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Archival Report

Central Histamine Boosts Perirhinal Cortex Activity and Restores Forgotten Object Memories

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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. Because histamine H_3 receptor inverse agonists upregulate histamine release, the inverse agonists may enhance learning and memory. However, whether the inverse agonists promote the retrieval of forgotten long-term memory has not yet been determined.

METHODS: Here, we employed multidisciplinary methods, including mouse behavior, calcium imaging, and chemogenetic manipulation, to examine whether and how the histamine H_3 receptor inverse agonists, thioperamide and betahistine, 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 betahistine treatment promotes memory retrieval in humans.

RESULTS: The treatment of H_3 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, which activated the histamine H_2 receptor. Histamine depolarized perirhinal cortex neurons, enhanced their spontaneous activity, and facilitated the reactivation of behaviorally activated neuronal ensembles. A human clinical trial revealed that treatment of H_3 receptor inverse agonists is 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.

Keywords: Histamine H_3 receptor, Memory recovery, Object recognition memory, Perirhinal cortex, Retrieval, Stochastic resonance

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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. Long-term 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 H_3 receptors are located primarily in the axon terminals and somata of neurons, and they inhibit the presynaptic release of histamine and other neurotransmitters and negatively regulate histamine synthesis (7). Because histamine H_3 receptors are constitutively active, their inverse agonists upregulate histamine release (8). Therefore, histamine H_3 receptor inverse agonists may enhance learning and memory. Indeed, several pioneering studies have found that histamine H_3 receptor inverse agonists enhance memory performance (9–15). However, whether H_3 receptor inverse agonists promote the retrieval of forgotten long-term memory has not yet been determined, as indicated by the following reasoning. First, because many of the previous studies administered H_3 receptor inverse agonists before or shortly after training, their results demonstrate the drug effect on memory acquisition

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and/or consolidation, but not retrieval (9,10). Second, in some studies examining the drug effect on memory retrieval, basal memory performance was high without administration of H_3 receptor inverse agonists because they employed aversive learning tasks (11,12). Thus, they could not examine the drug effect on forgotten memories. Third, the other studies successfully examined the drug effect on retrieval of forgotten memories, but they targeted short-term memories (1–2 hours) and not long-term memories (24 hours or longer) (13–15). Fourth, 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 H_3 receptor inverse agonists are effective long after training and forgetting. More important, it is unclear whether H_3 receptor inverse agonists affect human long-term memory. Previous studies have shown that H_3 receptor inverse agonists have no effect on the performance in memory-related tasks (16–18). Taken together, it is unclear whether and how H_3 receptor inverse agonists promote retrieval of forgotten long-term memory.

In the current study, we examined whether the histamine H_3 receptor inverse agonists, thioperamide and betahistine, 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, betahistine treatment enhanced the retrieval of object recognition memory.

METHODS AND MATERIALS

Animals

Animal experiments were performed with the approval of the animal experiment ethics committee at the University of Tokyo (Approval No. 24-10) and Hokkaido University (Approval No. 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 minutes. In a test session, the mice explored the open field for 5 minutes in the presence of one familiar object and one novel object. A discrimination ratio was calculated for each mouse as the ratio $(T2 - T1)/(T1 + T2)$, where T1 is time spent exploring the familiar object and T2 is 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 University Hospital Medical Information Network Clinical Trials Registry (No. UMIN000015110), and carried out in accordance with the Code of Ethics of the World Medical Association. The experimental design was a randomized double-blind, 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 eighth day, the participants in group A were orally administered nine capsules of betahistine mesilate (total of 108 mg) and those in group B were orally administered nine capsules of placebo. Next, 30 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 eighth day, 30 minutes after the oral administration.

Object Recognition Task in Humans

On the training day, the participants viewed 128 pictures of objects and decided whether each picture depicted an indoor item or outdoor item. On the test days, the participants were shown the same 32 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 the Supplement.

RESULTS

Histamine H_3 Receptor Inverse Agonists Induce the Recall of Forgotten Memories

We employed the novel object recognition task, in which the test session mice were presented with a novel object and a familiar object that was presented during the training session. A different set of mice was 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 H_3 receptor inverse agonist, promotes the retrieval of forgotten object memories, we intraperitoneally administered thioperamide (10 or 20 mg/kg) to mice 30 minutes 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 an open field test or on anxiety-like behavior in an elevated plus maze (Supplemental Figure S2E, F).

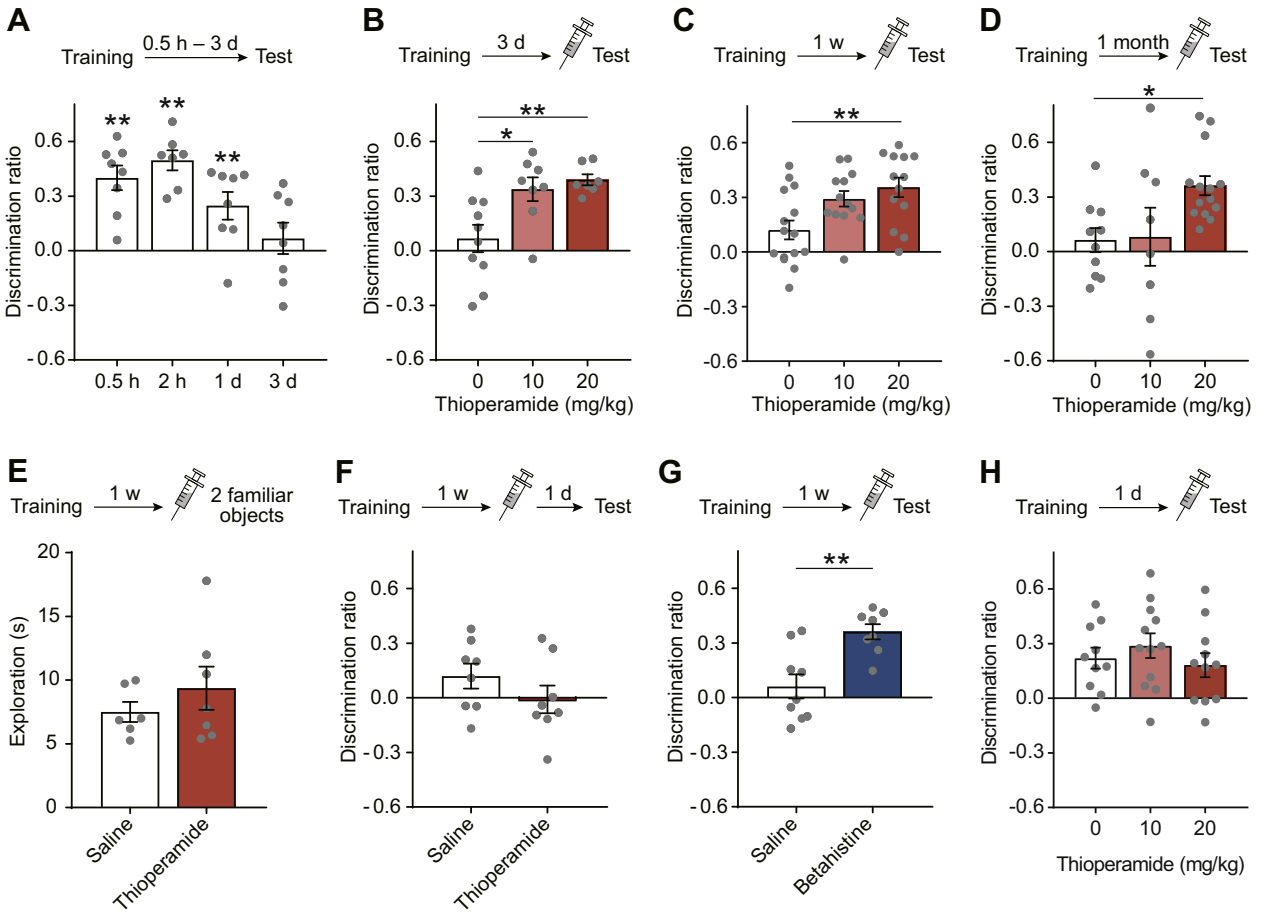

Figure 1. Histamine H_3 receptor inverse agonists induce the recall of forgotten memories. **(A)** The discrimination ratio in the novel object recognition task decreased over time. Tukey's test after one-way analysis of variance, $n = 7–8$ mice. **(B–D)** Intraperitoneal administration of thioperamide 30 minutes before the recall test increased the discrimination ratio. Tukey's test after one-way analysis of variance, $n = 7–14$ mice. **(E)** Exploration for two familiar objects was not affected by thioperamide injection. **(F)** Thioperamide-induced memory recovery was within 1 day. **(G)** Pretest injection of betahistine 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. $^*p < .05$, $^{**}p < .01$. Values are reported as mean ± SEM. See also Supplemental Figures S1 and S2 and Supplemental Table S1.

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 betahistine, another structurally irrelevant H_3 receptor inverse agonist with a weak H_1 receptor–stimulating effect. Mice that received betahistine injection 30 minutes before the test significantly discriminated between the novel and familiar objects 1 week after the training (Figure 1G and Supplemental Figures S1F and 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 and Supplemental Figures S1G and S2E). Taken together, the treatment of histamine H_3 receptor inverse agonists transiently promotes the retrieval of the forgotten 3-day-old, 1-week-old, and 1-month-old object memories in mice.

Thioperamide activates histamine release from the axon terminals by antagonizing H_3 receptors (8). The PRh is a critical brain region for the novel object recognition task (19), and a radioligand binding assay suggests H_3 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. First, we inhibited PRh activity through an intra-PRh injection of 250 ng muscimol, a gamma-aminobutyric acid type A receptor agonist, at the same time when mice received an intraperitoneal thioperamide injection. The PRh inhibition prevented thioperamide-induced memory retrieval (Figure 2A and Supplemental Figure S3A). Second, intraperitoneal thioperamide injection increased the extracellular concentration of histamine in the PRh 30 minutes after the injection (Figure 2B). Third, intra-PRh injection of thioperamide mimicked the effect of intraperitoneal thioperamide injection at the 1-week test (Figure 2C and Supplemental Figure S3B). Fourth, an intra-PRh injection of ranitidine (H_2 receptor antagonist) blocked

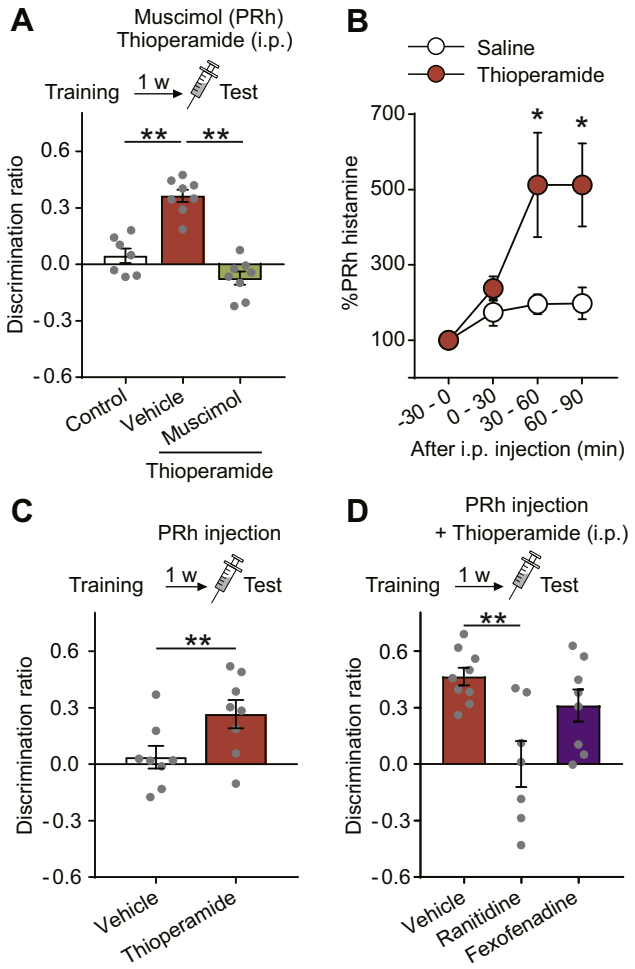

Figure 2. Histamine H_2 receptor in the perirhinal cortex (PRh) mediates thioperamide-induced memory recovery. **(A)** Local injection of muscimol into the PRh prevented memory retrieval induced by intraperitoneal (i.p.) 20 mg/kg thioperamide. Tukey's test, $n = 7–8$ mice. **(B)** Thioperamide injection increased histamine release in the PRh. Sidak's test after two-way repeated-measures analysis of variance, $n = 4$ mice. **(C)** Intra-PRh injections of thioperamide drove memory retrieval. Student's t test, $n = 8$ mice. **(D)** Ranitidine (histamine H_2 receptor antagonist) but not fexofenadine (H_1 receptor antagonist) prevented thioperamide-induced memory recovery. Tukey's test, $n = 7–9$ mice. $^*p < .05$, $^{**}p < .01$. Values are reported as mean ± SEM. See also Supplemental Figure S3 and Supplemental Table S1.

intraperitoneal thioperamide-induced memory retrieval, whereas an intra-PRh injection of fexofenadine (H_1 receptor antagonist) did not (Figure 2D and Supplemental Figure S3C). Therefore, the activation of H_2 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. Because the effect of H_3 receptor inverse agonists on histamine release in vitro is much lower compared with the effect in vivo (21), we applied histamine instead of thioperamide to the brain slices. 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-2-acetoxymethyl ester (Figure 3C, D). Histamine perfusion enhanced the overall rate of calcium transients, an effect that was blocked by 2 μM ranitidine (Figure 3E, F).

We also recorded the calcium activity evoked by the field stimulation of the PRh cortical layer II/III every 20 seconds. Histamine perfusion did not increase the percentage of neurons responsive to the stimulation (baseline: 31 ± 1.6%; histamine: 28 ± 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 were dimension-reduced using multidimensional scaling (MDS) and were 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 was able to assign 30-trial data points accurately to the corresponding datasets with an F1 score as high as 0.90 ± 0.05 (mean ± SEM of 8 slices). More important, 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 with 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). Thus, we 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 minutes. The percentage of $dVenus^+$ neurons was higher in the training group compared with 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 μM 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). The synergistic effect of

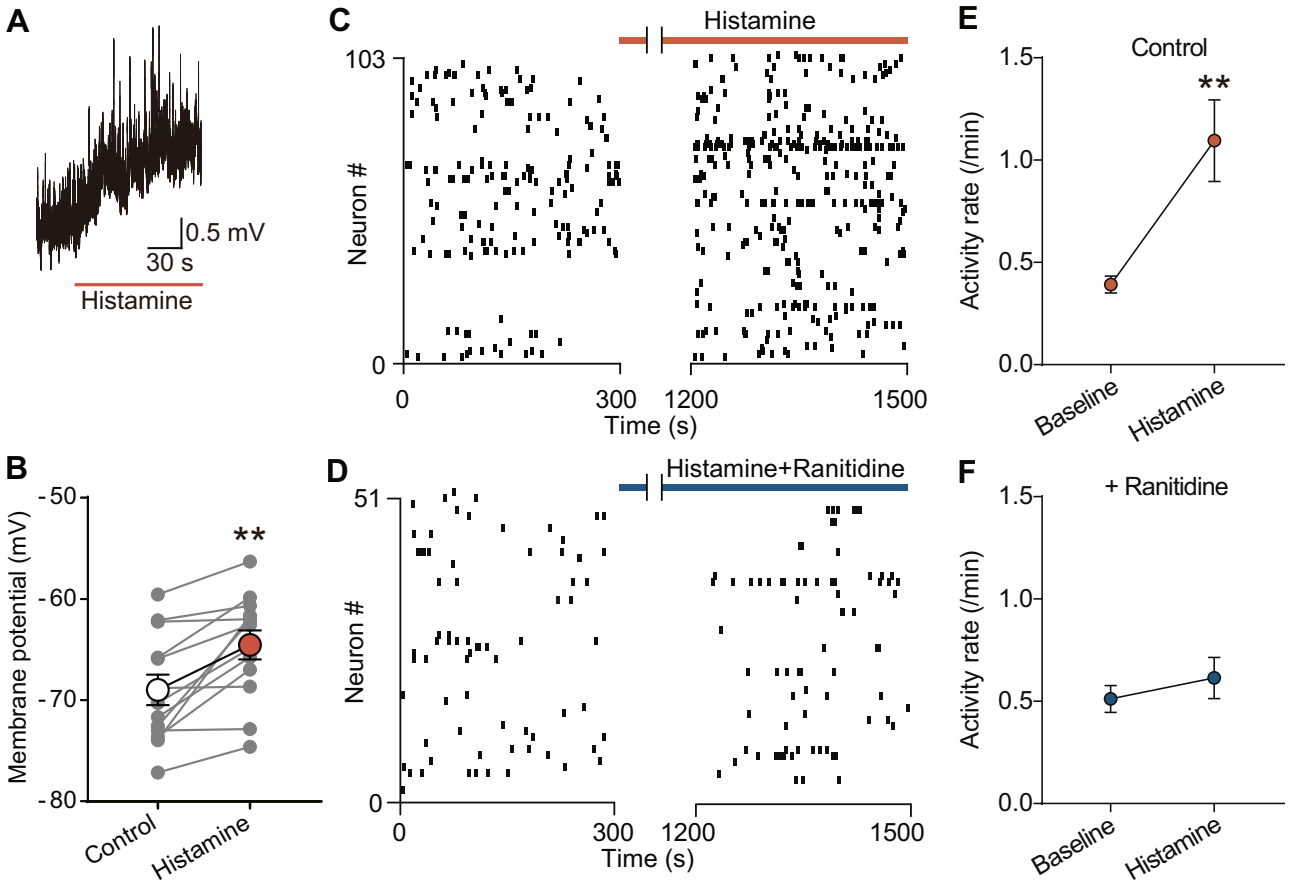

Figure 3. Histamine enhances the spontaneous activity in perirhinal cortex neurons. **(A, B)** Bath application of 10 μM histamine depolarized the membrane potential of the perirhinal cortex layer V neurons. Paired *t* test, $n = 13$ cells. **(C, D)** Raster plots of spontaneous calcium transients in individual perirhinal cortex neurons before and during the bath application of 10 μM histamine **(C)** or 10 μM histamine + 2 μM 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 μM ranitidine **(F)**. Nested analysis of variance where the cells were nested under the slices, $n = 423$ cells from 6 slices **(E)**, 273 cells from 6 slices **(F)**. $^{**}p < .01$. Values are reported as mean ± SEM. See also Supplemental Table S1.

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 Promotes 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 (DREADDs) (29), to PRh neurons by intra-PRh injection of adeno-associated virus (AAV)-hSyn-hM3Dq-IRES-mCitrine (Figure 5A). The percentage of mCitrine$^+$ neurons was 44.5 ± 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 on bath application of 10 μM 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 with control mice (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 H_3 Receptor Inverse Agonists Enhance 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.

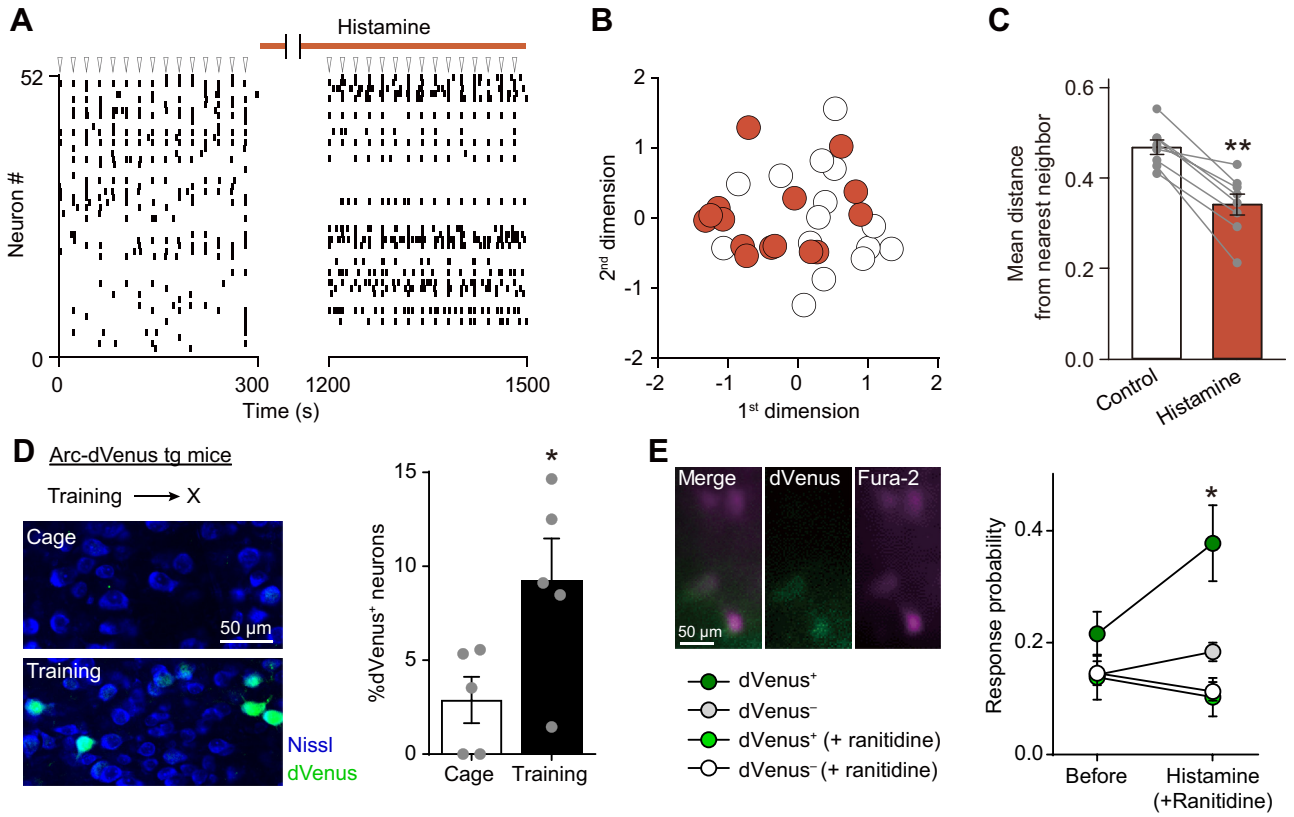

Figure 4. Histamine enhances reactivation of behaviorally activated neurons ex vivo. **(A)** Raster plots of transient calcium events in perirhinal cortex neurons in response to field stimulation of the layer II/III (gray triangles at the top). **(B)** Stimulus-evoked responses of individual neurons were dimension reduced using multidimensional 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 are from a representative slice. **(C)** Pooled data of all experiments (n = 8 slices). The mean interval from the nearest neighbors in multidimensional scaling 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 perirhinal cortex 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 perirhinal cortex 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 coapplication of ranitidine. Tukey's test after two-way repeated-measures analysis of variance, n = 4–6 slices. $^*p < .05$, $^{**}p < .01$. Values are reported as mean ± SEM. See also Supplemental Figure S4 and Supplemental Table S1.

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 days after the training (days 8 and 10, respectively). The participants were asked whether they had seen the target items during the training. They were administered 108 mg betahistine or placebo orally 30 minutes 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 using a binomial distribution to fit the number of correct items with N as the number of participants and drug (placebo vs. betahistine). The generalized linear model confirmed that betahistine treatment enhanced the overall correct ratio (odds ratio = 1.11) (Figure 6B) and that there is a significant participant × drug interaction effect. To further analyze the participant × drug interaction effect, the participants were divided into six groups according to the correct rate during placebo treatment. We computed another generalized linear model using a binomial distribution on the number of correct items with group and drug. There was a significant group × drug interaction. Specifically, betahistine enhanced the correct rate of subjects who 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 six difficulty levels according to a correct rate that was obtained when the participants received placebo treatment. Specifically, betahistine improved the correct rate for difficult items (Figure 6D). In contrast, betahistine reduced the correct rate of subjects who had better performance under placebo treatment and subjects with low and high IQ, and it reduced the rate for easy items.

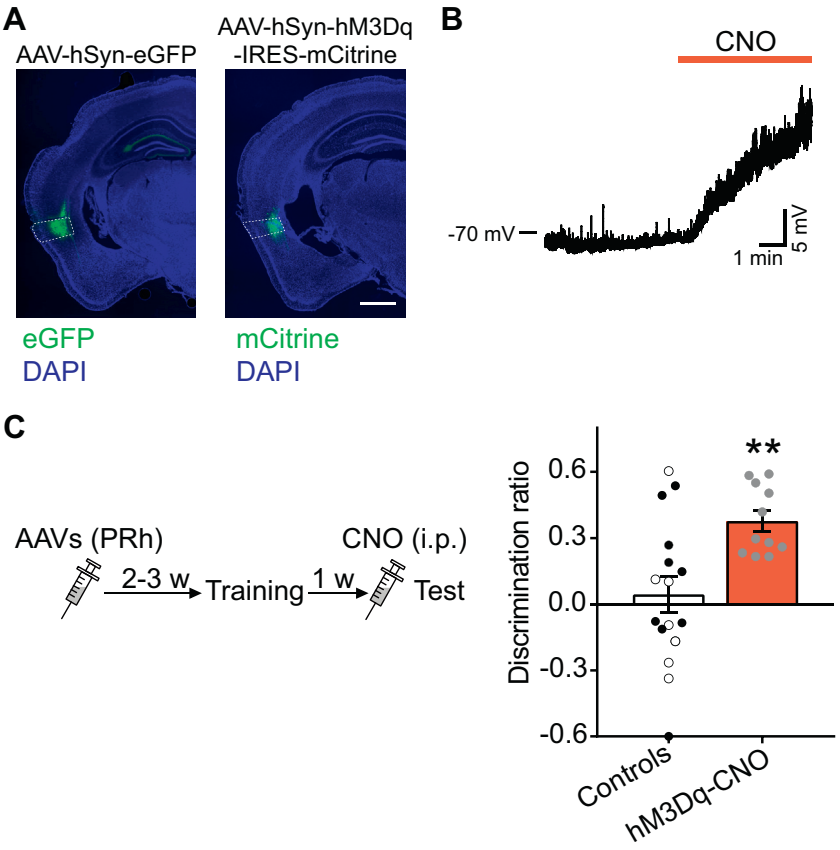

Figure 5. Chemogenetically increased spontaneous activity in the perirhinal cortex (PRh) neurons enhances memory recovery. **(A)** Either adeno-associated virus (AAV)-hSyn-enhanced green fluorescent protein (eGFP) or AAV-hSyn-hM3Dq was injected into the PRh. **(B)** A representative patch clamp recording from a single PRh neuron. Bath application of clozapine-*N*-oxide (CNO) depolarized PRh neurons in brain slices. **(C)** Pretest chemogenetic activation of PRh neurons via intraperitoneal (i.p.) 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. $^{**}p < .01$. Values are reported as mean ± SEM. See also Supplemental Figure S5 and Supplemental Table S1.

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 amnestic 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 H_3 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 in humans as well as mice.

The upregulated histamine release and the following activation of histamine H_2 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 H_2 receptor expression in the PRh (30). Histamine perfusion depolarized a membrane potential (Figure 3) and decreases calcium-activated potassium conductance in an H_2 receptor-dependent manner (31), both of which contribute to histamine's excitatory effect. Histamine H_2 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 H_2 receptor and promotes retrieval of the apparently forgotten memories. The increase in PRh spontaneous activity is sufficient to promote memory retrieval because CNO injection in mice that received AAV-hSyn-hM3Dq in the PRh improved memory retrieval (Figure 5).

Reactivation of memory engram neurons underlies memory retrieval. Neurons activated during memory formation are reactivated during the memory test (24,25,32). The reactivation is observed in the cerebral cortex as well as in the hippocampus and amygdala (33,34). The ratio of the reactivation correlates with performance at the memory test (24,25,35). In addition, artificial reactivation of neurons activated during training triggers 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 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

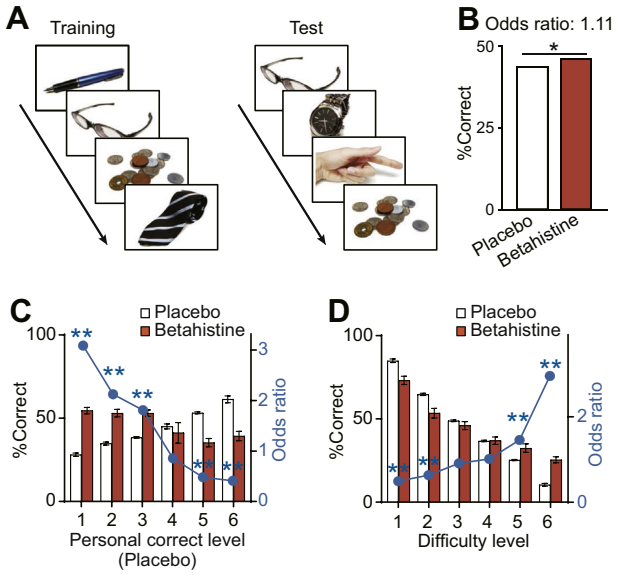

Figure 6. Histamine H_3 receptor inverse agonist enhances memory retrieval in humans. **(A)** Object recognition task in humans. **(B)** Betahistine treatment increased the overall correct ratio in the object recognition task. χ^2 test, n = 38 subjects. **(C)** Participants who had poor performance under placebo treatment were more sensitive to betahistine treatment. χ^2 test. **(D)** Items that were difficult to remember were more sensitive to betahistine treatment. χ^2 test. $^*p < .05$, $^{**}p < .01$. Values are reported as mean ± SEM. See also Supplemental Figure S6 and Supplemental Table S1.

future, long-lasting labeling of memory-related neurons would be better to perform physiological experiments 3 to 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. Stochastic resonance is a phenomenon where adding nonzero 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 stochastic resonance is as follows. First, 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). Second, however, the activity of engram neurons exceeds a threshold level with support of enhanced background activity, leading to successful recall. Third, the activity of nonengram 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 the increase in spontaneous activity promoted the retrieval of forgotten memories. In theory of stochastic resonance, adding nonzero noise to a subthreshold signal allows the signal to reach threshold, while adding 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 subjects originally remember the items.

It is important to note that histamine and histamine H_3 receptor inverse agonists 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 supports 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 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 H_3 receptor inverse agonists enhances the attentional state (41). However, the memory recovery in our study is unlikely to be due to the enhanced attentional state. First, local injection of thioperamide into the PRh enhanced retrieval of forgotten memories. Second, intra-PRh injection of an H_2 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 the human symbol coding task.

Histamine H_3 receptor is a promising target for treating cognitive dysfunction. Accordingly, previous studies examined effects of histamine H_3 receptor inverse agonists 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 seven times as much as a typical single dose, because we estimated that this dose is required to achieve the concentration of 1 nM betahistine, which is necessary for maximal H_3 receptor activation. Second, in the object recognition memory task, betahistine treatment enhanced retrieval performance of items that are more difficult to remember and in 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 the possibility that H_3 receptor inverse agonists affect other types of memory because histamine neurons send fiber projections

to nearly all parts of the brain and because H_2 and H_3 receptors are distributed in many brain regions (3).

In conclusion, we propose 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 H_3 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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ARTICLE INFORMATION

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