* extracellular field potential recording at the rat Schaffer collateral-CA1 synapse using the eLab system. MethodsX 2024; 12:102544.
14. Azimzadeh M, Noorbakhshnia M. Maternal linalool treatment protects against radiofrequency wave-induced deteriorations in adolescent rats: A behavioral and electrophysiological study. Sci Rep 2024; 14:17257-17271.
15.Paxinos G, Watson C. The rat brain in stereotaxic coordinates: Hard cover edition: Elsevier; 2007.
16. Arrang JM, Garbarg M, Lancelot JC, Lecomte JM, Pollard H, Robba M, *et al*. Highly potent and selective ligands for histamine H3-receptors. Nature 1987; 327:117-123.
17. Brabant C, Charlier Y, Quertemont E, Tirelli E. The H3 antagonist thioperamide reveals conditioned preference for a context associated with an inactive small dose of cocaine in C57BL/6J mice. Behav Brain Res 2005; 160:161-168.
18. Ligneau X, Landais L, Perrin D, Piriou J, Uguen M, Denis E, *et al*. Brain histamine and schizophrenia: Potential therapeutic applications of H3-receptor inverse agonists studied with BF2.649. Biochem Pharmacol 2007; 73:1215-1224.
19. Fox GB, Esbenshade TA, Pan JB, Radek RJ, Krueger KM, Yao BB, *et al*. Pharmacological properties of ABT-239 [4-(2-{2-[(2 R)-2-methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzonitrile]: II. neurophysiological characterization and broad preclinical efficacy in cognition and schizophrenia of a potent and selective histamine H3 receptor antagonist. J Pharmacol Exp Ther 2005; 313:176-190.
20. Ligneau X, Lin J, Vanni-Mercier G, Jouvet M, Muir JL, Ganellin CR, *et al*. Neurochemical and behavioral effects of ciproxifan, a potent histamine H3-receptor antagonist. J Pharmacol Exp Ther 1998; 287:658-666.
21. Brown RE, Stevens DR, Haas HL. The physiology of brain histamine. Prog Neurobiol 2001; 63:637-672.
22. Brabant C, Charlier Y, Tirelli E. The histamine H_3-receptor inverse agonist pitolisant improves fear memory in mice. Behav Brain Res 2013; 243:199-204.
23. Ligneau X, Perrin D, Landais L, Camelin JC, Calmels TP, Berrebi-Bertrand I, *et al*. BF2.649 [1-{3-[3-(4-Chlorophenyl) propoxy]propyl}piperidine, hydrochloride], a nonimidazole inverse agonist/antagonist at the human histamine H3 receptor: Preclinical pharmacology. J Pharmacol Exp Ther 2007; 320:365-375.
24. Medhurst AD, Atkins AR, Beresford IJ, Brackenborough K, Briggs MA, Calver AR, *et al*. GSK189254, a novel H3 receptor antagonist that binds to histamine H3 receptors in Alzheimer's disease brain and improves cognitive performance in preclinical models. J Pharmacol Exp Ther 2007; 321:1032-1045.
25. Mahdy AM, Webster NR. Histamine and antihistamines. Anaest Intens Care Med 2011; 12:324-329.
26. Tuomisto L, Lozeva V, Valjakka A, Lecklin A. Modifying effects of histamine on circadian rhythms and neuronal excitability. Behav Brain Res 2001; 124:129-135.
27. Selbach O, Brown RE, Haas HL. Long-term increase of hippocampal excitability by histamine and cyclic AMP. Neuropharmacology 1997; 36:1539-1548.
28. Chang M, Saito H, Abe K. Histamine H3 receptor-mediated inhibition of excitatory synaptic transmission in the rat dentate gyrus *in vivo*. Jpn J Pharmacol 1998; 77:251-255.
29. Andersson R, Lindskog M, Fisahn A. Histamine H3 receptor activation decreases kainate-induced hippocampal gamma oscillations *in vitro* by action potential desynchronization in pyramidal neurons. J Physiol 2010; 588:1241-1249.
30. Schlicker E, Kathmann M. Role of the histamine H(3) receptor in the central nervous system. Handb Exp Pharmacol 2017; 241:277-299.
31. Varaschin RK, Rosenberg MJ, Hamilton DA, Savage DD. Differential effects of the histamine H(3) receptor agonist methimepip on dentate granule cell excitability, paired-pulse plasticity and long-term potentiation in prenatal alcohol-exposed rats. Alcohol Clin Exp Res 2014; 38:1902-1911.
32. Köhler CA, da Silva WC, Benetti F, Bonini JS. Histaminergic mechanisms for modulation of memory systems. Neural Plast 2011; 2011:328602.
33. Martin SJ, Grimwood PD, Morris RG. Synaptic plasticity and memory: An evaluation of the hypothesis. Annu Rev Neurosci 2000; 23:649-711.
34. Luo T, Wang Y, Qin J, Liu ZG, Liu M. Histamine H3 receptor antagonist prevents memory deficits and synaptic plasticity disruption following isoflurane exposure. CNS Neurosci Ther 2017; 23:301-309.
35. Dai H, Fu Q, Shen Y, Hu W, Zhang Z, Timmerman H, *et al*. The histamine H3 receptor antagonist clobenpropit enhances GABA release to protect against NMDA-induced excitotoxicity through the cAMP/protein kinase A pathway in cultured cortical neurons. Eur J Pharmacol 2007; 563:117-123.
36. Schlicker E, Betz R, Göthert M. Histamine H3 receptor-mediated inhibition of serotonin release in the rat brain cortex. Naunyn Schmiedebergs Arch Pharmacol 1988; 337:588-590.
37. Introini-Collison IB, Dalmaz C, McGaugh JL. Amygdala beta-noradrenergic influences on memory storage involve cholinergic activation. Neurobiol Learn Mem 1996; 65:57-64.
38. Vazdarjanova A, McGaugh JL. Basolateral amygdala is involved in modulating consolidation of memory for classical fear conditioning. J Neurosci 1999; 19:6615-6622.
39. Maurer SV, Williams CL. The cholinergic system modulates memory and hippocampal plasticity via its interactions with non-

neuronal cells. Front Immunol 2017; 8:1489-1502.
40. Tsetsenis T, Broussard JI, Dani JA. Dopaminergic regulation of hippocampal plasticity, learning, and memory. Front Behav Neurosci 2022; 16:1092420.
41. Ellenbroek BA, Ghiabi B. The other side of the histamine H3 receptor. Trends Neurosci 2014; 37:191-199.
42. Trofimiuk E, Braszko JJ. Single dose of H3 receptor antagonist--ciproxifan--abolishes negative effects of chronic stress on cognitive processes in rats. Psychopharmacology (Berl) 2014; 231:209-219.
43. Goncalves-Garcia M, Davies S, Savage DD, Hamilton DA. The histamine H(3) receptor inverse agonist SAR-152954 reverses deficits in long-term potentiation associated with moderate prenatal alcohol exposure. Alcohol 2024; 118:45-55.
44. Zou Y, Yang L, Zhu J, Fan J, Zheng H, Liao X, *et al*. Pitolisant alleviates brain network dysfunction and cognitive deficits in a mouse model of Alzheimer's disease. Transl Psychiatry 2025; 15:126-140.
45. Hara K, Harris RA. The anesthetic mechanism of urethane: The effects on neurotransmitter-gated ion channels. Anesth Analg 2002; 94:313-318.
46. Sherin JE, Elmquist JK, Torrealba F, Saper CB. Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. J Neurosci 1998; 18:4705-4721.
47. Jiang S, Li H, Zhang L, Mu W, Zhang Y, Chen T, *et al*. Generic diagramming platform (GDP): A comprehensive database of high-quality biomedical graphics. Nucleic Acids Res 2024; 53:D1670-D1676.