


Histamine H3 Receptor Antagonist Prevents Memory Deficits and Synaptic Plasticity Disruption Following Isoflurane Exposure

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Keywords

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SUMMARY

Background: Exposure to pharmacological concentration of inhaled anesthetics such as isoflurane can cause short- or long-term cognitive impairments in preclinical studies. The selective antagonists of the histamine H3 receptors are considered as a promising group of novel therapeutic agents for the treatment of cognitive disorders. In this study, we investigated whether ciproxifan, a nonimidazole antagonist of H3 histamine receptors, could overcome the functional and electrophysiological sequela associated with isoflurane anesthesia. **Methods:** Adult male Sprague Dawley rats were exposed to 1.4% isoflurane or vehicle gas for 2 h. The memory tests (novel object recognition and passive avoidance) as well as in vivo hippocampal excitatory synaptic potentials were recorded 24 h postanesthesia. Locomotor activity, anxiety, and nociception 24 h after isoflurane were also examined. The drugs (ciproxifan 3 mg/kg or saline) were intraperitoneally injected 30 min prior to the behavioral tests or long-term potentiation induction. **Results:** Animals that were previously (24 h) exposed to 1.4% isoflurane for 2 h displayed no preference for novel objects and had impaired retention of a passive avoidance response at 1 h after sample phase. Treating isoflurane-exposed rats with ciproxifan significantly improved the memory performance, as evidenced by an increased discrimination ratio in objects recognition and prolonged retention time in passive avoidance test. Accordingly, hippocampus long-term potentiation was reduced in animals that received isoflurane, while administration of ciproxifan completely abolished the effect of isoflurane exposure on synaptic plasticity. Neither isoflurane nor ciproxifan altered motor performance, anxiety, and nociceptive responses. **Conclusion:** These results suggest that H3R in the CNS, probably in the hippocampus, may serve as therapeutic target for improvement of anesthesia-associated cognitive deficits.

Implication Statement

Systemic administration of ciproxifan, a histamine H3 receptor antagonist/inverse agonist, attenuated memory deficit and hippocampal synaptic plasticity impairment following isoflurane exposure. This finding suggests that H3R may serve as a therapeutic target against anesthesia-associated cognitive deficits.

Introduction

Early postoperative cognitive dysfunction, confusion, and delirium are well-known complications after major surgery [1,2]. General anesthesia has often been mentioned as a possible cause of these problems, because it affects brain function at multiple

levels—from single neurons to neural networks. Although the recovery of consciousness from general anesthesia is usually rapid, several studies in humans and rodents indicate that the amnesic effects of general anesthetics such as isoflurane may persist beyond the time of anesthesia, leading to impairment of cognitive functions [1–7]. The cellular changes contributing to these effects

have yet to be fully understood. Consequently, progress toward developing pharmacotherapeutic agents that could ameliorate anesthetic-induced cognitive deficits has been limited.

Histamine and its receptors in the brain are associated with the regulation of basic homeostatic and higher functions, including arousal, cognition, circadian, and feeding rhythms [8]. Among the four identified histamine receptors, H3 receptors are predominantly expressed in the central nervous system, act as autoreceptors as well as heteroreceptors, and control presynaptic release of histamine and other neurotransmitters, including acetylcholine, serotonin, noradrenaline, and dopamine [8–10]. Modulation of the H3R is expected to affect vigilance and sleep–wake regulation [11]. In recent years, selective antagonists of the H3R are considered as a promising group of new potential drugs for the treatment of cognitive deficits such as Alzheimer's disease, attention deficit hyperactivity disorder, and schizophrenia [12].

Long-term potentiation (LTP), a cellular model of activity-dependent synaptic plasticity, is believed to be the major cellular mechanism that underlies learning and memory [13]. Several studies have suggested that the memory impairments of general anesthetics are, in part, linked to physiological alterations that diminish the enhancement of synaptic neurotransmission in the hippocampus *in vitro* [14–17]. Histamine, on the other hand, has been shown to increase or even evoke LTP of synaptic transmission [8,18]. Antagonists of H3R have procognitive actions and therefore enhance the potentiation of synaptic transmission [19].

Using a mouse model, we previously showed that a robust deficit in short-term object recognition memory occurred 1 day after exposure to isoflurane (1.3%) for 2 h [20]. In this study, we asked the question as to whether ciproxifan [21], a well-characterized nonimidazole antagonist of H3 histamine receptors, could overcome the functional and electrophysiological sequela associated with isoflurane anesthesia. We first assessed the effect of ciproxifan on memory deficit following exposure to isoflurane in behavioral paradigms including the novel object recognition and passive avoidance test. Next, we examined whether H3 receptor inhibition could rescue impairment of synaptic plasticity in the hippocampal CA1 region *in vivo* of isoflurane-treated rats.

Materials and Methods

Animals

Experiments were performed on adult male Sprague Dawley rats (235–280 g). All animals were given water and regular rat chow *ad libitum* and housed under climate-controlled conditions with a 12-hour light/dark cycle (lights on at 7:00 am). All procedures were approved by the Institutional Animal Care and Use Committee.

Novel Object Recognition Test

The novel object recognition was conducted as previously reported to evaluate nonspatial hippocampal-mediated memory [22]. The apparatus consists of an open field (40 × 80 cm²) with two adjacently located imaginary circular zones. The zones are equally spaced in the center of the square and designated as “old object” and “novel object” zones. The old object used was square-

shaped, whereas the novel object was L-shaped and assembled by building blocks from Lego toys, which were clearly distinct in shape and color. Two 5-minute trials were performed. The first (training phase) trial was performed with two identical objects in both zones and the second (testing phase) trial with one old object and one novel object present in the respective zones of the open field. There was an intertrial interval of 1 h, during which the animals were returned to their home cages. A video camera was mounted on the ceiling above the chamber and connected to a monitor in an adjoining room. Object exploration was defined as sniffing or touching the object with the nose and/or forepaws. The time spent with each object was recorded by two trained investigators, uninformed of drug treatment, whose inter-rater reliability was within 1 second of one another. Because rats inherently prefer to explore novel objects, a preference for the novel object indicates intact memory for the familiar object. The “discrimination index” for the second trial was calculated as: discrimination index = time spent in novel object zone / (time spent in old object zone + time spent in novel object zone).

Passive Avoidance Test

The passive avoidance test was used to evaluate nonspatial, hippocampus-mediated contextual memory, and fear-related amygdala-dependent emotional memory, as previously described [23]. The one-trial step-through passive avoidance apparatus consisted of two adjoining compartments, one lighted and one darkened, divided by a guillotine door. The floor of each compartment consists of steel rods that deliver an electric foot shock to the rats. In the training phase of the passive avoidance test, a rat was placed in the lighted compartment, and when the rat entered the dark compartment, the guillotine door was closed manually by the experimenter and an electric foot shock of 0.5 mA for 3 seconds was delivered. The rats were confined in the dark compartment for 20 seconds after the electric shock and were then removed from the apparatus and returned to their home cages. In the testing phase performed 1 h later, the rats were tested for the retention of the passive avoidance response by placing them in the lighted compartment and recording the latency to enter into the previously shocked dark compartment. No shock was given during the test phase. If the rat did not enter into the dark compartment within 5 min, it was assigned a latency of 300 seconds.

Locomotor Activity

Spontaneous locomotor activity was tested using the open-field method. The animals were individually placed in a corner facing the wall of the open-field chamber (50 × 50 × 40 cm) and allowed to freely explore the chamber for 10 min. Total moving distance and mean speed were measured by computer-based Any-Maze automated video tracking system (Stoelting Co.)

Elevated Plus Maze Task

Elevated plus maze task (EPM) is one of the most widely used assessments to evaluate anxiety-like behavior in rodents. The EPM apparatus comprised two open arms and two closed arms (50 × 10 cm), which extended from a common central platform

(10 × 10 cm). Each arm was attached to a sturdy leg and elevated 50 cm from the ground. The animal was placed in the center of the maze and allowed to explore freely for 5 min. The time spent in the center and in the open and closed arms was measured.

Tail Flick

The nociceptive threshold was taken as the reaction time to remove the tail from a source of noxious heat stimulus using a hot water bath (49°C). The tip of the tail was inserted to approximately 1 cm depth into a bath of water. Latency for the rat to remove its tail from the water was recorded and used as an indication of nociception.

In Vivo Electrophysiology

In vivo electrophysiology in freely moving animals was performed as described previously [24]. Briefly, the Sprague Dawley rats were anesthetized with sodium pentobarbital 60 mg/kg intraperitoneally and stereotaxically implanted with electrodes in the hippocampus. A pair of recording electrodes was implanted to straddle the hippocampal cell layer of CA1 (in mm: P4.6 and L2.8), and stimulating electrodes were placed in CA1 stratum oriens on the same side anterior to the recording electrodes (in mm: P3.2 and L1.7). Photoisolated current stimulus pulses (0.2 ms duration) were delivered cathodally to one stimulating electrode, and the evoked potentials were recorded from another pair of electrodes. The animals were allowed to recover from surgery for 14 days before experiments were conducted.

The evoked basal-dendritic field excitatory postsynaptic potentials (fEPSPs) were recorded during awake immobility, and LTP was induced during behavioral exploration. After recording baseline fEPSPs for 1 h, a high-frequency stimulus train (tetanus) was used for LTP induction. The train consisted of 100 pulses at 200 Hz (5-ms interpulse interval), with a stimulus intensity of 0.8–1.5× the fEPSP threshold. fEPSPs were recorded with test pulses of 1.5× threshold intensity. The maximal slope of the negative fEPSP, recorded in stratum oriens, was measured during the falling phase (over 2 ms interval). The last six recordings before the tetanus were averaged to determine the baseline value, which was normalized to 100%. The enhanced synaptic plasticity was recorded at “fixed” times of 5, 10, 15, 20, 30, 60, 90, 120, 150, and 180 min after the tetanus. For each experiment, the response after tetanus was normalized to the baseline average. During baseline and at 1 and 2 h after tetanus, four different stimulus intensities (1×, 1.2×, 2×, and 4× fEPSP threshold) were used to generate input–output curves.

Experimental Design and Drug Treatment

Experiments were conducted between 9:00 and 16:00 h during the day. Animals were randomly divided into four experimental groups as follows: (1) vehicle gas plus saline injection (naïve–saline), (2) isoflurane plus saline injection (isoflurane–saline), (3) vehicle gas plus ciproxifan injection (naïve–ciproxifan), and (4) isoflurane plus ciproxifan injection (isoflurane–ciproxifan). Animals were exposed to 1.4% isoflurane or vehicle gas for 2 h, and the behavioral tests as well as electrophysiological

recordings were performed 24 h postanesthesia. Ciproxifan hydrochloride (Sigma, Shanghai, China) was used in the experiments and administered at the dose of 3 mg/kg body weight i.p. in a volume of 1 mL of sterile saline 30 min prior to the training phase of behavioral tests or LTP induction. Control rats received 1 mL of sterile saline as a vehicle. Animals were used only once for the novel object recognition and passive avoidance tests, respectively. However, in the study of electrophysiological recordings, each rat was used in paired ciproxifan and saline treatments, in a random order with 7 days between experiments. Additional groups of rats were used to evaluate locomotor activity, anxiety level, and pain perception in the order of open-field, EPM task and tail-flick test, respectively.

Rats randomized to the anesthesia groups received 1.4% isoflurane in 30% oxygen for 2 h in a Plexiglas anesthetizing chamber, whereas the vehicle gas groups received 30% oxygen without any inhalational anesthetic for 2 h. The 1.4% concentration was chosen because it represents one minimum alveolar concentration (the concentration at which 50% of animals do not have a motor response to painful stimuli) of isoflurane in rats. The isoflurane concentration, oxygen, and carbon dioxide levels in the chamber were continuously monitored with an agent gas monitor, and the temperature of the Plexiglas anesthetizing chamber was controlled with a heating pad to maintain rectal temperature at 37°C ± 0.5°C. To determine adequacy of ventilation and blood pressure, a separate set of rats (n = 6) were implanted with a femoral arterial catheter for continuous measurement of mean arterial blood pressure (MAP) and heart rate. Arterial blood gases were measured before and at the end of the two-hour anesthetic exposure.

Statistical Analysis

All data are expressed as mean ± SEM. The data were tested for normality before parametric analysis. If a measurement variable did not fit a normal distribution in different groups, a data transformation was performed followed by parametric analysis. In the novel object recognition test, differences in the mean exploration time spent with the two objects were compared using a Student's *t*-test, and comparison of the discrimination index between groups was performed using one-way ANOVA followed by the Student–Newman–Keuls test. Statistical differences in the passive avoidance test among groups were analyzed using the one-way ANOVA, followed by post hoc Student–Newman–Keuls test. Statistical differences in the open-field, EPM, and tail-flick tests among groups were analyzed using the one-way ANOVA. Electrophysiological data were analyzed by a two-way repeated-measures ANOVA. The data were analyzed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA, USA). *P* < 0.05 was considered to be significant.

Results

Ciproxifan Ameliorates Object Recognition Memory Deficits After Isoflurane

Hippocampus-mediated nonspatial learning and memory following isoflurane anesthesia was assessed by the novel object

recognition test. As shown in Figure 1A, during the training phase, animals from all four groups showed an equal preference to the identical objects. During the choice phase performed 1 h after training, rats in the vehicle gas plus saline injection group (naïve–saline) spent more time than chance with the novel object ($n = 12$ per group, $t = 8.133$, $df = 11$, $P < 0.0001$; Figure 1B), indicating intact memory, whereas the rats exposed to isoflurane only (isoflurane–saline) spent similar times with the novel and familiar objects, exhibiting memory deficits ($t = 1.932$, $df = 11$, $P = 0.0795$, Figure 1B). Treating isoflurane-exposed rats with ciproxifan (isoflurane–ciproxifan) increased preference to the novel object ($n = 12$ per group, $t = 6.178$, $df = 11$, $P < 0.0001$, Figure 1B), indicating that treatment restored recognition memory in isoflurane–ciproxifan injection rats. Furthermore, one-way ANOVA analysis showed a significant effect of treatment on

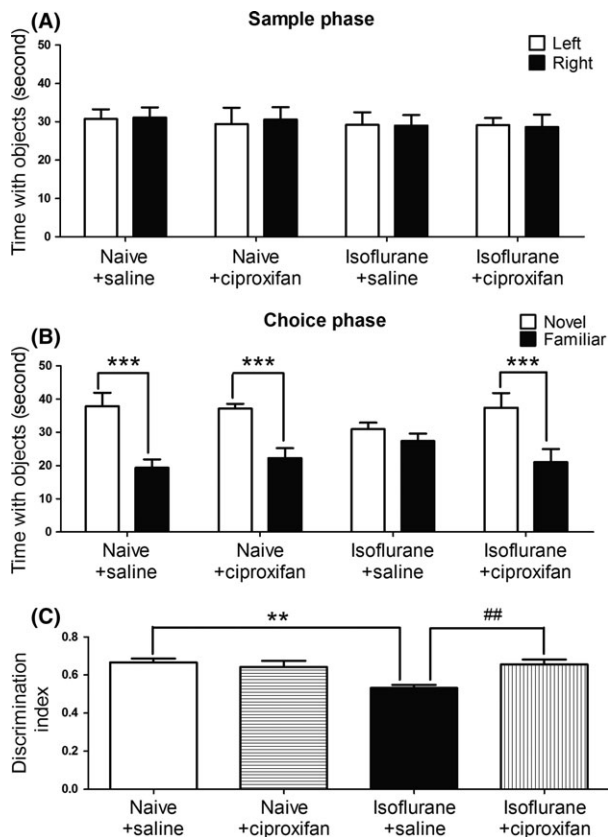


Figure 1 Ciproxifan ameliorates object recognition memory deficits after isoflurane. Animals were exposed to 1.4% isoflurane or vehicle gas for 2 h, and the novel object recognition test was performed 24 h postanesthesia. The drugs (ciproxifan 3 mg/kg or saline) were intraperitoneally injected in a volume of 1 mL 30 min prior to the training phase. (A) During the training phase, two identical objects were placed in two defined sites of the chamber. Animals from all four groups showed an equal preference to identical objects. (B) During the test phase performed 1 h after the training phase, one of the objects was replaced by a new object with a different shape. Time spent with the novel and familiar objects during test phase was recorded. (C) Discrimination ratio from the test phase for each treatment group. Data are expressed as mean \pm SEM. (** $P < 0.01$, *** $P < 0.001$ and ## $P < 0.01$, respectively, $n = 12$ per group).

discrimination ratio ($n = 12$ per group, $F = 6.264$; $P = 0.0012$). Specifically, post hoc Student–Newman–Keuls analysis revealed that the isoflurane–ciproxifan injection group had a significant higher discrimination ratio when compared with the isoflurane–saline injection group ($P < 0.01$). Significant differences were also observed between naïve–saline injection and isoflurane–saline injection groups ($P < 0.01$). Without exposure to isoflurane, ciproxifan injection alone did not improve object preference, as compared to saline injection.

Ciproxifan Ameliorates Passive Avoidance Memory Deficits After Isoflurane

As a second memory test, we determined isoflurane-induced cognitive deficits using a passive avoidance test. As shown in Figure 2A, there were no significant differences in the latency to enter the dark compartment in the training phase among the four groups of animals. One hour after training (Figure 2B), a significant effect of treatment on transfer times to the dark compartment was observed ($n = 12$ per group, $F = 4.358$; $P = 0.0090$). The retention time in the isoflurane–saline group (161.4 ± 33.3 seconds) was significantly shorter than that of the control naïve–saline injection group (244.6 ± 24.88 seconds, $P < 0.05$). There was no significant difference between treatment with ciproxifan (270.1 ± 9.794 seconds, $P > 0.05$) and the naïve group. In contrast, treatment with ciproxifan (257.0 ± 19.49 seconds, $P < 0.05$) showed significant improvement of the isoflurane-induced cognitive deficit in this task.

Neither Isoflurane Nor Ciproxifan Alters Motor Performance, Anxiety, and Nociceptive Responses

Locomotor activity was studied with open-field test 24 h after exposure to isoflurane or vehicle gas. As shown in Figure S1A, animals in the groups of naïve–saline (5.37 ± 0.25 m), isoflurane–saline (5.74 ± 0.37 m), naïve–ciproxifan (5.15 ± 0.35 m), and isoflurane–ciproxifan (5.63 ± 0.35 m) displayed similar distance traveled during the 10 min of testing. There were no significant locomotor impairments in each of the groups when compared to the naïve–saline group ($n = 12$ per group, $F = 0.6283$, $P = 0.60$). No differences in mean speed were also observed among the four groups ($n = 12$ per group, $F = 0.5532$, $P = 0.65$, Figure S1B).

In the EPM task (Figure S1C–E), the proportion of time spent in open arms was not different among the four groups ($n = 12$ per group, $F = 0.7067$, $P = 0.55$): naïve–saline ($34.08 \pm 1.06\%$), isoflurane–saline ($33.11 \pm 0.66\%$), naïve–ciproxifan ($32.47 \pm 0.89\%$), and isoflurane–ciproxifan ($33.86 \pm 0.85\%$). The time spent exploring the closed arms and the central area of the maze was not statistically different among the four groups, suggesting that isoflurane/ciproxifan treatment did not alter anxiety measures.

Altered pain perception following anesthesia could also affect nonassociative fear learning. Therefore, nociception was studied using the tail-flick assay 24 h after exposure to isoflurane or vehicle gas. No differences were detected in the latency to tail flick ($n = 12$ per group, $F = 0.1075$, $P = 0.9553$, Figure S1F), suggesting that all groups of animals perceived the same foot shock stimulus strength.

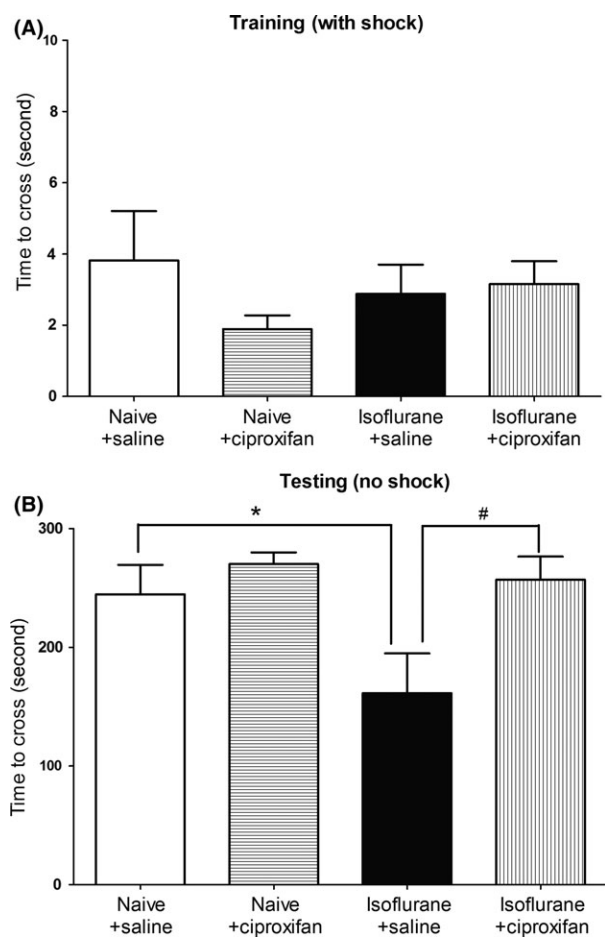


Figure 2 Ciproxifan ameliorates passive avoidance memory deficits after isoflurane. Animals were exposed to 1.4% isoflurane or vehicle gas for 2 h, and the passive avoidance test was performed 24 h postanesthesia. The drugs (ciproxifan 3 mg/kg or saline) were intraperitoneally injected in a volume of 1 mL 30 min prior to the training phase. **(A)** The rats were placed in a transfer chamber, and a foot shock was delivered upon entry into the dark compartment from the bright compartment. The rats from each group showed similar transfer times. **(B)** An hour later, the rats were placed in the transfer chamber again and the transfer time was recorded without foot shock. Data are expressed as mean \pm SEM. (* $P < 0.05$ and # $P < 0.05$, respectively, $n = 12$ per group).

Neither Isoflurane Nor Ciproxifan Alters Baseline Input/Output Responses

Figure 3 depicts input/output curves for fEPSPs slope in control and isoflurane-exposed rats treated with either saline or 3 mg/kg of ciproxifan. The fEPSPs evoked by stimuli ranging from one to four times threshold intensity collected before LTP induction were not affected by isoflurane exposure. Two-way repeated-measures ANOVA confirmed a lack of significant difference between the fEPSPs slope in naïve–saline group and that of isoflurane–saline group ($F_{(1,30)} = 0.81$; $P = 0.3902$), and there was no significant group \times time interaction ($F_{(3,30)} = 1.71$; $P = 0.1856$). Furthermore, ciproxifan treatment did not

affect input/output curves compared with those of saline-treated rats. Two-way repeated-measures ANOVA confirmed a lack of significant difference between the fEPSPs slope in naïve–saline group and that of naïve–ciproxifan group ($F_{(1,30)} = 0.01$; $P = 0.9176$), and there was no significant group \times time interaction ($F_{(3,30)} = 0.42$; $P = 0.7430$). Thus, the ionotropic glutamate synaptic transmission was not altered by isoflurane anesthesia and/or ciproxifan treatment.

Ciproxifan Rescues Hippocampal Synaptic Plasticity After Isoflurane

Hippocampal CA1 basal-dendritic LTP was quantified by an increase in fEPSPs slope from the baseline. As shown in Figure 4, in isoflurane-free rats, ciproxifan did not enhance fEPSP slope compared with saline-treated controls (Figure 4A, B). Two-way repeated-measures ANOVA confirmed a lack of significant difference between the LTP induced in naïve–ciproxifan and that induced in naïve–saline ($F_{(1,160)} = 0.05$; $P = 0.8161$), and there was no significant treatment \times time interaction ($F_{(15,160)} = 0.23$; $P = 0.999$). Basal-dendritic LTP in animals that received isoflurane was smaller than that of naïve rats. Repeated-measures ANOVA showed a significant effect of isoflurane ($F_{(1,160)} = 17.11$; $P < 0.0001$), a significant time effect ($F_{(15,160)} = 17.76$; $P < 0.0001$), but no significant treatment \times time interaction ($F_{(15,160)} = 0.87$; $P = 0.5992$). When administered 30 min before tetanic stimulation, ciproxifan did not enhance the baseline fEPSPs slope compared with saline-treated controls. However, the fEPSP responses after tetanic stimulation, in ciproxifan-treated isoflurane rats, were significantly greater than those in saline-treated isoflurane rats (Figure 4C, D). Repeated-measures ANOVA showed a significant effect of ciproxifan ($F_{(1,160)} = 18.21$; $P < 0.0001$), a significant time effect ($F_{(15,160)} = 25.27$; $P < 0.0001$), but no significant treatment \times time interaction ($F_{(15,160)} = 1.02$; $P = 0.4352$).

Isoflurane Anesthesia Does Not Change Physiological Parameters

As hypotension, hypercarbia, and hypoxia are potential causes of cognitive deficit, we analyzed the blood gas levels before and at the end of isoflurane exposure in a separate set of rats ($n = 6$), which revealed normal oxygenation (0 h/2 h: 132 ± 3.2 mmHg/ 136 ± 2.1 mmHg), adequate PaCO₂ values (0 h/2 h: 40 ± 1.32 mmHg/ 43.2 ± 1.22 mmHg), and no acidosis (0 h/2 h: pH: 7.36 ± 0.00 / 7.35 ± 0.02). MAP and heart rate were also within the physiological range and remained stable over the course of the anesthesia (Table 1).

Discussion

We demonstrated that ciproxifan is capable of reducing memory deficits caused by isoflurane anesthesia and attenuating isoflurane-induced suppression of hippocampal LTP induction in vivo. To the best of our knowledge, this is the first preclinical work that supports the potential use of a H3R antagonist for treatment of general anesthesia-associated cognitive disorders.

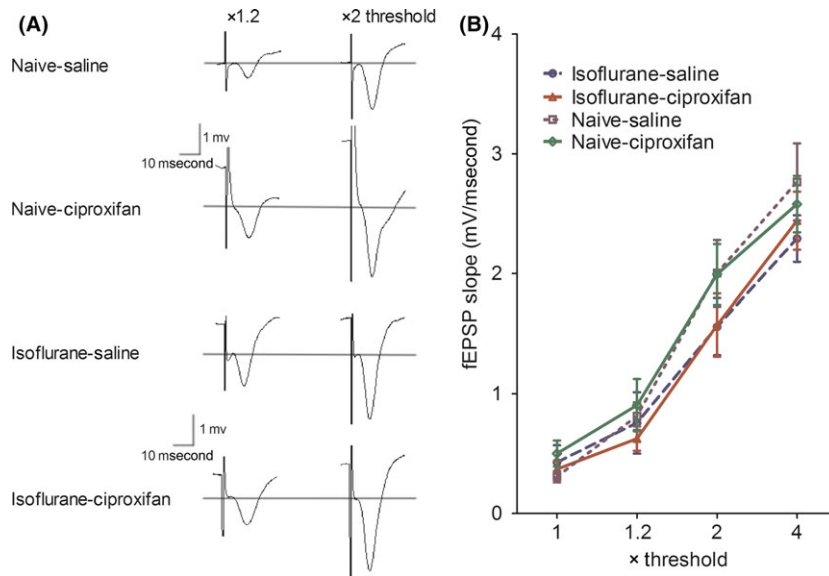


Figure 3 Isoflurane and ciproxifan do not alter hippocampal fEPSPs input/output responses. **(A)** Representative fEPSP traces at the basal-dendritic electrode with stimulus intensities 1.2x and 2x test pulse. **(B)** Normalized basal-dendritic fEPSPs responses in isoflurane/vehicle gas-exposed rats in the presence or absence of ciproxifan. Data are expressed as mean \pm SEM. A two-way repeated-measures ANOVA revealed no significant effect of isoflurane versus vehicle gas, and no significant effect of ciproxifan versus saline for input/output responses, $n = 6$ per group.

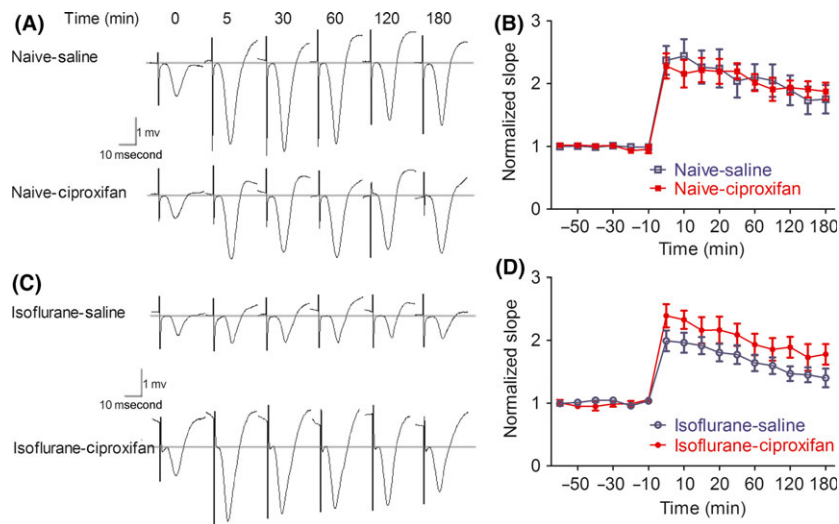


Figure 4 Ciproxifan rescues hippocampal synaptic plasticity after isoflurane. **(A, C)**, fEPSP traces at the basal-dendritic electrode of representative rats at 0 (baseline), 5, 30, 60, 120, and 180 min after tetanus, with tetanus given 30 min after i.p administration of either saline or ciproxifan in vehicle gas **(A)** and isoflurane **(C)**-exposed rats. **(B)** Normalized fEPSP slopes with LTP induced after vehicle gas exposure and administration of either saline or ciproxifan 30 min before tetanus. **(D)** Normalized fEPSP slopes with LTP induced after isoflurane exposure and administration of either saline or ciproxifan 30 min before tetanus. Data are expressed as mean \pm SEM. A two-way repeated-measures ANOVA revealed a significant effect of isoflurane versus vehicle gas for LTP induction, and a significant effect of ciproxifan versus saline for LTP induction in isoflurane-exposed rats, $n = 6$ per group.

We performed behavioral tests including the novel object recognition and passive avoidance to assess the cognitive outcomes of rats following isoflurane anesthesia. Each task has a different role in investigating effects on memory. Novel object recognition is known to evaluate the function of nonspatial hippocampal memory [25]. The passive avoidance is related to the amygdala-

hippocampus complex, which involves both contextual memory and amygdala-dependent emotional memory [26]. In the present study, animals that were previously (24 h) exposed to 1.4% isoflurane for 2 h displayed no preference for the novel object and had impaired retention of a passive avoidance response at 1 h after sample phase. This is unlikely to be a consequence of

Table 1 Effect of isoflurane on physiological parameter

	0 h	2 h	<i>P</i> value
pH	7.36 ± 0.02	7.35 ± 0.02	>0.05
PaO ₂	132 ± 3.2	136 ± 2.1	>0.05
PaCO ₂	40.0 ± 1.32	43.2 ± 1.22	>0.05
HCO ₃ ⁻	23.2 ± 0.79	22.7 ± 0.61	>0.05
MAP	105 ± 2	100 ± 2	>0.05
HR	334 ± 7.3	327 ± 8.5	>0.05

Values are mean ± SEM. There were no group differences in all measures (*n* = 6 per group).

physiological changes associated with general anesthesia, because blood pressure and arterial blood gases remained within the physiological range during anesthesia. It should also be noted that memory was the only remarkable deficit 24 h after exposure to isoflurane as no obvious impairment in motor, anxiety, and sensory function had been identified.

A number of preclinical studies indicate that exposure to volatile anesthetics, such as isoflurane, cause temporary or sustained impairments in learning and memory. In support with our finding, several studies reported that exposure of 2- to 4-month-old mice to 1–1.3% isoflurane impaired the memory performance when they were tested within 7 days after isoflurane exposure [7,27,28]. Similarly, isoflurane (1 minimum alveolar concentration, 4 h) impaired retention memory for Morris water maze 1 week after exposure in young adult rats and resulted in a delayed although weak impairment at 4 weeks in middle-aged rats [29]. In contrast to these findings, another recent study demonstrated that two-month-old rats did not have impairment in spatial reference learning and fear conditioning test but had improved long-term memory at 4 months after isoflurane anesthesia [30]. The discrepancies of these findings remain unclear but may be related to animal species and strains, developmental stage, anesthetic agent, dosage, and duration of exposures.

Isoflurane exposure-induced memory dysfunction was overcome by treatment with ciproxifan. The histamine H3 receptor antagonists have been found to improve performance in a variety of rodent models of memory impairment. Ciproxifan is able to restore the cognitive performance impaired by scopolamine and prolonged administration of corticosterone [31,32]. Single administration of ciproxifan potently prevents the deleterious effects of chronic restraint stress on cognitive functions tested with Morris water maze or Barnes maze [32]. Furthermore, ciproxifan effectively improves the performance in the water maze and the object recognition test in Alzheimer's APP_{Tg2576} mice [33]. The procognitive effect of ciproxifan involves enhanced release of neurotransmitters of such as histamine, acetylcholine, dopamine, and glutamate [34–36]. At the molecular level, the sustained increase in α 5GABA(A)R activity contributes for impaired memory performance and synaptic plasticity in the hippocampus [17]. α 5GABAA receptor-selective inverse agonist L-655,708 effectively prevents memory deficit after anesthesia [27]. Histamine has been shown to act through allosteric sites on GABAA receptor channels, depending upon subunit composition [37,38]. However, evidence

for histamine-GABA cross-talk in native neuronal contexts remains to be elucidated [37].

Both novel object recognition and passive avoidance are sensitive to impairments in hippocampal function [39,40]. We therefore used an *in vivo* paradigm of LTP to investigate the putative hippocampal site of action for the cognitive effects observed with isoflurane exposure. Our finding demonstrated that hippocampal synaptic plasticity in freely moving rats is impaired after isoflurane anesthesia and that the LTP deficit can be ameliorated by treatment with ciproxifan. Moreover, ciproxifan at the 3 mg/kg dose rescued synaptic potentiation without increasing baseline input/output responses, suggesting that the histamine H3 receptor system affects synaptic potentiation at doses that do not affect baseline neurotransmission. It is of interest to note that LTP was not enhanced by the administration of ciproxifan in control rats, possibly because LTP induced during behavioral exploration had already maximized the degree of synaptic potentiation [24,41]. This also supports the finding that behavioral performance on the tasks could not be improved any further when ciproxifan was given to animals without isoflurane exposure.

Histaminergic neurons, located exclusively in the tuberomammillary nucleus of the posterior hypothalamus, are involved in the wake-sleep regulation. They enhance arousal via diffuse projections to the cerebral cortex [8]. There is growing evidence that the neural histamine system is influenced by many general anesthetic agents [42–44]. Inhalation of halothane dose dependently reduces endogenous histamine levels from the anterior hypothalamic area of rats [42]. Depletion of brain histamine content by selective lesions of the histaminergic tuberomammillary nucleus facilitates anesthesia maintenance and delays anesthesia emergence by isoflurane [43]. Moreover, exogenous administration of histaminergic agents modulates the depth of anesthesia and emergence time [44].

Memory impairment was found 24 h after isoflurane exposure in the present study. The 24-hour time interval from anesthetic discontinuation is sufficient to avoid the confounding effects of residual anesthetic on neurobehavioral performance [27,45]. Moreover, it has been demonstrated that exposure to isoflurane does not alter subsequent levels of wakefulness [46]. Thus, our finding suggests that H3R may serve as therapeutic target for improvement of anesthesia-associated cognitive deficits. However, it cannot exclude the possibility that ciproxifan enhances cognition by improving vigilance. Indeed, recent evidence suggests that ciproxifan enhances vigilance and wake [11, 47–49]. The arousal effect mainly depends on the activation of histaminergic systems through H1R [47, 48]. A functional imaging study demonstrated that fronto-cortical areas involved in higher cognitive function, and the network of pro-arousing areas can be activated simultaneously by cognitive enhancing reagent [50]. Further investigations are also required to determine the effect of ciproxifan on cortical activation, as well as acquisition, consolidation and retrieval of memory processes following anesthesia specifically.

The results of this study must be viewed in light of potential limitations. First, ciproxifan amelioration of memory impairment does not mean that the underlying mechanism of isoflurane-induced memory dysfunction is mediated through the central histamine system *per se*. The inhibition of presynaptic H3 receptors by ciproxifan leads to the release of neurotransmitters including

histamine, acetylcholine, noradrenaline, dopamine, and serotonin [34–36]. The extent to which each of these putative mechanisms of ciproxifan action may have contributed to prolonged synaptic potentiation and memory enhancing needs further investigation. Direct measuring-related neurotransmitter levels would help solving this question. Second, only two hippocampus-related memory tasks and electrophysiological changes were investigated in this study. In addition to its high expression in the hippocampus, H3 receptors are also widely distributed in brain regions including the cerebral cortex, basal ganglia, globus pallidus, and hypothalamus that are implicated in learning and memory processes [8]. Future research may reveal whether the effects found here can be generalized to nonhippocampal-dependent cognitive functions. Third, this study was performed in young adult animals, which cannot be translated to postoperative cognitive impairments seen in elderly humans in clinical situations. Further investigations with evaluating the effect of ciproxifan in animal model of postoperative cognitive dysfunction will be of more clinical significance.

In summary, we have observed that systemic administration of ciproxifan attenuated memory deficit and hippocampal synaptic plasticity impairment following isoflurane exposure. It is worth noting that in March 2016, the European Commission granted a

marketing authorization for pitolisant (as the first representative of the H3R inverse agonists) for the treatment of narcolepsy [51]. The waking action of Pitolisant in experimental animals and healthy human volunteers is associated with a shift of the power distribution pattern of the cortical EEG in favor of the high-frequency rhythms, which are known to accompany cognitive activities such as attention or learning [52]. A future investigation evaluating the efficacy of pitolisant in improving arousal and cognition after general anesthesia may be of greater clinical significance.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

The following supplementary material is available for this article:

Figure S1. Isoflurane and ciproxifan do not affect motor function, anxiety, and nociception in rats.