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Central histamine boosts perirhinal cortex activity and restores forgotten object memories

Short title: Histamine restores forgotten memories.

Authors

Hiroshi Nomura^{1,2*}, Hiroto Mizuta³, Hiroaki Norimoto^{1§}, Fumitaka Masuda¹, Yuki Miura¹, Ayame Kubo², Hiroto Kojima¹, Aoi Ashizuka³, Noriko Matsukawa³, Zohal Baraki¹, Natsuko Hitora-Imamura^{1,2}, Daisuke Nakayama¹, Tomoe Ishikawa¹, Mami Okada¹, Ken Orita¹, Ryoki Saito², Naoki Yamauchi², Yamato Sano⁴, Hiroyuki Kusunohara⁴, Masabumi Minami², Hidehiko Takahashi^{3*}, Yuji Ikegaya^{1,5}

Affiliations

¹Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan

²Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, 060-0812, Japan

³Department of Psychiatry, Kyoto University Graduate School of Medicine, Kyoto, 606-8501, Japan

⁴Laboratory of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, the University of Tokyo, Tokyo, 113-0033, Japan

⁵Center for Information and Neural Networks, National Institute of Information and Communications Technology, Osaka, 565-0871, Japan

*Correspondence to: hnomura@pharm.hokudai.ac.jp (H.N.); hidehiko@kuhp.kyoto-u.ac.jp (H.T.)

§Current address: Max Planck Institute for Brain Research, Frankfurt am Main, 60438, Germany

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Abstract

Background: A method that promotes the retrieval of lost long-term memories has not been well established. Histamine in the central nervous system is implicated in learning and memory, and treatment with antihistamines impairs learning and memory. Since histamine H₃ receptor inverse agonists upregulate histamine release, histamine H₃ receptor inverse agonists may enhance learning and memory. However, whether H₃ receptor inverse agonists promote the retrieval of forgotten long-term memory has not yet been determined.

Methods: Here, we employed multidisciplinary methods including the mouse behavior, calcium imaging and chemogenetic manipulation to examine whether and how the histamine H₃ receptor inverse agonists, thioperamide and betahistidine, promote the retrieval of a forgotten long-term object memory in mice. In addition, we conducted a randomized double-blind, placebo-controlled crossover trial in healthy adult participants to investigate whether a betahistidine treatment promote memory retrieval in humans (registration number: UMIN000015110).

Results: The treatment of H₃ receptor inverse agonists induced the recall of forgotten memories even 1 week and 1 month after training in mice. The memory recovery was mediated by the disinhibition of histamine release in the perirhinal cortex (PRh), which activated the histamine H₂ receptor. Histamine depolarized PRh neurons, enhanced their spontaneous activity, and facilitated the reactivation of behaviorally activated neuronal ensembles. A human clinical trial revealed that treatment of H₃ receptor inverse agonists are specifically more effective for items that are more difficult to remember and subjects with poorer performance.

Conclusions: These results highlight a novel interaction between the central histamine signaling and memory engrams.

Introduction

Forgotten memories may occasionally be recollected spontaneously. Even after the memories fade over time, the forgotten memories may persist latently in the brain. Therefore, reinforcement of positive modulators for retrieval of long-term memory may recover the ostensibly forgotten memories. Indeed, very few animal studies have successfully recovered retrograde amnesia in animals. Chronic treatment with a histone deacetylase inhibitor recovers forgotten fear memory (1). Optogenetic activation of memory engram neurons also restores forgotten fear memory (2). However, these studies needed long-term and/or highly invasive manipulation. Thus, a clinically applicable method that promotes the retrieval of forgotten long-term memories has not yet been established.

Histamine in the central nervous system is produced mainly in the tuberomammillary nucleus, and is implicated in learning and memory as well as sleep and wakefulness, feeding and drinking, and neuroendocrine regulation (3). For instance, treatment with antihistamines not only produces drowsiness but also impairs learning and memory (4–6). Histamine H₃ receptors are located primarily in the axon terminals and somata of neurons, and inhibit the presynaptic release of histamine and other neurotransmitters, and negatively regulate histamine synthesis (7). Since histamine H₃ receptors are constitutively active, their inverse agonists upregulate histamine release (8). Therefore, histamine H₃ receptor inverse agonists may enhance learning and memory. Indeed, several pioneering studies have found that histamine H₃ receptor inverse agonists enhance memory performance (9–15). However, whether H₃ receptor inverse agonists promote the retrieval of forgotten long-term memory has not yet been determined, as indicated by the following reasoning. Firstly, since many of the previous studies administered H₃ receptor inverse agonists before or shortly after training, their results demonstrate the drug effect on memory acquisition and/or consolidation but not retrieval (9, 10). Secondly, in some studies examining the drug effect on memory retrieval, basal memory

performance was high without administration of H₃ receptor inverse agonists since they employed aversive learning tasks (11, 12). Thus, they could not examine the drug effect on forgotten memories. Thirdly, the other studies successfully examined the drug effect on retrieval of forgotten memories, but they targeted short-term (1–2 h), but not long-term (24 h or longer), memories (13–15). Fourthly, all the previous studies targeting memory retrieval have tested memory performance within only 1 day of training (11–15). From a clinical view, it is important to know whether H₃ receptor inverse agonist is effective long after training and forgetting. More importantly, it is unclear whether H₃ receptor inverse agonist affects human long-term memory. Previous studies have shown that H₃ receptor inverse agonists have no effect on the performance in memory-related tasks (16–18). Taken together, it is unclear as to whether and how H₃ receptor inverse agonists promote retrieval of forgotten long-term memory.

In the present study, we examined whether the histamine H₃ receptor inverse agonists, thioperamide and betahistidine, promote the retrieval of a forgotten long-term object memory in mice and humans. The treatment induced the recall of forgotten memories even 1 week and 1 month after training through disinhibition of histamine release in the perirhinal cortex (PRh) in mice. Histamine depolarized PRh neurons, enhanced their spontaneous activity, and facilitated the reactivation of behaviorally activated neurons. Moreover, in a human clinical trial, betahistidine treatment enhanced the retrieval of object recognition memory.

Materials and methods

Animals

Animal experiments were performed with the approval of the animal experiment ethics committee at the University of Tokyo (approval number: 24-10) and Hokkaido University (approval number: 16-0043) and according to the University of Tokyo and Hokkaido University guidelines for the care and use of laboratory animals.

Novel object recognition task

In a training session, mice were placed in the field, in which two identical objects were positioned. Mice were left to explore the objects for 15 min. In a test session, the mice explored the open field for 5 min in the presence of one familiar and one novel object. A discrimination ratio was calculated for each mouse as the ratio $(T2-T1)/(T1+T2)$ [$T1$ = time spent exploring the familiar object, $T2$ = time spent exploring the novel object].

Human study design and treatments

This study was approved by the Committee on Medical Ethics of Kyoto University, registered with the UMIN Clinical Trials Registry (UMIN000015110) and was carried out in accordance with The Code of Ethics of the World Medical Association. The experimental design was a double-blind randomized placebo-controlled crossover trial ($n = 38$ subjects). On the first day, all participants received training on the paired-associate learning task and object recognition behavior task. On the 8th day, the participants in Group A were orally administered 9 capsules of betahistine mesilate (total 108 mg), and those in Group B were orally administered 9 capsules of placebo. Thirty minutes after the drug administration, they underwent the paired-associate learning task, the object recognition behavior task, the digit sequencing task, and the symbol coding task. On the 10th day, conversely, the participants in Group A were

administered the placebo and those in Group B were administered betahistine mesilate. They underwent the same tasks as on the 8th day, 30 min after the oral administration.

Object recognition task in human

On the training day, the participants viewed 128 pictures of objects and decided whether the picture depicted an indoor or an outdoor item. On the test days, the participants were shown the 32 same images that they viewed on the training day, 32 new images and 32 images that were similar but not identical to previously shown images. They were instructed to decide whether each image was "old," "new," or "similar." A different list of images was administered on a different test day.

Additional information is provided in Supplementary information.

Results

Histamine H₃ receptor inverse agonists induce the recall of forgotten memories.

We employed the novel object recognition task, wherein the test session mice were presented with a novel and a familiar object that was presented during the training session. A different set of mice were exposed to the test session at the different time points after the training session. When an interval between the training and test sessions was within 1 day, mice preferentially explored the novel object (Figure 1A and Supplemental Figure S1A). At an interval of 3 days, however, they were unable to discriminate the novel object from the familiar one. Therefore, in following experiments, we used 3 days or longer as an interval between training and test.

To examine whether a treatment of thioperamide, an H₃ receptor inverse agonist, promotes the retrieval of forgotten object memories, we intraperitoneally administered thioperamide (10 or 20 mg/kg) to mice 30 min before the test session on day 3, 14 or 28 after training. Thioperamide enhanced the discrimination ratio between the novel and familiar objects in a dose-dependent manner, and this retrieval-enhancing effect of thioperamide was observed on days 3, 14, and 28 after the training (Figure 1B-D and Supplemental Figure S1B-D). Distance moved during the test session was comparable across groups (Supplemental Figure S2A-C). To test whether thioperamide injection increases general exploration time, mice underwent another test session where two identical familiar objects were presented. Thioperamide injection had no effect on total exploration time (Figure 1E). Thioperamide treatment also had no effect on locomotor activity in open field test and anxiety-like behavior in elevated plus maze (Supplemental Figure S2E, F). The retrieval-enhancing effect of thioperamide was transient because mice did not discriminate the novel and familiar objects 1 day after thioperamide injection (Figure 1F and Supplemental Figure S1E). We also examined the effect of betahistidine, another structurally irrelevant H₃ receptor inverse agonist with a weak

H₁ receptor-stimulating effect. Mice that received betahistidine injection 30 min before the test significantly discriminated between the novel and familiar objects 1 week after the training (Figure 1G, S1F, S2D). To examine the thioperamide effect on unforgotten memories, we administered vehicle or thioperamide to mice 1 day after training. The vehicle-injected mice discriminated between the novel and familiar objects, and thioperamide injection had no effect on the discrimination ratio (Figure 1H, S1G, S2E). Taken together, the treatment of histamine H₃ receptor inverse agonist transiently promote the retrieval of the forgotten 3-days-old, 1-week-old and 1-month-old object memory in mice.

Thioperamide activates histamine release from the axon terminals by antagonizing H₃ receptors (8). The perirhinal cortex (PRh) is a critical brain region for the novel object recognition task (19), and a radioligand binding assay suggests H₃ receptor expression in the PRh (20). Indeed, we identified that the activation of histamine receptor signaling in the PRh mediated thioperamide-induced memory retrieval, based on the following four observations: i) we inhibited PRh activity through an intra-PRh injection of 250 ng muscimol, a GABA_A receptor agonist, at the same time when mice received an intraperitoneal thioperamide injection. The PRh inhibition prevented the thioperamide-induced memory retrieval (Figure 2A and Supplemental Figure S3A). ii) Intraperitoneal thioperamide injection increased the extracellular concentration of histamine in the PRh 30 min after the injection (Figure 2B). iii) Intra-PRh injection of thioperamide mimicked the effect of intraperitoneal thioperamide injection at 1-week test (Figure 2C and Supplemental Figure S3B). iv) An intra-PRh injection of ranitidine (H₂ receptor antagonist) blocked the intraperitoneal thioperamide-induced memory retrieval, whereas an intra-PRh injection of fexofenadine (H₁ receptor antagonist) did not (Figure 2D and Supplemental Figure S3C). Therefore, the activation of H₂ receptor in the PRh is responsible for thioperamide-induced memory retrieval.

Histamine enhances the spontaneous neuronal activity and reactivation of behaviorally activated neurons *in vitro*.

To examine the effect of histamine on the electrophysiological properties of PRh neurons, we performed whole-cell patch-clamp recordings of PRh neurons in acute neocortical slices. Since the effect of H₃ receptor inverse agonist on histamine release *in vitro* is much lower compared to *in vivo* (21), we applied histamine to the brain slices instead of thioperamide. Bath application of histamine depolarized the membrane potential (Figure 3A, B). In addition, we optically imaged the spike-triggered somatic calcium transients in PRh neurons in acute neocortical slices that received a bulk injection of Fura-2AM (Figure 3C, D). Histamine perfusion enhanced the overall rate of calcium transients, an effect that was blocked by 2 μ M ranitidine (Figure 3E, F).

We also recorded the calcium activity evoked by the field stimulation of the PRh cortical layer II/III every 20 s. Histamine perfusion did not increase the percentage of neurons responsive to the stimulation (baseline: $31 \pm 1.6\%$, histamine: $28 \pm 1.7\%$); however, it modulated the patterns of stimulus-evoked neuronal ensembles (Figure 4A). We quantified the stimulus-to-stimulus variability in stimulus-evoked ensembles by calculating the Euclidean distances between the vectors of the active cells. The matrix dataset of the Euclidean distances was dimension-reduced using multi-dimensional scaling (MDS) and was plotted in the two-dimensional space (Figure 4B). The MDS plot revealed that histamine altered the patterns of stimulus-evoked activity because the activity datasets under control conditions and histamine perfusion were separated in the MDS space; note that support vector machine with a Gaussian kernel were able to assign 30-trial data points accurately to the corresponding datasets with a F1 score as high as 0.90 ± 0.05 (mean $\pm$ SEM of 8 slices). More importantly, the evoked activity datasets under histamine perfusion were less dispersed in the MDS space than those under control conditions (Figure 4C). In addition, we computed correlations of stimulus-evoked

neuronal ensembles between stimulation trials. Histamine perfusion enhanced the correlations, and this correlation enhancement did not depend on the trial distance (Supplemental Figure S4). Taken together, repetitive stimulation stably recruited a more specific neuronal ensemble in the presence of histamine, compared to the control conditions, possibly through the enhanced spontaneous background activity (22).

Previous reports have demonstrated that the specific reactivation of neuronal ensembles that were activated during training leads to memory retrieval (23–25). We, thus, hypothesized that histamine facilitates the reactivation of behaviorally activated neurons. Using Arc-dVenus transgenic mice, in which a destabilized version of the fluorescent protein Venus was expressed in a neuronal activity-dependent manner (26), we probed the neurons that were active while the mice explored novel objects for 10 min. Percentage of dVenus⁺ neurons was higher in the training group compared to the home cage group (Figure 4D). We prepared neocortical slices and imaged the somatic calcium transients from dVenus⁺ and dVenus⁻ PRh neurons (Figure 4E). Under the control conditions, dVenus⁺ and dVenus⁻ neurons responded to the layer II/III stimulation with equal probability; however, during bath application of histamine, dVenus⁺ neurons were more frequently activated by the stimulation than dVenus⁻ neurons (Figure 4E). This preferential reactivation of dVenus⁺ neurons was prevented by 2 μ M ranitidine. Prior experience enhances synaptic strength and/or intrinsic excitability in specific neurons, which may contribute to the reactivation of memory-related neurons (27, 28). Synergistic effect of experience-dependent and cell-specific plastic changes and histamine-induced general activity enhancement may cause reactivation of dVenus⁺ neurons.

Chemogenetically increased spontaneous activity in the PRh neurons promote the retrieval of the forgotten memories

We asked whether increased baseline of neuronal activity in the PRh is sufficient for the improvement of memory retrieval. We virally targeted hM3Dq, the G_q-coupled excitatory designer receptor exclusively activated by designer drugs (DREADD) (29), to PRh neurons by intra-PRh injection of AAV-hSyn-hM3Dq-IRES-mCitrine (Figure 5A). The percentage of mCitrine⁺ neurons was $44.5 \pm 7.2\%$. Clozapine-N-oxide (CNO) selectively binds to hM3Dq and activates neurons through G_q signaling pathways. We confirmed that the membrane potentials in PRh neurons in neocortical slices were depolarized upon bath application of 10 μ M CNO (Figure 5B). To determine whether the activation of PRh neurons promotes the retrieval of a forgotten memory, mice that received an intra-PRh injection of either AAV-hSyn-hM3Dq or AAV-hSyn-EGFP were trained in the novel object recognition task and tested 1 week after the training. Intraperitoneal CNO injection (1 mg/kg) in mice that received AAV-hSyn-hM3Dq led to a significant increase in the discrimination between novel and familiar objects as compared to controls (Figure 5C and Supplemental Figure S5). The CNO injection presumably enhanced general network activity in the PRh because hM3Dq expression was in a random subset of PRh neurons but not targeted to those activated by learning.

Histamine H₃ receptor inverse agonist enhances retrieval of more difficult items and in subjects with poorer performance in humans.

Finally, we investigated whether the activation of the histaminergic system promotes memory retrieval in humans. We employed betahistine mesilate because it is widely prescribed for the clinical treatment of vestibular disorders. We conducted a randomized double-blind, placebo-controlled crossover trial in healthy adult participants.

During the training for the object recognition task, 38 participants incidentally studied serial images of 128 objects (Figure 6A). The recognition performance was tested 7 and 9 d

after the training (days 8 and 10, respectively). The participants were asked if they had seen the target items during the training. They were administered 108 mg betahistine or placebo orally 30 min before the tests started. Half of the participants were administered placebo on day 8 and betahistine on day 10, whereas the other half were administered betahistine on day 8 and placebo on day 10. Because the data within the treatment groups were not different between days 8 and 10, we pooled all results. We computed a generalized linear model (GLM) using a binomial distribution to fit the number of correct items with N as the number of participants and drug (placebo vs. betahistine). The GLM confirmed that betahistine treatment enhanced the overall correct ratio (odds ratio = 1.11) (Figure 6B) and that there is a significant participant $\times$ drug interaction effect. To further analyze the participant $\times$ drug interaction effect, the participants were divided into 6 groups according to the correct rate during placebo treatment. We computed another GLM using a binomial distribution on the number of correct items with group and drug. There was a significant group $\times$ drug interaction. Specifically, betahistine enhanced the correct rate of subjects that had poor performance under placebo treatment (Figure 6C). We also found that betahistine enhanced the correct rate of subjects with middle-range IQ (Supplemental Figure S6A). To analyze an effect of difficulty of a target item on a drug effect, we divided the target items into 6 difficulty levels according to a correct rate that was obtained when the participants received placebo. Specifically, betahistine improved the correct rate for difficult items (Figure 6D). In contrast, betahistine reduced the correct rate of subjects that had better performance under placebo treatment and subjects with low and high IQ, and it reduced the rate for easy items. Incidentally, betahistine did not alter working memory, attention or paired associate memory (Supplemental Figure S6B-D).

Discussion

Memories persist latently in the brain even after they fade out due to the passage of time, treatment of amnesic drugs or neurodegeneration. Although a few animal studies have shown that several experimental manipulations recover the forgotten memories (1, 2), they need long-term and/or highly invasive manipulation. In this study, we found that a treatment of histamine H₃ receptor inverse agonists promotes retrieval of apparently forgotten memories. A single treatment followed by retrieval test was sufficient for the improvement of memory retrieval. The treatment with betahistine mesilate has a high level of safety and was effective to humans as well as mice.

The upregulated histamine release and the following activation of histamine H₂ receptor contribute to the increase in PRh spontaneous activity, which promotes memory retrieval. We showed that thioperamide enhances histamine release in the PRh (Figure 2). *In situ* hybridization data (Allen Mouse Brain Atlas) reveals histamine H₂ receptor expression in the PRh (30). Histamine perfusion depolarized a membrane potential (Figure 3) and decreases calcium-activated potassium conductance in an H₂ receptor-dependent manner (31), both of which contribute to the histamine's excitatory effect. Histamine H₂ receptor antagonist blocked histamine-induced increase in spontaneous neuronal activity (Figure 4) and thioperamide-induced memory retrieval (Figure 2). Taken together, these findings suggest that thioperamide increases PRh spontaneous activity through upregulated histamine release and activation of histamine H₂ receptor and promotes retrieval of the apparently forgotten memories. The increase in PRh spontaneous activity is sufficient to promote the memory retrieval because CNO injection in mice that received AAV-hSyn-hM3Dq in the PRh improved the memory retrieval (Figure 5).

Reactivation of memory engram neurons underlie memory retrieval. Neurons activated during memory formation are reactivated during memory test (24, 25, 32). The reactivation is

observed in the cerebral cortex as well as the hippocampus and amygdala (33, 34). Ratio of the reactivation correlates with performance at the memory test (24, 25, 35). In addition, artificial reactivation of neurons activated during training triggers the memory retrieval (23). In this study, we found that histamine perfusion increased reactivation of PRh neurons that were activated during training (dVenus⁺ neurons). The PRh neurons that were not activated during training were not sensitive to histamine perfusion. Boosting the reactivation could underlie thioperamide-induced memory retrieval. Although our physiological analysis in brain slices provided an experimental evidence showing that histamine enhances the reactivation of memory-related neurons, we note that there is a gap between the behavioral and physiological experiments. In future, long-lasting labelling of memory-related neurons would be better to perform physiological experiments 3-10 days after training, in which mice are not able to discriminate between novel and familiar objects.

Stochastic resonance (36) is a possible mechanism by which enhanced spontaneous activity promotes the retrieval of forgotten memories. The stochastic resonance is a phenomenon where adding non-zero noise to a subthreshold signal boosts detecting the signal in nonlinear physical and biological systems, including neuronal circuits (37). The possible mechanism by which enhanced spontaneous activity promotes the retrieval of forgotten memories through the stochastic resonance is as follows: (1) long after training, a recall cue is no longer strong enough to activate engram neurons, and this subthreshold activity of engram neurons is not enough for memory retrieval (24); (2) however, the activity of engram neurons exceeds a threshold level with support of enhanced background activity, leading to successful recall; (3) the activity of non-engram neurons does not exceed the threshold level because they do not receive an input of the recall cue. Indeed, we found that histamine perfusion increases overall spontaneous activity and concurrently enhances reactivation of behaviorally activated neurons and that both activation of histamine signaling and increase in spontaneous activity

promoted the retrieval of forgotten memories. In theory of stochastic resonance, adding non-zero noise to a subthreshold signal allows the signal to reach threshold while adding the noise to a suprathreshold signal leads to a low signal-to-noise ratio, which is conceptually consistent with our findings in the human object recognition task. Betahistine treatment enhanced retrieval of items that are more difficult to remember and in subjects with poorer performance. In contrast, betahistine treatment deteriorated retrieval of easier items and in subjects with better performance. The retrieval-enhancing effect is likely to depend on how they originally remember the items.

It is important to note that histamine and histamine H₃ receptor inverse agonist by themselves have no specificity to reactivate specific memories. In the test session of our novel object recognition task, mice were presented with novel and familiar objects in an open field. The specificity to reactivate the specific object memories is based on the exposure of the familiar object in the open field. Histamine boosts the overall neuronal activity in the PRh and probably support the recall cues to reactivate the memory-related neurons.

It has not been determined how object memories are stored and retrieved in neuronal ensembles in the PRh because most of studies for memory engram neurons have targeted the hippocampus and amygdala using fear conditioning. A long-standing view is that reduction of firing rate in the PRh encodes object familiarity on the basis of the findings that the firing rates are higher when a stimulus is novel (19). On the other hand, several newer studies showed that neuronal activity does not decrease over stimulus repetition in rats and monkeys (38–40). In addition, they reported that a subset of PRh neurons respond to specific objects (40). These object-selective neurons might be responsible for the storage and retrieval of object memories, although it cannot be concluded without manipulation of these neurons.

Histamine modulates an attentional state, which might affect performances in the object recognition test. Indeed, systemic injection of H₃ receptor inverse agonists enhances the

attentional state (41). However, the memory recovery in our study is unlikely to be due to the enhanced attentional states. First, local injection of thioperamide into the PRh enhanced retrieval of the forgotten memory. Second, intra-PRh injection of an H₂ receptor antagonist blocked the retrieval-enhancing effect of the systemic thioperamide treatment. Third, systemic thioperamide injection did not affect general exploration or locomotor activity. Moreover, betahistine treatment did not alter attention in human symbol coding task.

Histamine H₃ receptor is a promising target for treating cognitive dysfunction. Accordingly, previous studies examined effects of histamine H₃ receptor inverse agonist on human learning and memory but found little effect (16–18). The drug effects may depend on dose, task difficulty and memory type. First, we employed 108 mg betahistine mesilate, which is about 7 times as much as typical single dose, because we estimated that this dose is required to achieve the concentration of 1 nM betahistine, which is necessary for maximal H₃ receptor activation. Second, in object recognition memory task, betahistine treatment enhanced retrieval performance of items that are more difficult to remember and subjects with poorer performance possibly through stochastic resonance as discussed above. Third, object recognition memory was sensitive to betahistine treatment, whereas paired associate memory and working memory were not affected by the treatment. However, we do not exclude a possibility that H₃ receptor inverse agonists affect other types of memory because histamine neurons send fiber projections to almost all parts of the brain and because H₂ and H₃ receptors are distributed in many brain regions (3).

In conclusion, we propose the central histamine signaling as a potential target for reactivating forgotten object memories. Betahistine has an advantage of high safety (42); however, it also has disadvantages, including mixed inverse agonism/agonism and low efficacy (43). Currently, several new histamine H₃ receptor antagonists or inverse agonists are being

developed (44–46). These new drugs may improve memory retrieval impairments observed in various neuropsychiatric disorders.

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Disclosures

The authors report no biomedical financial interests or potential conflicts of interest.

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Figure legends

Figure 1. Histamine H₃ receptor inverse agonists induce the recall of forgotten memories.

(A) The discrimination ratio in the novel object recognition task decreased over time. Tukey's test after one-way ANOVA, $n = 7-8$ mice. (B-D) Intraperitoneal administration of thioperamide 30 min before the recall test increased the discrimination ratio. Tukey's test after one-way ANOVA, $n = 7-14$ mice. (E) Exploration for 2 familiar objects was not affected by thioperamide injection. (F) Thioperamide-induced memory recovery was within 1 day. (G) Pre-test injection of betahistidine also increased the discrimination ratio. (E-G) Student's t -test, $n = 6-9$ mice. (H) Thioperamide injection had no effect on the discrimination ratio before forgetting. $n = 10-12$ mice. $**P < 0.01$, $*P < 0.05$. Values are reported as mean $\pm$ SEM. See also Supplemental Figures S1, S2 and Supplemental Table S1.

Figure 2. Histamine H₂ receptor in the PRh mediates thioperamide-induced memory recovery.

(A) Local injection of muscimol into the perirhinal cortex (PRh) prevented memory retrieval induced by intraperitoneal 20 mg/kg thioperamide. Tukey's test, $n = 7-8$ mice. (B) Thioperamide injection increased histamine release in the PRh. Sidak's test after two-way repeated measures ANOVA, $n = 4$ mice. (C) Intra-PRh injections of thioperamide drove memory retrieval. Student's t -test, $n = 8$ mice. (D) Ranitidine (histamine H₂ receptor antagonist) but not fexofenadine (H₁ receptor antagonist) prevented thioperamide-induced memory recovery. Tukey's test, $n = 7-9$ mice. $**P < 0.01$, $*P < 0.05$. Values are reported as mean $\pm$ SEM. See also Supplemental Figure S3 and Supplemental Table S1.

Figure 3. Histamine enhances the spontaneous activity in PRh neurons.

(A, B) Bath application of 10 μ M histamine depolarized the membrane potential of the PRh layer V neurons. Paired t -test, $n = 13$ cells. (C, D) Raster plots of spontaneous calcium transients in individual PRh neurons before and during the bath application of 10 μ M histamine (C) or 10 μ M histamine + 2 μ M ranitidine (D) from a single representative slice. (E-F) Histamine enhanced the activity

frequency of individual neurons (E); however, it had no effect in the presence of 2 μ M ranitidine (F). Nested ANOVA where the cells were nested under the slices, $n = 423$ cells from 6 slices (E), 273 cells from 6 slices (F). $**P < 0.01$. Values are reported as mean $\pm$ SEM. See also Supplemental Table S1.

Figure 4. Histamine enhances reactivation of behaviorally activated neurons *ex vivo*. (A) Raster plots of transient calcium events in PRh neurons in response to field stimulation of the layer II/III (gray triangles at the top). (B) Stimulus-evoked responses of individual neurons were dimension-reduced using multi-dimensional scaling (MDS) and were plotted in the two-dimensional space. Each circle indicates a single stimulus trial. Open and closed circles indicate data before and during histamine application respectively. Note that closed circles are less dispersed than open circles. The data were from a representative slice. (C) Pooled data of all experiments ($n = 8$ slices). The mean interval from the nearest neighbors in MDS was reduced by histamine perfusion, indicating that histamine decreases the variability in stimulus-driven neuronal ensembles. Paired t -test. (D) More dVenus⁺ neurons were observed in the PRh of Arc-dVenus mice after training of novel object recognition test. Student's t -test, $n = 5$ mice. (E) Confocal imaging of Fura-2-loaded PRh slices of Arc-dVenus mice. (F) dVenus⁺ neurons participated more frequently in the stimulus-responsive neuronal ensembles than dVenus⁻ neurons during histamine perfusion, which was blocked by the co-application of ranitidine. Tukey's test after two-way repeated measures ANOVA, $n = 4-6$ slices. $**P < 0.01$, $*P < 0.05$. Values are reported as mean $\pm$ SEM. See also Supplemental Figure S4 and Table S1.

Figure 5. Chemogenetically increased spontaneous activity in the PRh neurons enhances memory recovery. (A) Either AAV-hSyn-eGFP or AAV-hSyn-hM3Dq was injected into the PRh. (B) A representative patch clamp recording from a single PRh neuron. Bath application of CNO depolarized PRh neurons in brain slices. (C) Pre-test chemogenetic activation of PRh neurons via intraperitoneal CNO injection increased the discrimination ratio. In the controls,

white symbols indicate the hM3Dq/saline group, and black symbols indicate the eGFP/CNO group. Student's *t*-test, *n* = 12-16 mice. *****P* < 0.01**. Values are reported as mean ± SEM. See also Supplemental Figure S5 and Supplemental Table S1.

Figure 6. Histamine H₃ receptor inverse agonist enhances memory retrieval in humans.

(A) Object recognition task in humans. (B) Betahistidine treatment increased the overall correct ratio in the object recognition task. χ^2 test, *n* = 38 subjects. (C) Participants that had poor performance under placebo treatment were more sensitive to betahistidine treatment. χ^2 test. (D) Items that were difficult to remember were more sensitive to betahistidine treatment. χ^2 test. *****P* < 0.01**, ****P* < 0.05**. Values are reported as mean ± SEM. See also Supplemental Figure S6 and Supplemental Table S1.

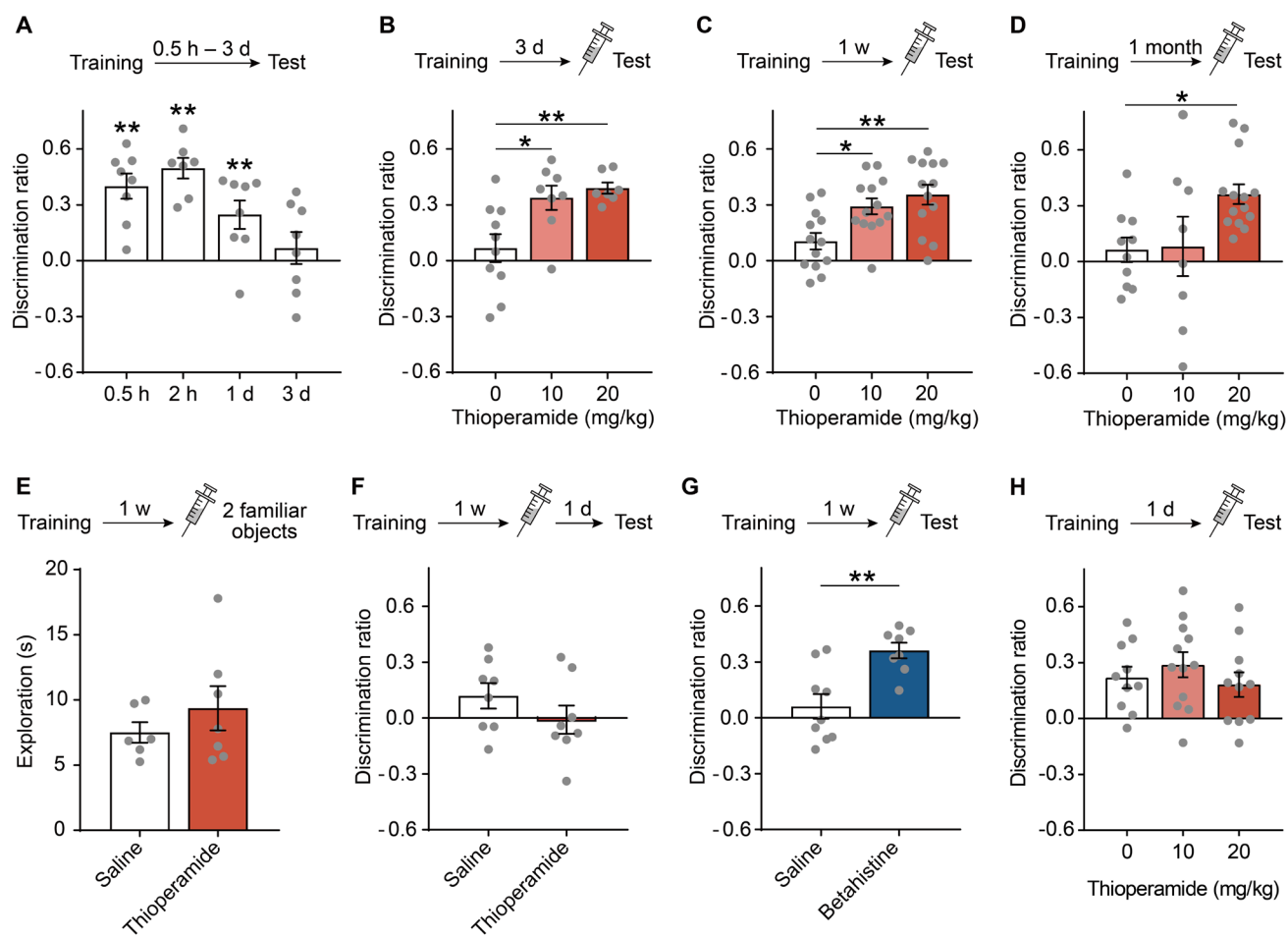


Figure 1

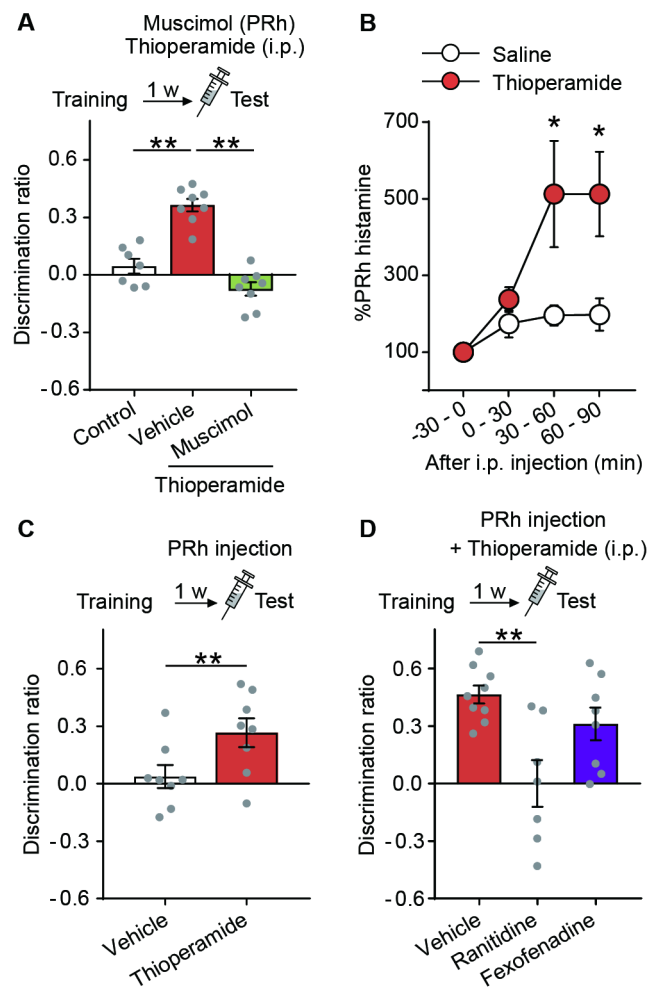


Figure 2

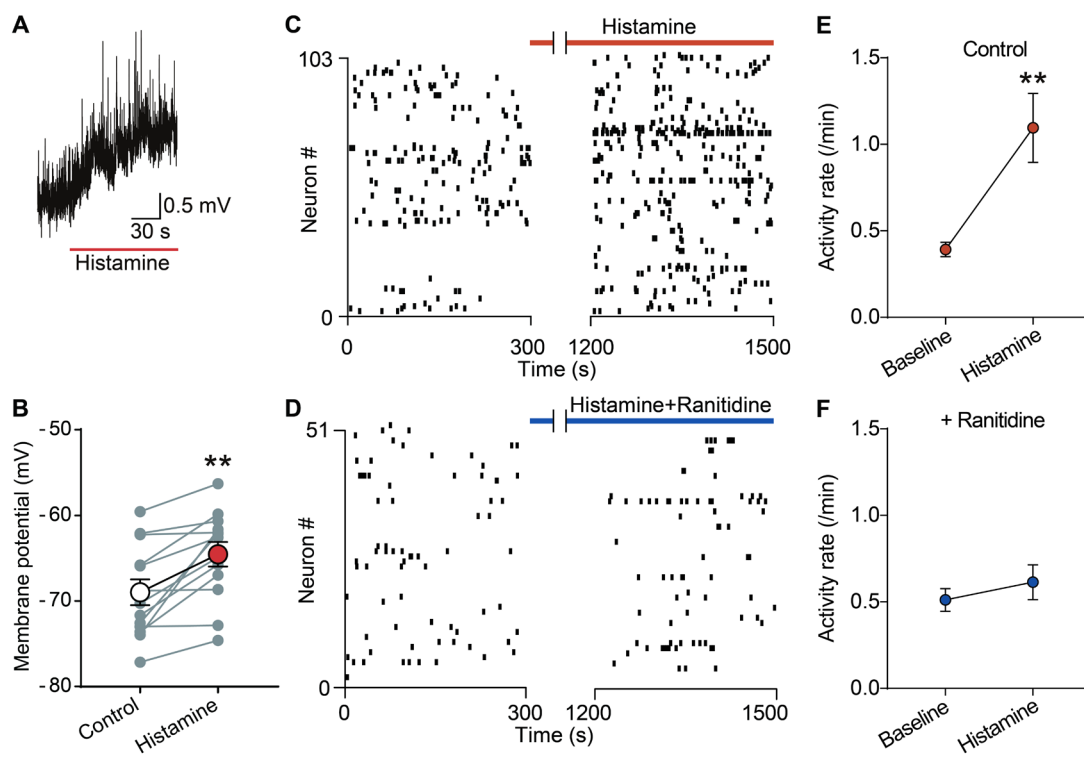


Figure 3

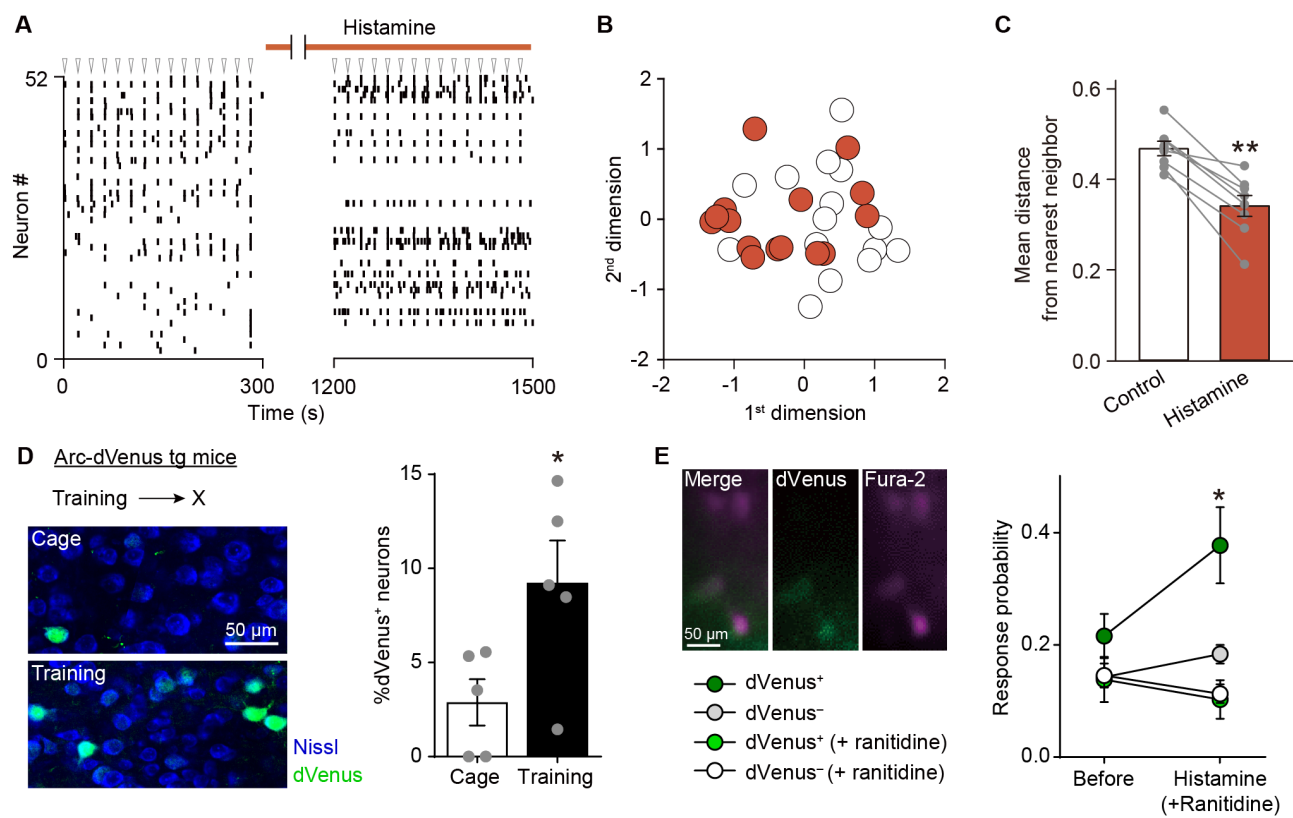


Figure 4

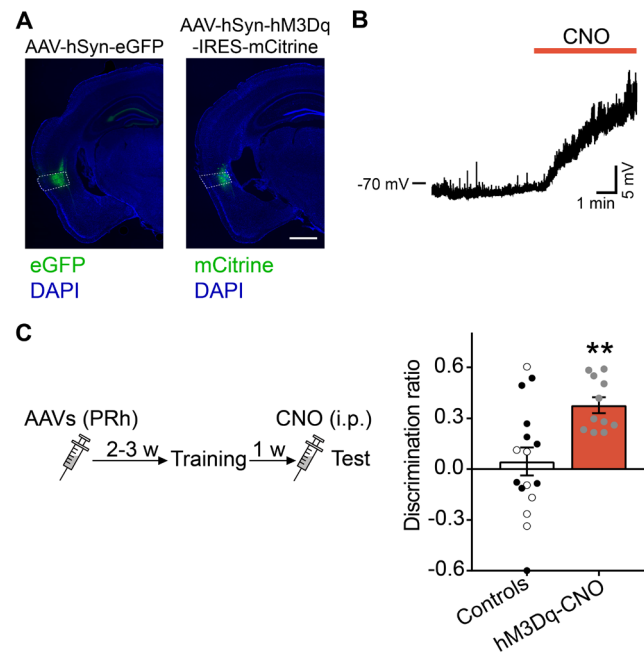


Figure 5

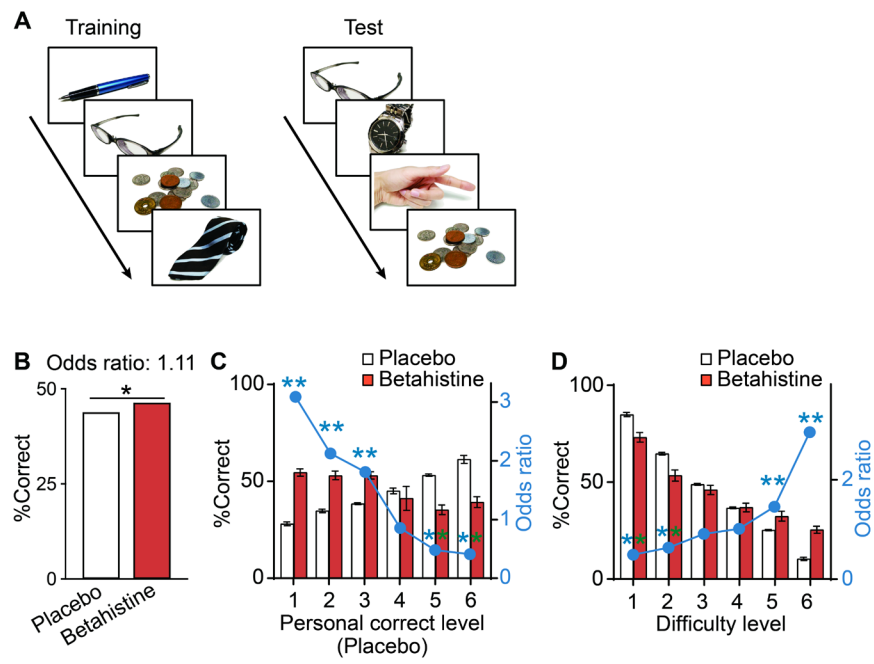


Figure 6

Central histamine boosts perirhinal cortex activity and restores forgotten object memories

Supplemental information

Supplemental Methods

Animals

Experimental protocols were carried out in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Ministry of Education, Culture, Sports, Science and Technology, Notice No. 71 of 2006), the Standards for Breeding and Housing of and Pain Alleviation for Experimental Animals (Ministry of the Environment, Notice No. 88 of 2006), and the Guidelines on the Method of Animal Disposal (Prime Minister's Office, Notice No. 40 of 1995).

Mice were housed 2–4 per cage and kept in a 12-h light/dark cycle (lights on from 7 a.m. to 7 p.m.). Adult male C57BL/6J mice (Japan SLC Inc., Shizuoka, Japan), weighing 20–30 g and aged 8–13 weeks, were used for the behavioral experiments. Male C57BL/6J mice aged 8–13 weeks were used for the electrophysiology experiments. Juvenile male or female C57BL/6J mice aged 3–4 weeks were used for the calcium imaging experiments. Juvenile male or female Arc-dVenus transgenic mice (1) aged 4 weeks were used for calcium imaging from behaviorally activated neurons. Mice were randomly assigned to different experimental groups without regard to any of their characteristics.

Novel object recognition task

The procedures for novel object recognition task were similar to those described previously (2). Mice underwent habituation sessions for 3 consecutive days, in which they explored the open field (47 cm × 47

cm × 47 cm) for 15 min. In a training session, mice were placed in the field, where two identical objects were placed in two adjacent corners, 5 cm from the walls. Mice were left to explore the objects for 15 min. In a test session, the mice explored the open field for 5 min in the presence of one novel and one familiar object. The two plastic objects had similar sizes but distinctive shapes. The objects used as novel or familiar ones were counterbalanced across mice. We ensured the lack of difference between the times spent exploring each object (exploration time for object A: 39.9 ± 4.9 s, exploration time for object B: 41.4 ± 4.9 s; Student *t*-test, *P* = 0.84). A discrimination ratio was calculated for each mouse as the ratio $(T1-T2)/(T1+T2)$ [T1 = time spent exploring the novel object, T2 = time spent exploring the familiar object]. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the object was not considered exploration. All trials were recorded by a camera, and the exploration time was measured by an experimenter blind to the treatment conditions.

In the experiment for Figure 1E, mice underwent a test session where two identical familiar objects were presented 1 week after the training for novel object recognition task. They received saline or thioperamide (20 mg/kg) injection 30 min before the test session.

In the experiment for Figure 1F, mice received saline or thioperamide (20 mg/kg) injection 1 week after the training for novel object recognition task. One day later, they were exposed to the normal test session.

In the experiment for Figure 4D, Arc-dVenus mice were killed either 5 h after the training for novel object exploration task or immediately after removal from the home cage.

Open-field test

The procedures for open-field test was similar to those described previously (2). Immediately after placement of a mouse in the center of a square, white acrylic box (46 cm in width, 46 cm in length, and

25 cm in depth), its position was recorded with a camera that was installed above the center of the field. The total distance moved were calculated by Ethovision software (Noldus Information Technologies). In the experiment for Supplemental Figure S2E, mice received saline or thioperamide (20 mg/kg) injection 30 min before the open-field test.

Elevated plus maze test

An animal was placed in the center of a maze with four arms arranged in the shape of a plus sign. The maze consisted of a central quadrangle (8 cm wide and 8 cm long), two opposing open arms (8 cm wide and 25 cm long), and two opposing closed arms of the same size, equipped with 25-cm-high walls at the sides and the far end. At the beginning of each trial, an animal was placed on the central quadrangle facing a closed arm. The animal movements during a 5-min test period were recorded by a camera positioned above the center of the maze. The time spent in the open arms was determined by Ethovision software. Entry into an arm was defined as placement of all four paws in that arm. In the experiment for Supplemental Figure S2F, mice received saline or thioperamide (20 mg/kg) injection 30 min before the elevated plus maze test.

Drugs

For intraperitoneal (i.p.) injection of thioperamide (10 or 20 mg/kg, Sigma-Aldrich) and betahistine dihydrochloride (1 mg/kg, Sigma-Aldrich), they were dissolved in saline. Twenty mg/kg thioperamide was injected to mice unless otherwise specified. Clozapine N-oxide (CNO, Enzo Life) was dissolved in 0.5% dimethyl sulfoxide (DMSO)/saline. For microinfusion experiments, muscimol, BODIPY TMR-X Conjugate (0.25 µg per side, Invitrogen), ranitidine (16 µg per side, Sigma-Aldrich) and thioperamide (10 µg per side) were dissolved in phosphate buffered saline (PBS).

Microinfusions

Mice were anesthetized with pentobarbital (2.5 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), and 26-gauge stainless steel guide cannulas (Plastics One) were implanted in the perirhinal cortex (PRh) (A/P: –3.05 mm, L/M: ± 4.55 mm, D/V: –2.8 mm relative to the bregma). Cannulas were secured to the skull using a mixture of acrylic and dental cement, and 33-gauge dummy cannulas were then inserted into each guide cannula to prevent clogging. Mice were administered at least 7 d of postoperative recovery time. The solutions (0.5 μ L per side) were infused with a pump over 2 min, and the infusion cannulas (28 gauge, extending 0.5-mm below the guide cannula) were left in place for at least 2 min afterward to facilitate the diffusion of solutions.

In vivo microdialysis

Under pentobarbital and xylazine anesthesia, guide cannula (CXG-X, Eicom, Kyoto, Japan) were unilaterally implanted aimed at the PRh. These were secured to the skull using a mixture of acrylic and dental cement, and dummy cannulas were then inserted into each guide cannula to prevent clogging. Mice were recovered for at least 7 days postoperatively. After recovery, the microdialysis probe (CX-I-6-01, membrane length 1 mm, Eicom, Kyoto, Japan) was implanted. The PRh was perfused with Ringer's solution containing: 147 mM NaCl, 4 mM KCl, and 2.25 mM CaCl₂ at 2 μ L/min. Immediately after the implantation, the probe was perfused for 120 min to stabilize the histamine release. Then, fractions were collected every 30 min for 2 h prior to drug infusion to confirm the stability of the histamine concentration, and the last fraction was used to calculate the baseline histamine concentration. Thioperamide (20 mg/kg) or saline were administered intraperitoneally, and fractions were collected for additional 90 min. For *in vitro* assay, the probe was immersed in Ringer's solution containing 3 μ M histamine and 300 nM

thiopiperamide. Fractions were collected for 30 min at 37°C with the perfusion rate 2 μ L/min. The histamine content was determined by an LC-MS/MS method. An AB SCIEX QTRAP 5500 mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a Prominence LC system (Shimadzu, Kyoto, Japan), operated in the electron spray ionization mode, was used for the analysis. Chromatographic separation was achieved with a PC HILIC column (150 mm $\times$ 2.0 mm, particle size 3 μ m), supplied by SHISEIDO (Tokyo, Japan). The flow rate was 0.3 mL/min; eluent A was 20 mM ammonium acetate/0.2% formic acid, and eluent B was acetonitrile. The elution gradient was started at 90% of eluent B, decreasing it to 30% for 1.25 min, maintained at 30% for 3.25 min, then increased to 90% in a 0.1 min, and maintained at 90% for 1.4 min. Histamine-d4 was used as an internal standard. For the selection of the precursor ions in MS, the following m/z values were used; histamine: 112.2, histamine-d4: 116.1. After the selection, the product ions from target compound were detected with the following m/z values; histamine: 95.1, histamine-d4: 99.0.

Chemogenetic activation of PRh neurons

AAV8-hSyn-HA-hM3D(Gq)-IRES-mCitrine (2×10^{12} vg/mL) and AAV8-hSyn-EGFP (3.9×10^{12} vg/mL) were purchased from the UNC Vector Core service (The University of North Carolina Gene Therapy Center). AAV8-hSyn-hM3D(Gq)-mCherry (2×10^{12} vg/mL) was a gift from Bryan Roth (Addgene viral prep #50474-AAV8). The group “AAV-hSyn-hM3Dq” were injected with either AAV8-hSyn-HA-hM3D(Gq)-IRES-mCitrine or AAV8-hSyn-hM3D(Gq)-mCherry. Mice were anesthetized using pentobarbital and xylazine or isoflurane and placed in a stereotaxic apparatus. The virus (0.3 μ L/site) was injected into the bilateral PRh (A/P: -2.2 mm, L/M: $\pm$ 4.1 mm, D/V: -3.8 mm and A/P: -3.3 mm, L/M: $\pm$ 4.5 mm, D/V: -3.8 mm relative to the bregma) at a rate of 0.1 μ L/min. The infusion cannulas (33 gauge) were left in place for at least 10 min afterward to facilitate the diffusion of solutions. In the

experiment for Figure 5 and S5, mice received intraperitoneal injections of either CNO (1 mg/kg in 0.5% DMSO/saline) or vehicle (0.5% DMSO/saline) 30 min before the start of behavioral experiments.

Immunohistochemistry

Mice were deeply anesthetized with pentobarbital and perfused intracardially with PBS followed by 4% paraformaldehyde. The brains were removed and stored in the same fixative for 24 h at 4°C and subsequently immersed in 15% and 30% sucrose for 24 h and 48 h respectively at 4°C. The immunohistochemical staining was performed on 40- μ m thick free-floating sections that were prepared using a cryostat (CM3050X, Leica).

The sections were incubated with 0.1% Triton X-100 for 15 min and with 2% BlockAce (DS Pharma Biomedical, Osaka, Japan) for 1 h. mCitrine was visualized with an anti-green fluorescent protein primary antibody (#ab6673, Abcam) and AlexaFluor-488 donkey anti-goat IgG secondary antibody (#A-11055, Thermo Fisher Scientific). Nuclei were counterstained with DAPI (#H-1500, Vector Laboratories). Images were acquired using a fluorescence microscope (BZ-X710, Keyence) at 4X.

Slice preparation

Mice were deeply anesthetized with diethyl ether and decapitated. The brains were removed quickly, and coronal slices (400 μ m thick) containing the PRh were prepared with a vibratome (VT 1200S, Leica) in ice-cold, oxygenated cutting solution consisting of (in mM) 222.1 sucrose, 27 NaHCO₃, 1.4 NaH₂PO₄, 2.5 KCl, 1 CaCl₂, 7 MgSO₄, and 0.5 ascorbic acid. Slices were allowed to recover for at least 30 min submerged in a chamber filled with oxygenated artificial cerebrospinal fluid (aCSF) at 34°C. aCSF consisted of (in mM) 127 NaCl, 1.6 KCl, 1.24 KH₂PO₄, 1.3 MgSO₄, 2.4 CaCl₂, 26 NaHCO₃, and 10 D-

glucose for electrophysiology experiments or 127 NaCl, 3.5 KCl, 1.24 KH₂PO₄, 1.2 MgSO₄, 2.0 CaCl₂, 26 NaHCO₃, and 10 D-glucose for calcium imaging experiments.

Electrophysiology

Slices were transferred to a recording chamber, and superfused aCSF bubbled with 95% O₂/5% CO₂ (30–33°C, 1–2 mL/min). Whole-cell recordings were performed from visually identified, pyramidal neurons located in the layer V of the PRh using infrared differential interference contrast (IR/DIC) technique. Patch pipettes (3–6 MΩ) were fabricated from borosilicate glass and filled with a solution containing the following (in mM): 120 K-gluconate, 5 KCl, 10 HEPES, 1 MgCl₂, 10 phosphocreatine-Na₂, 2 MgATP, 0.1 Na₂GTP, and 0.2 EGTA (pH 7.2–7.3, 280–295 mOsm). Histamine (10 μM) was applied for 10 min, and its effect on membrane potential was examined before and during the histamine application. Data were sampled at 20 kHz and filtered at 2 kHz.

To confirm the chemogenetic activation of PRh neurons, PRh slices were prepared from mice that received an intra-PRh injection of AAV8-hSyn-hM3D(Gq)-mCherry. Membrane potentials were monitored in whole-cell configuration, and CNO (10 μM) was applied.

Optical recording

The procedures for optical recording were similar to those described previously (3). Functional multineuron calcium imaging from the layer V neurons in the PRh was conducted locally loading with Fura-2AM. Fura-2AM was dissolved in DMSO containing 10% Pluronic F-127 to yield a concentration of 200 μM. Fluorophores were excited at 405 nm with a laser diode and visualized using 507-nm-long pass emission filters. Videos were recorded at 50 frames/s using a 16X objective (0.8 numerical aperture, Nikon), a spinning-disk confocal microscope (CSU-X1; Yokogawa Electric, Tokyo, Japan), a cooled CCD

camera (iXonEM+DV897; Andor Technology, Belfast, UK), and an upright microscope (Eclipse FN1; Nikon, Tokyo, Japan). The fluorescence change was measured as $(F_t - F_0)/F_0$, where F_t is the fluorescence intensity at time t and F_0 is the fluorescence intensity averaged from -10 to 10 s relative to t . Spike-elicited calcium transients were semiautomatically detected with a custom-written program in Visual Basic (4).

To examine the effect of histamine perfusion on spontaneous neuronal activity, a baseline activity was recorded for 5 min, and then 10 μ M histamine was applied. The activity during histamine perfusion was recorded from 15 min to 20 min after the initiation of histamine perfusion. In experiments for testing whether the histamine effect on spontaneous activity depended on H_2 receptor activation, 2 μ M ranitidine was applied 5 min before histamine perfusion.

To examine the histamine effect on the precision of evoked activities, monopolar rectangle electrical pulses (50 μ s, 100–300 μ A) were delivered every 20 s using a borosilicate glass pipette filled with aCSF. An electrode was placed in the layer II/III of the PRh. After a baseline evoked activity was recorded for 5 min, 10 μ M histamine was applied, and the activity during histamine perfusion was recorded from 15 min to 20 min after the initiation of histamine perfusion. We had 15 stimulation trials before histamine perfusion and 15 stimulation trials during histamine perfusion. A vector of evoked activities consisted of the responses of individual neurons to the stimulation (active or silent). The bin size of the active/silent evaluation was 1 sec. We collected 15 vectors for a basal level and 15 vectors for histamine perfusion from each brain slice. We calculated the Euclidean distance between each pair of vector's Z-score and applied the conventional nonmetric multidimensional scaling (MDS), a dimension reduction technique for illustration purpose, to these pairwise distances. The MDS plot (Figure 4B) indicates the relative pairwise distances between the vectors. Each point in Figure 4B corresponds to a single vector which indicates evoked activities in a single stimulation trial. The distance from the nearest neighbor of individual trials was calculated and averaged across trials in each slice. For each slice, all 30 trials were labelled as 0

(control) or 1 (histamine) and were classified using nonlinear support vector machine with a Gaussian kernel, a supervised machine learning. The data separation was expressed by the F1 score. Figure 4C indicates the mean distance from all slice experiments ($n = 8$ slices). For Supplemental Figure S4, we computed correlations between each pair of the vectors, calculated the mean of the correlations for each slice across different trial distances, and plotted the data from all slice experiments.

In experiments for optical recording from behaviorally activated neurons, Arc-dVenus transgenic mice were exposed to novel objects and were allowed to explore for 10 min. They were subsequently returned to their home cages. After 120 min, acute slices were prepared. dVenus was excited at 488 nm and visualized using a 520/535-nm band-pass emission filter. Monopolar rectangle electrical pulses (50 μ s, 100–300 μ A) were delivered every 20 s using a borosilicate glass pipette filled with aCSF. An electrode was placed in the layer II/III of the PRh. After a baseline evoked activity was recorded for 5 min, histamine (10 μ M) was applied, and the activity during histamine perfusion was recorded from 15 min to 20 min after initiation of histamine perfusion. Ranitidine was perfused 5 min before the initiation of histamine perfusion. The response probability of individual dVenus⁺ and dVenus⁻ neurons was compared between the baseline and histamine perfusion.

Human subjects

Forty healthy volunteers participated in the study. After the complete description of the study, written informed consent was obtained from each participant. Experienced physicians assessed the participants and confirmed that none of them met the exclusion criteria. Intelligence Quotient (IQ) was measured by the Japanese Version of the National Adult Reading Test short form, and all participants' IQ were confirmed to be more than 80. The exclusion criteria were as shown below:

- 1) Active peptic ulcer, bronchial asthma, pheochromocytoma, Meniere's disease, Meniere's syndrome, vertigo, blood disorders, liver failure, kidney failure, and neurological and psychiatric disorders
- 2) History of peptic ulcer, drug dependence, and alcoholism
- 3) Administration of the drug within a week before the test
- 4) Pregnant or suspected of being pregnant female individuals, breast-feeding, male/female individuals who did not agree to the terms of contraception during the study
- 5) Drinking a fruit juice containing grapefruit, orange, or apple, between the first day and the 10th day of the test period
- 6) Participated in Phase 1 clinical trials of new drug substances and received the drug dosing within four months before the test
- 7) Seropositive of HBs antigen, HCV antibody, HIV antigen-antibody, syphilis serum (TP antibody, RPR method)
- 8) History of allergies to food or drugs
- 9) An individual who was judged by the investigator as an inappropriate subject of the study

Human study design and treatments

The experimental design was a double-blind randomized placebo-controlled crossover trial. Forty participants were randomly assigned to two groups with Fisher-Yates Shuffling algorithm. One group (Group A) consisted of 20 participants, with 7 female and 13 male subjects (mean age 25.0 ± 1.5 years, IQ 109.4 ± 1.7). Another group (Group B) consisted of 20 participants. Two participants dropped out due to the withdrawal of consent. Consequently, Group B included 18 participants with 9 female and 9 male subjects (mean age 24.8 ± 1.5 years, IQ 108.2 ± 2.0).

On the first day, all participants received training on the paired-associate learning task and object recognition behavior task. On the 8th day, the participants in Group A were orally administered 9 capsules of betahistine mesilate (total 108 mg), and those in Group B were orally administered 9 capsules of placebo. Thirty minutes after the drug administration, they underwent the paired-associate learning task, the object recognition behavior task, the digit sequencing task, and the symbol coding task. On the 10th day, conversely, the participants in Group A were administered the placebo and those in Group B were administered betahistine mesilate. They underwent the same tasks as on the 8th day, 30 min after the oral administration.

Betahistine plasma concentration in humans is not available in the published literature. Instead, the plasma concentration of a betahistine metabolite, (2-pyridyl)acetic acid, have been monitored (5, 6). Both the maximum plasma concentrations ($C_{\max}$) and area under the plasma concentration time curve of (2-Pyridyl)acetic acid show linearity at 50, 100, and 200 mg (5). According to the unpublished information by Muntendam A (ABL B.V.) in the EBF 5th Open Meeting (Barcelona, Spain, 2012), which is available online

(http://bcn2012.europeanbioanalysisforum.eu/slides/day%203/viii%20ba%20clinic/4_muntendam.pdf), the $C_{\max}$ of betahistine and (2-pyridyl)acetic acid was stated to be approximately 200 pg/mL and 1000 ng/mL, respectively. Assuming this ratio is conserved, the dose of betahistine mesilate required to achieve the concentration of 1 nM betahistine was estimated to be 104 mg.

Object recognition task in human

On the training day, the participants viewed 128 pictures of objects and decided whether the picture depicted an indoor or an outdoor item. Each picture was displayed for 2 s. On the test days, the participants were shown the 32 same images that they viewed on the training day, 32 new images and 32 images that

were similar but not identical to previously shown images. They were instructed to decide whether each image was "old," "new," or "similar." Each image was displayed for 3 s. A different list of images was administered on a different test day.

Working memory task in human

We assessed the working memory using the digit sequencing task (7). Participants were presented with 28 clusters of numbers. The length of the numbers increased from 2 to 8 in 4 clusters. They were instructed to tell the experimenter the numbers in order from the lowest to highest. The number of correct responses was measured.

Attention task in human

We assessed attention with symbol coding task (7). Participants were presented with a table showing a relationship between numerals 1-9 and symbols. They were instructed to write numerals corresponding to the symbols on a response sheet. The number of correct numerals in 90 s was measured.

Paired-associate learning task in human

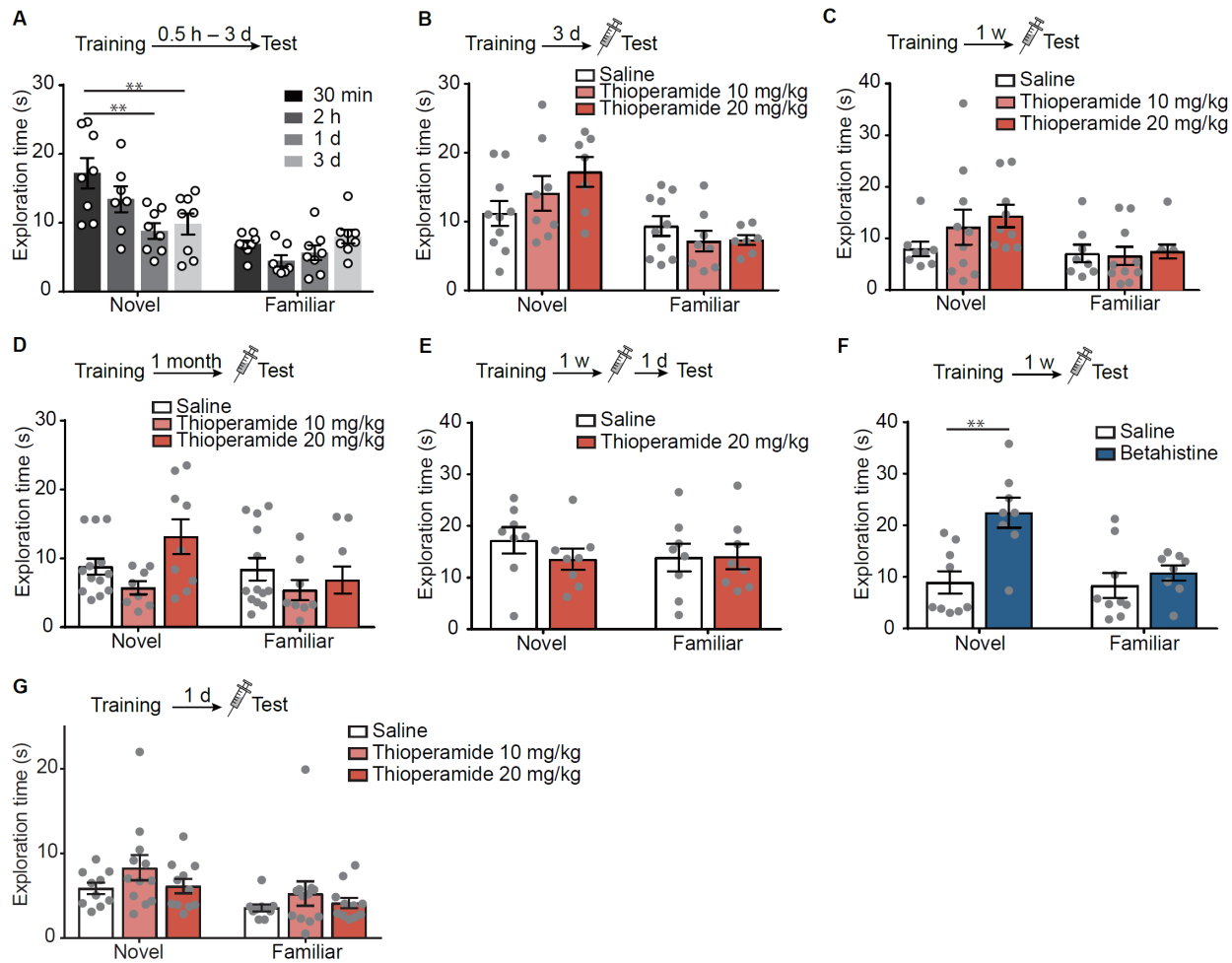
On the training day, the participants studied 70 word pairs with the instruction to remember the pairs. Each pair was displayed for 5 s. The first 2 pairs and last 2 pairs were excluded from the following tests. After the participants had completed their training, they underwent a test session, in which they were presented with a word from the pairs and instructed to input the paired word on the keyboard without a time limit. After they input the word, the correct word was displayed for 2 s. When participants completed 66 pairs, and the number of correct pairs was less than 53 (80% of total pairs), they were administered another round of the test session with a list of word pairs that they had answered incorrectly. They repeated

the test sessions until the total number of correct pairs reached 53 or more. On the test days, the participants were presented with a word from 33 of the pairs that they studied on the training day. The different list of word pairs was administered on a different test day. The correct answer was not displayed.

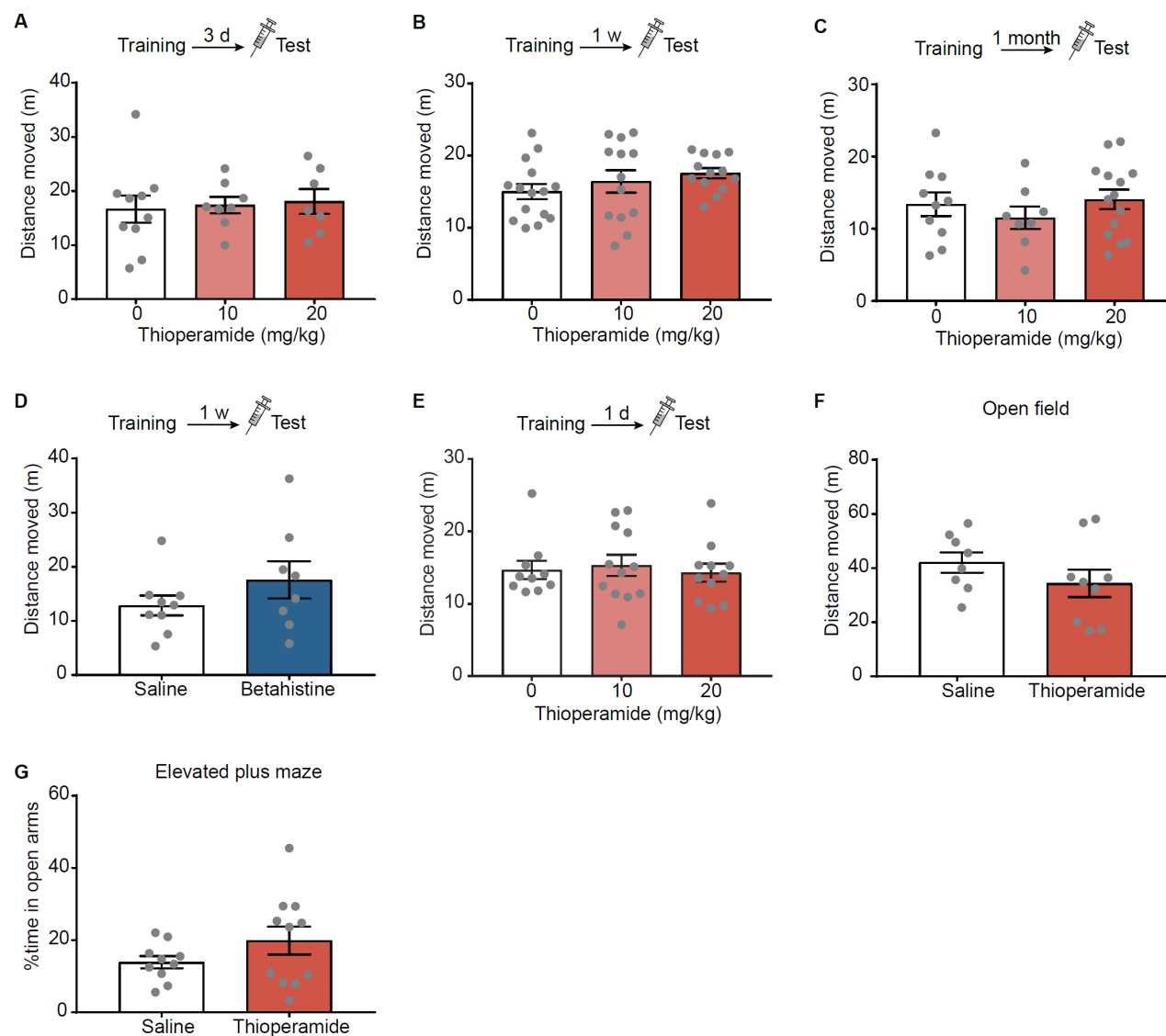
Statistical analysis

Values are reported as mean $\pm$ SEM (standard error of mean) unless otherwise indicated. Statistical analysis was performed using one-way analysis of variance (ANOVA), repeated-measures ANOVA, Tukey's test, Sidak's test, two-sided Student's *t*-test, two-sided paired *t*-test, and Chi-square test, where appropriate. The detailed statistics was described at Table S1. Parametric tests were used when distribution was assumed to be normal and variance was assumed to be similar, but these were not formally tested. The sample sizes were chosen to approximately match those of previous work, as there was no pre-specified effect size. To analyze a drug effect on the performance on human object recognition task, we computed a generalized linear model (GLM) (binominal logistic regression analysis) on the number of correct items.

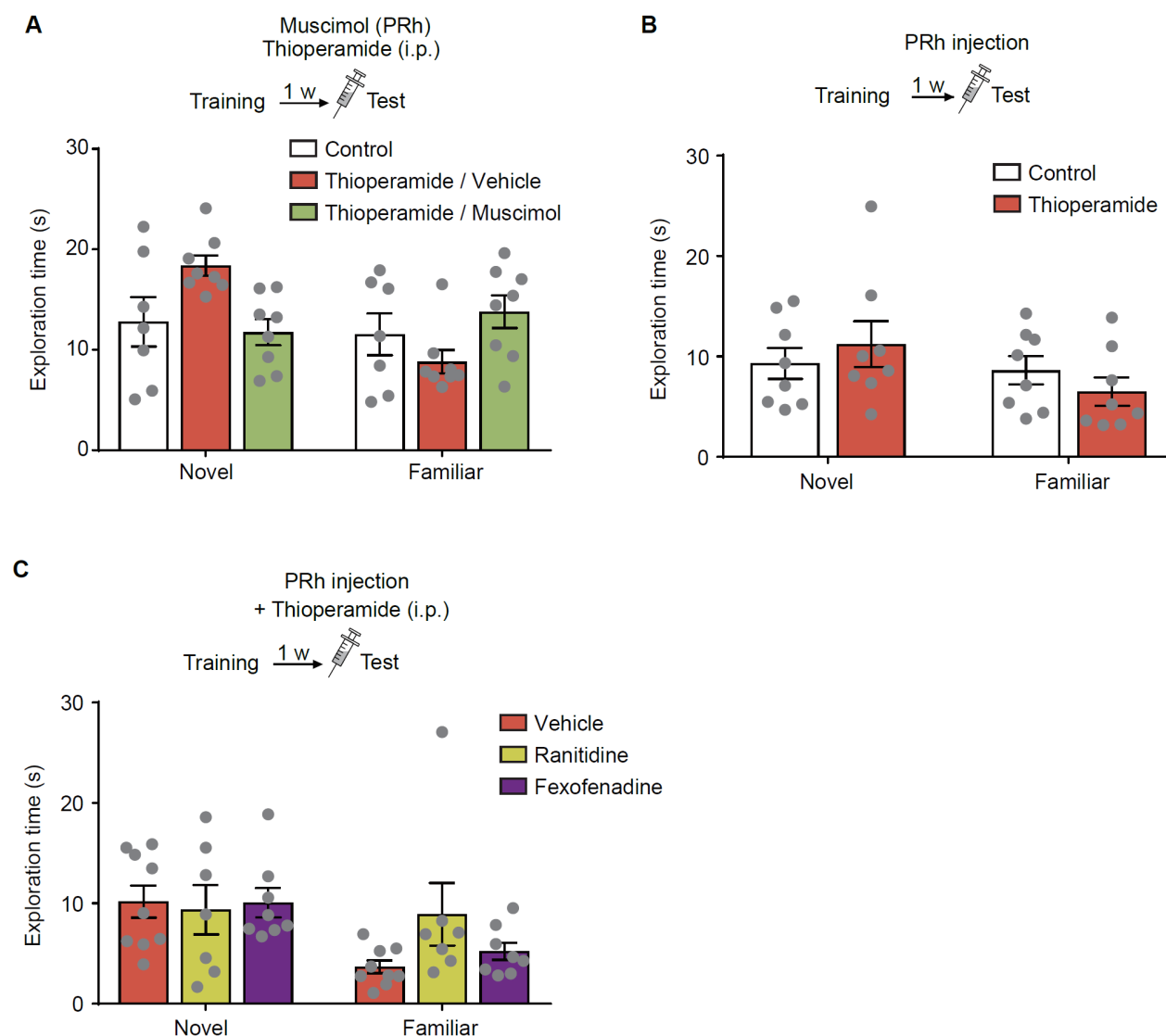
Supplemental Figures



Supplemental Figure S1 Exploration time for novel and familiar objects, Related to Figure 1. (A) Exploration time was measured at the test 0.5 h, 2 h, 1 d or 3 d after the training (related to Figure 1A). (B-D) Exploration time was measured after saline or thioperamide injection at 3 d (B), 1 w (C) and 1 month (D) test (related to Figure 1B, C, D). (E) Exploration time was measured 1 day after saline or thioperamide injection (related to Figure 1F). (F) Exploration time was measured after saline or betahistine injection at 1 w test (related to Figure 1G). (G) Exploration time was measured after saline or thioperamide injection at 1 d test (related to Figure 1H). Sidak's test after two-way repeated measures ANOVA. ** $P < 0.01$. Values are reported as mean $\pm$ SEM.

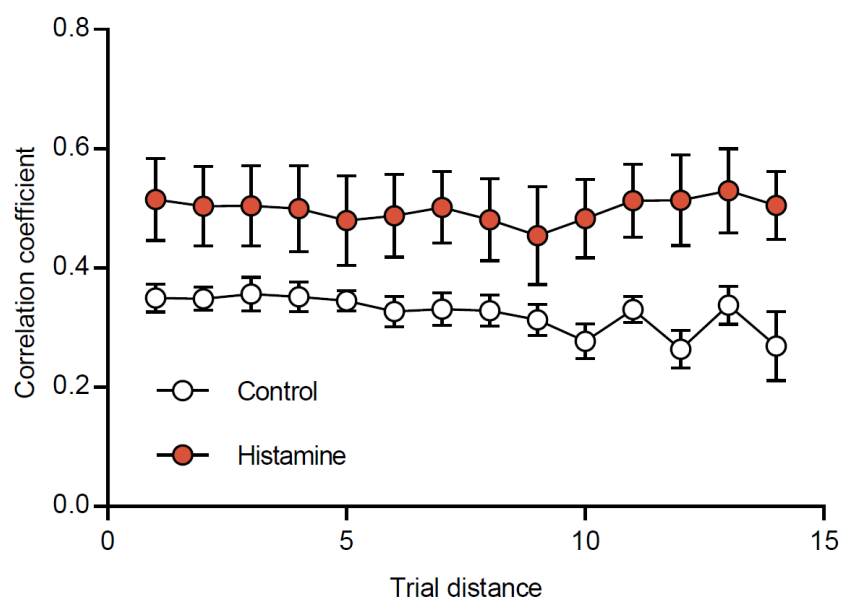


Supplemental Figure S2 Effects of thioperamide and/or betahistine on distance moved and anxiety-like behavior, Related to Figure 1. (A-C) Distance moved at novel object recognition test after saline or thioperamide injection (related to Figure 1B-D). (D) Distance moved at novel object recognition test after saline or betahistine injection (related to Figure 1G). (E) Distance moved at novel object recognition test after saline or thioperamide injection (related to Figure 1H). (F) Distance moved at open field test after saline or thioperamide injection (20 mg/kg). (G) Time spent in open arms at elevated plus maze after saline or thioperamide injection (20 mg/kg). Values are reported as mean $\pm$ SEM.

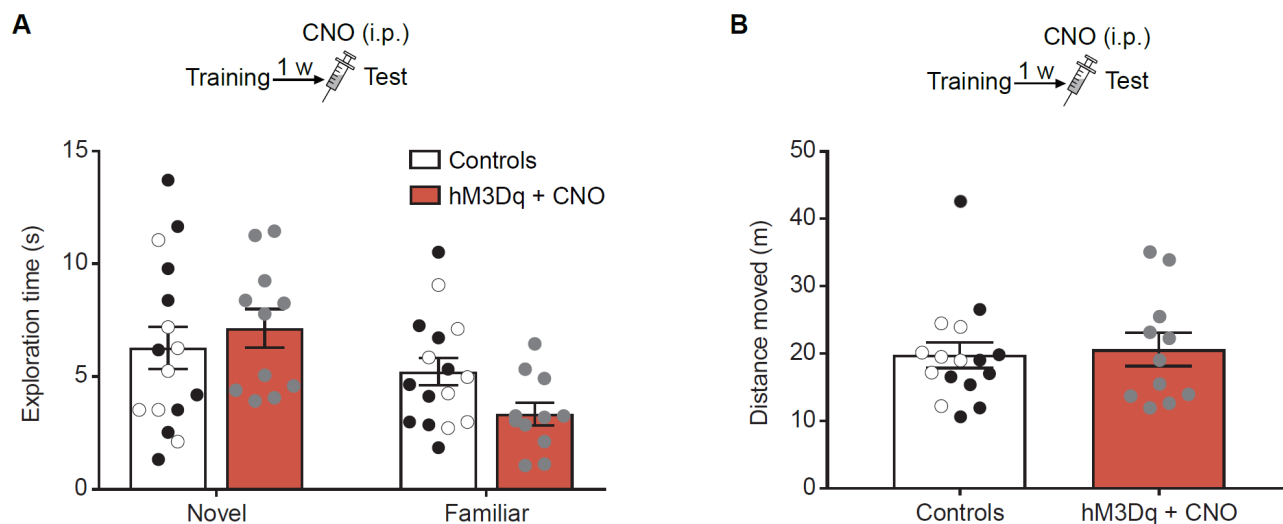


Supplemental Figure S3 Exploration time for novel and familiar objects, Related to Figure 2. (A)

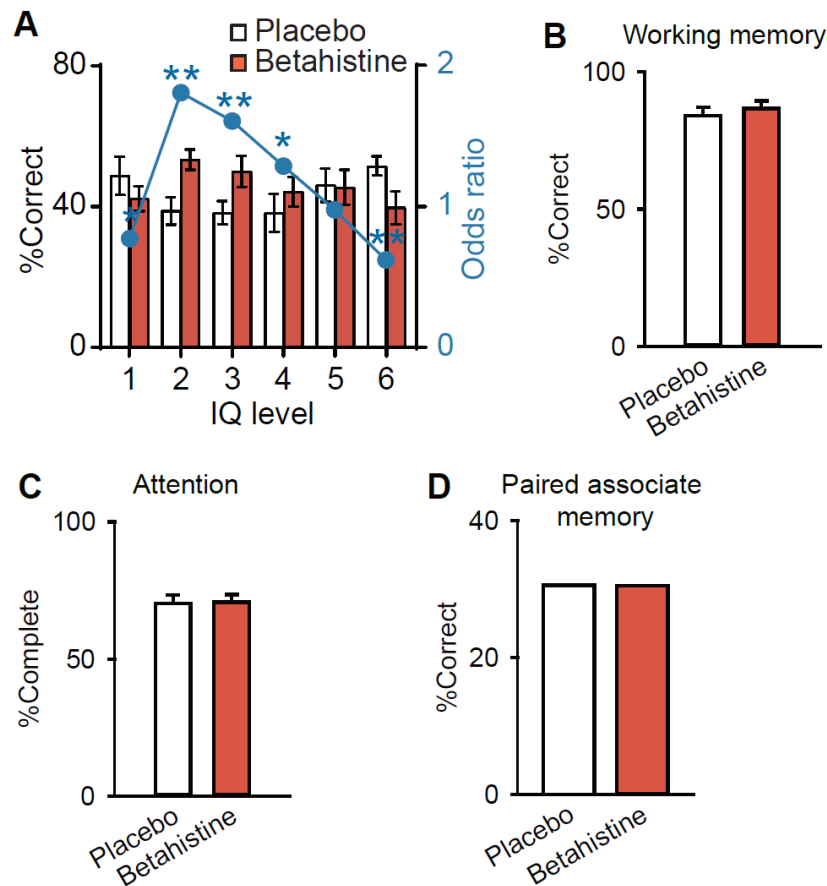
Exploration time was measured after saline, thiopiperamide, vehicle and/or muscimol injections (related to Figure 2A). (B) Exploration time was measured after vehicle or thiopiperamide injection into the PRh (related to Figure 2C) (C) Exploration time was measured after systemic thiopiperamide injection and intra-PRh injection of vehicle, ranitidine or fexofenadine (related to Figure 2D). Values are reported as mean $\pm$ SEM.



Supplemental Figure S4 Histamine perfusion enhances correlations of stimulus-responsive neuronal ensembles between stimulation trials, Related to Figure 4. The correlation coefficient of each pair of the vectors which indicate the responses of individual neurons to the stimulation was computed. The correlation enhancement did not depend on the trial distance. 2-way repeated measures ANOVA. $n = 357$ cells from 8 slices. Values are reported as mean $\pm$ SEM.



Supplemental Figure S5 The behavioral characteristics that is induced by chemogenetically increased PRh activity, Related to Figure 5. (A-B) Either AAV-hSyn-eGFP or AAV-hSyn-hM3Dq was injected into the PRh. (A) Exploration time was measured after saline or CNO injection (related to Figure 5C). (B) Distance moved at novel object recognition test after saline or CNO injection (related to Figure 5C). In the controls, white symbols indicate the hM3Dq/saline group, and black symbols indicate the eGFP/CNO group. Values are reported as mean $\pm$ SEM.



Supplemental Figure S6 Effect of betahistine on human long-term memory, working memory and attention, Related to Figure 6. (A) Participants with middle-range IQ were more sensitive to betahistine treatment. χ^2 test. * $P < 0.05$, ** $P < 0.01$. (B) Working memory was assessed with a digit sequencing task. Betahistine had no effect on the percentage of correct responses. (C) Attention was assessed with a symbol coding task. Betahistine had no effect on the percentage of correct numerals. (D) Betahistine had no effect on the percentage of correct responses in the paired-associate learning task. Values are reported as mean $\pm$ SEM.

Table S1. Statistical analysis, Related to Figure 1-6 and Supplemental Figure S1-6.

Figure 1A	Tukey's test after one-way ANOVA, $F_{3,27} = 6.42$, $P = 0.0020$ 0.5 h vs. 3 d: $*P = 0.015$, 2 h vs. 3 d: $**P = 0.020$ 0.5 h: $n = 8$, 2 h: $n = 7$, 1 d: $n = 8$, 3 d: $n = 8$
Figure 1B	Tukey's test after one-way ANOVA, $F_{2,22} = 7.41$, $P = 0.0035$ 0 mg/kg vs. 10 mg/kg: $*P = 0.017$, 0 mg/kg vs. 20 mg/kg: $**P = 0.0062$ 0 mg/kg: $n = 10$, 10 mg/kg: $n = 8$, 20 mg/kg: $n = 7$
Figure 1C	Tukey's test after one-way ANOVA, $F_{2,36} = 7.58$, $P = 0.0018$ 0 mg/kg vs. 10 mg/kg: $*P = 0.022$, 0 mg/kg vs. 20 mg/kg: $**P = 0.0018$ 0 mg/kg: $n = 13$, 10 mg/kg: $n = 13$, 20 mg/kg: $n = 13$
Figure 1D	Tukey's test after one-way ANOVA, $F_{2,29} = 4.17$, $P = 0.026$ 0 mg/kg vs. 20 mg/kg: $*P = 0.042$ 0 mg/kg: $n = 10$, 10 mg/kg: $n = 8$, 20 mg/kg: $n = 14$
Figure 1E	Student's t -test. $t_{11} = 0.94$. Saline: $n = 6$, Thioperamide: $n = 7$
Figure 1F	Student's t -test. $t_{14} = 1.26$. Saline: $n = 8$, Thioperamide: $n = 8$
Figure 1G	Student's t -test. $t_{15} = 3.72$, $**P = 0.0021$. Saline: $n = 9$, Betahistine: $n = 8$
Figure 1H	One-way ANOVA, $F_{2,30} = 0.72$, $P = 0.50$
Figure 2A	Tukey's test after one-way ANOVA, $F_{2,20} = 42.43$, $P < 0.0001$ Control vs. vehicle + thioperamide: $**P < 0.0001$, vehicle + thioperamide vs. muscimol + thioperamide: $**P < 0.0001$ Control: $n = 7$, vehicle + thioperamide: $n = 8$, muscimol + thioperamide: $n = 8$
Figure 2B	Sidak's test after two-way repeated measures ANOVA Time: $F_{3,18} = 12.65$, $P = 0.0001$; Group: $F_{1,6} = 5.66$, $P = 0.055$; Time x Group interaction: $F_{3,18} = 5.64$, $P = 0.0066$ Saline vs. Thioperamide; 30-60 min: $*P = 0.011$, 60-90 min: $*P = 0.012$ Saline: $n = 4$, thioperamide: $n = 4$
Figure 2C	Student's t -test. $t_{14} = 2.36$, $*P = 0.033$. Vehicle: $n = 8$, Thioperamide: $n = 8$
Figure 2D	Tukey's test after one-way ANOVA, $F_{2,21} = 7.65$, $P = 0.0032$ Vehicle vs. Ranitidine: $**P = 0.0024$ Vehicle: $n = 9$, Ranitidine: $n = 7$, Fexofenadine: $n = 8$
Figure 3A, B	Paired t -test. $t_{12} = 4.15$, $**P = 0.0013$. $n = 13$ cells.
Figure 3E	Nested ANOVA (cells were nested under slices) Drug: $F_{1,422} = 13.82$, $P = 0.00028$ $n = 423$ cells from 6 slices
Figure 3F	Nested ANOVA (cells were nested under slices) Drug: $F_{1,272} = 0.89$, $P = 0.35$

	$n = 273$ cells from 6 slices
Figure 4A-C	Paired t -test. $t_7 = 6.75$, $**P = 0.0003$ $n = 357$ cells from 8 slices
Figure 4D	Student's t -test. $t_8 = 2.48$, $*P = 0.038$. Cage: $n = 5$, Training: $n = 5$
Figure 4E	Tukey's test after two-way repeated measures ANOVA Group: $F_{3, 311} = 6.16$, $P = 0.0004$; Histamine (Before vs. During histamine): $F_{1, 311} = 0.072$; Group $\times$ Histamine interaction: $F_{3, 311} = 7.04$, $P = 0.0001$ dVenus ⁺ , Histamine vs. others (during histamine): $**P < 0.0001$ Histamine: $n = 23$ dVenus ⁺ and 178 dVenus ⁻ cells from 6 slices Histamine + ranitidine: $n = 13$ dVenus ⁺ and 105 dVenus ⁻ cells from 4 slices
Figure 5C	Student's t -test. $t_{25} = 3.11$, $*P = 0.0047$. Controls: $n = 16$ (hM3Dq + Saline: $n = 7$, eGFP + CNO: $n = 9$), hM3Dq + CNO: $n = 11$
Figure 6B	Generalized linear model (binominal logistic regression analysis) with subject and drug Drug: $\chi^2 = 4.63$, $P = 0.031$; Subject $\times$ Drug: $\chi^2 = 303.8$, $P < 2.2 \times 10^{-16}$ $n = 38$ subjects (96 photo items for placebo, 96 photo items for betahistine per subject)
Figure 6C	Generalized linear model (binominal logistic regression analysis) with group and drug Group $\times$ Drug: $\chi^2 = 252.9$, $P < 2.2 \times 10^{-16}$ $n = 38$ subjects Subjects were divided into 6 groups according to the correct rate during placebo treatment (Group 1: 6 subjects, Group 2: 7 subjects, Group 3: 6 subjects, Group 4: 6 subjects, Group 5: 7 subjects, Group 6: 6 subjects). Post-hoc chi-squared test. Personal correct level 1: $\chi^2 = 83.4$, $P < 0.0001$. Personal correct level 2: $\chi^2 = 45.7$, $P < 0.0001$. Personal correct level 5: $\chi^2 = 43.4$, $P < 0.0001$. Personal correct level 6: $\chi^2 = 56.0$, $P < 0.0001$.
Figure 6D	Generalized linear model (binominal logistic regression analysis) with difficulty level and drug Difficulty level $\times$ Drug: $\chi^2 = 79.7$, $P = 9.9 \times 10^{-16}$ $n = 38$ subjects Post-hoc chi-squared test. Difficulty level 1: $\chi^2 = 22.8$, $P < 0.0001$. Difficulty level 2: $\chi^2 = 14.7$, $P = 0.00013$. Difficulty level 5: $\chi^2 = 8.96$, $P = 0.0028$. Difficulty level 6: $\chi^2 = 49.4$, $P < 0.0001$.
Supplemental Figure S1A	Sidak's test after 2-way repeated measures ANOVA Interval: $F_{3, 27} = 3.47$, $P = 0.030$; Object (novel vs. familiar): $F_{1, 27} = 52.2$, $P < 0.0001$; Interval $\times$ Object interaction: $F_{3, 27} = 6.35$, $P = 0.0021$ Novel: 30 min vs. 1 d, $P = 0.0003$; 30 min vs. 3 d, $P = 0.0019$

	0.5 h: $n = 8$, 2 h: $n = 7$, 1 d: $n = 8$, 3 d: $n = 8$
Supplemental Figure S1B	2-way repeated measures ANOVA Dose: $F_{2,22} = 0.43$, $P = 0.65$; Object (novel vs. familiar): $F_{1,22} = 38.11$, $P < 0.0001$; Dose x Object interaction: $F_{2,22} = 5.75$, $P = 0.0098$ 0 mg/kg: $n = 10$, 10 mg/kg: $n = 8$, 20 mg/kg: $n = 7$
Supplemental Figure S1C	2-way repeated measures ANOVA Dose: $F_{2,24} = 0.67$, $P = 0.52$; Object (novel vs. familiar): $F_{1,24} = 18.45$, $P = 0.0002$; Dose x Object interaction: $F_{2,24} = 2.89$, $P = 0.075$ 0 mg/kg: $n = 13$, 10 mg/kg: $n = 13$, 20 mg/kg: $n = 13$
Supplemental Figure S1D	Sidak's test after 2-way repeated measures ANOVA Dose: $F_{2,27} = 1.85$, $P = 0.18$; Object (novel vs. familiar): $F_{1,27} = 7.92$, $P = 0.0090$; Dose x Object interaction: $F_{2,27} = 5.61$, $P = 0.0092$ Novel: 10 mg/kg vs. 20 mg/kg, $P = 0.0179$ 0 mg/kg: $n = 10$, 10 mg/kg: $n = 8$, 20 mg/kg: $n = 14$
Supplemental Figure S1E	2-way repeated measures ANOVA Drug: $F_{1,14} = 0.34$, $P = 0.57$; Object (novel vs. familiar): $F_{1,14} = 0.67$, $P = 0.43$; Drug x Object interaction: $F_{1,14} = 1.19$, $P = 0.29$ Saline: $n = 8$, Thioperamide: $n = 8$
Supplemental Figure S1F	Sidak's test after 2-way repeated measures ANOVA Drug: $F_{1,15} = 6.84$, $P = 0.020$; Object (novel vs. familiar): $F_{1,15} = 31.78$, $P < 0.0001$; Drug x Object interaction: $F_{1,15} = 25.88$, $P = 0.0001$ Novel (Vehicle vs. Betahistine): $P = 0.0005$ Saline: $n = 9$, Thioperamide: $n = 8$
Supplemental Figure S1G	2-way repeated measures ANOVA Drug: $F_{2,30} = 1.2$, $P = 0.31$; Object (novel vs. familiar): $F_{1,30} = 38.46$, $P < 0.0001$; Drug x Object interaction: $F_{1,30} = 0.68$, $P = 0.51$ 0 mg/kg: $n = 10$, 10 mg/kg: $n = 12$, 20 mg/kg: $n = 11$
Supplemental Figure S2A	One-way ANOVA. $F_{2,22} = 0.10$, $P = 0.90$ 0 mg/kg: $n = 10$, 10 mg/kg: $n = 8$, 20 mg/kg: $n = 7$
Supplemental Figure S2B	One-way ANOVA. $F_{2,38} = 1.26$, $P = 0.29$ 0 mg/kg: $n = 15$, 10 mg/kg: $n = 13$, 20 mg/kg: $n = 13$
Supplemental Figure S2C	One-way ANOVA. $F_{2,29} = 0.67$, $P = 0.52$ 0 mg/kg: $n = 10$, 10 mg/kg: $n = 8$, 20 mg/kg: $n = 14$
Supplemental Figure S2D	Student's t -test. $t_{15} = 1.25$, $P = 0.23$. Saline: $n = 9$, Betahistine: $n = 8$
Supplemental Figure S2E	One-way ANOVA. $F_{2,30} = 0.15$, $P = 0.86$ 0 mg/kg: $n = 10$, 10 mg/kg: $n = 12$, 20 mg/kg: $n = 11$
Supplemental Figure S2F	Student's t -test. $t_{15} = 1.2$, $P = 0.25$. Saline: $n = 8$, Thioperamide: $n = 9$

Supplemental Figure S2G	Student's <i>t</i> -test. $t_{19} = 1.38$, $P = 0.18$. Saline: $n = 10$, Thioperamide: $n = 11$
Supplemental Figure S3A	Sidak's test after 2-way repeated measures ANOVA Drug: $F_{2,20} = 0.21$, $P = 0.81$; Object (novel vs. familiar): $F_{1,20} = 35.13$, $P < 0.0001$; Drug x Object interaction: $F_{2,20} = 51.35$, $P < 0.0001$ Novel (Vehicle + Thioperamide vs. Muscimol + Thioperamide): $P = 0.016$ Control: $n = 7$, vehicle + thioperamide: $n = 8$, muscimol + thioperamide: $n = 8$
Supplemental Figure S3B	2-way repeated measures ANOVA Drug: $F_{1,14} = 0.0014$, $P = 0.97$; Object (novel vs. familiar): $F_{1,14} = 8.88$, $P = 0.010$; Drug x Object interaction: $F_{1,14} = 4.97$, $P = 0.043$ Control: $n = 8$, Thioperamide: $n = 8$
Supplemental Figure S3C	2-way repeated measures ANOVA Drug: $F_{2,21} = 0.53$, $P = 0.60$; Object (novel vs. familiar): $F_{1,21} = 15.02$, $P = 0.0009$; Drug x Object interaction: $F_{2,21} = 3.07$, $P = 0.068$ Vehicle: $n = 9$, Ranitidine: $n = 7$, Fexofenadine: $n = 8$
Supplemental Figure S4	2-way repeated measures ANOVA Drug: $F_{1,7} = 9.19$, $P = 0.019$; Trial distance: $F_{13,91} = 1.43$, $P = 0.16$; Interaction: $F_{13,91} = 1.38$, $P = 0.18$ $n = 423$ cells from 6 slices
Supplemental Figure S5A	2-way repeated measures ANOVA Group: $F_{1,25} = 0.31$, $P = 0.58$; Object (novel vs. familiar): $F_{1,25} = 13.85$, $P = 0.0010$; Drug x Object interaction: $F_{1,25} = 4.41$, $P = 0.046$ Controls: $n = 16$ (hM3Dq + Saline: $n = 7$, eGFP + CNO: $n = 9$), hM3Dq + CNO: $n = 11$
Supplemental Figure S5B	Student's <i>t</i> -test. $t_{25} = 0.28$, $P = 0.78$. Controls: $n = 16$ (hM3Dq + Saline: $n = 7$, eGFP + CNO: $n = 9$), hM3Dq + CNO: $n = 11$
Supplemental Figure S6A	Generalized linear model (binominal logistic regression analysis) with IQ level and drug IQ level $\times$ Drug: $\chi^2 = 65.6$, $P = 8.6 \times 10^{-13}$ $n = 38$ subjects Subjects were divided into 6 groups according to the IQ level (Group 1: 6 subjects, Group 2: 7 subjects, Group 3: 6 subjects, Group 4: 6 subjects, Group 5: 7 subjects, Group 6: 6 subjects). Post-hoc chi-squared test. IQ level 1: $\chi^2 = 4.79$, $P = 0.029$ IQ level 2: $\chi^2 = 28.76$, $P < 0.0001$ IQ level 3: $\chi^2 = 15.80$, $P < 0.0001$ IQ level 4: $\chi^2 = 4.39$, $P = 0.036$ IQ level 6: $\chi^2 = 16.2$, $P < 0.0001$

Supplemental Figure S6B	Paired t -test. $t_{37} = 0.86$, $P = 0.39$. Placebo: $n = 38$, Betahistine: $n = 38$
Supplemental Figure S6C	Paired t -test. $t_{37} = 1.22$, $P = 0.23$. Placebo: $n = 38$, Betahistine: $n = 38$
Supplemental Figure S6D	Chi-squared test. $\chi^2 = 0.0019$, $P = 0.97$. Placebo: $n = 1254$ word pairs, Betahistine: $n = 1254$ word pairs.

Supplemental References

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