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Dried bonito broth improves cognitive function via the histaminergic system in mice

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ABSTRACT

Bonito extract, *i.e*., dried bonito broth (DBB), has been reported to counteract mental fatigue and to increase performance in a simple calculation task, but the mechanism by which DBB increases task performance is not known. The brain neurotransmitter histamine is biosynthesized only from histidine in the tuberomammillary nucleus. Histamine neurons are projected to almost all areas of the cerebral cortex, and histamine has various behavioral and neurobiological functions, particularly in recognition memory. Here we used a mouse model to investigate the effects of the oral ingestion of DBB, which contains abundant histidine, as well as the ingestion of histidine on cognitive function. In a retention trial of novel object recognition test, the administration of 1.6 g/kg of DBB and 500 mg/kg of histidine significantly increased the animals' exploratory behavior toward a novel object, and that these agents significantly increased the spontaneous alternation behavior ratio in a Y-maze under conditions of scopolamine-induced amnesia, which induced learning and memory impairment. These results suggested the improvement of spatial short-term working memory in a scopolamine amnesia model, as well as the strengthening of visual cognitive function by a single ingestion of DBB and histidine. Interestingly, the administration of αFMH, which is an inhibitor of histamine biosynthesis, eliminated the increase in the spontaneous alternation behavior ratio by DBB ingestion in the scopolamine-induced amnesia model, suggesting that DBB may improve working memory impairment via activation of the histaminergic neuron system.

Bonito (skipjack tuna, *Katsuwonus pelamis*) is known as *katsuo* in Japan and is very familiar to Japanese people from ancient times. Dried bonito broth (*katsuobushi-dashi*, DBB), a hot-water extract of dried bonito muscle, is ubiquitous in the Japanese diet, enhancing the taste and flavor of dishes (12, 19). DBB has also traditionally been considered a folk remedy for fatigue in the southern part of Japan. In previous works, DBB was confirmed to be effective against fatigue in animal and human studies (22, 32). We demonstrated that the daily ingestion of DBB by humans improves mood, especially by alleviating mental fatigue (31), and that it increases performance on a simple calculation task (21). However, the mechanisms underlying the ability of DBB to increase task performance are not yet known.

The brain neurotransmitter histamine is synthesized from histidine by tuberomammillary nuclei neurons of the posterior hypothalamus, and histaminergic neurons are projected to almost all areas of the cerebral cortex (34). Histamine is known to be involved in behavioral and neurobiological functions such as sleep-cycle maintenance and adjustment (16, 37), eating behavior and energy metabolism (17, 26, 36), stress response (38, 40), and learning and mem-

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ory (1, 8, 9). Therapeutic regimens have been developed to improve learning and memory performance by increasing histamine neurotransmission in animals and humans with reduced functions (6, 11, 18, 24). We hypothesized that DBB containing abundant histidine would directly improve cognitive performance without a fatigue recovery process, and in the present study we used a learning and memory model to investigate whether DBB changes cognitive function via histaminergic neurons. We also evaluated the changes of histidine and histamine levels within the hypothalamus over time after an oral administration of DBB. Based on the results, we discuss the effects of DBB on brain function.

MATERIALS AND METHODS

Animals. Six-week-old male ddY mice were purchased from Japan SLC and housed in cages under conditions of controlled temperature (23°C ± 2°C) and humidity (55% ± 15%). They were maintained under a 12-h lighting cycle (lights on: 07 : 00–19 : 00, lights off: 19 : 00–07 : 00) and given free access to water and AIN-93G formulated diet (Oriental Yeast Co., Itabashi, Japan). Each animal was used for only a single behavioral test, and all behavioral evaluations were performed between 09 : 00 and 14 : 00. The animal experiments were designed to comply with ethical regulations and were approved by the Animal Care Committee of Ajinomoto Co., Inc. The n-values of each group of mice are given in the figure legends.

Materials and reagents. Dried bonito broth (DBB) was prepared as described (21, 31). The composition is shown in Table 1. The DBB was diluted with distilled water (Otsuka Pharmaceutical Factory, Tokushima, Japan) and administered orally to mice at 1.6 g/kg. Histidine solution was prepared by dissolving L-histidine hydrochloride monohydrate (Ajinomoto Co., Kawasaki, Japan) in distilled water, adjusting the histidine concentration to 100 mg/mL, and administered orally to mice at 200 mg/kg or 500 mg/kg. Thioperamide, histamine H3 receptor antagonist, solution was prepared by dissolving thioperamide maleate (Tocris Cookson, Ellisville, MO) in saline containing 0.3% carboxymethylcellulose sodium; the thioperamide concentration was adjusted to 2 mg/mL and administered intraperitoneally to mice at 10 mL/kg.

Scopolamine was prepared by dissolving (−)-scopolamine hydrobromide trihydrate (Sigma-Aldrich Co., St. Louis, MO) in saline and administered intraperitoneally to mice at 1 mg/kg. Pargyline solution was prepared by dissolving pargyline hydrochloride (Sigma-Aldrich) in phosphate-buffered saline (PBS; Takara Bio, Shiga, Japan), adjusted to be pH 6.5, and administered intraperitoneally to mice at 0.5 mmol/kg. The inhibitor of histidine decarboxylase, α-fluoromethylhistidine (αFMH; Sumika Technoservice Corp., Takarazuka, Japan), was dissolved in saline, and 50 mg/kg was administered intraperitoneally to mice 16 h after treatment with the test sample. All test solutions were prepared at the time of use.

Measurement of histidine, histamine, and histamine metabolite levels. Blood and hypothalamus were collected from the mice at 15, 30, 45, 60, 90, and 120 min and 60, 120, 240, and 360 min after the ad-

Table 1 *Nutrient composition of dried bonito broth (DBB)*

Nutrient	DBB
Free amino acids	22.7
Alanine	0.8
Anserine	1.2
Arginine	0.4
Asparagine	0.3
Carnosine	0.4
Creatine	1.3
Cystine	0.1
Glutamic acid	0.5
Glycine	0.3
Histidine	10.9
Isoleucine	0.3
Leucine	0.6
Lysine	0.8
Methionine	0.2
Phenylalanine	0.3
Proline	0.5
Serine	0.3
Taurine	2.7
Threonine	0.2
Tryptophan	0.0
Tyrosine	0.3
Valine	0.3
Organic acid	
Lactic acid	11.7
Minerals	
Magnesium	0.3
Potasium	0.6
Sodium	4.1
Nucleic acids	
Inosic acid	0.6
Protein or unknown peptides	40.9

(g/100 g)

ministration of DBB or vehicle between 09 : 00–12 : 00 in the light period, respectively, and the plasma histidine concentration and the levels of histidine and histamine in the hypothalamus were measured. The whole brain was rapidly removed, and the hypothalamus was excised on ice using the method reported by Glowinski *et al.* (13). The histamine metabolite, tele-methylhistamine (t-MH), was measured in tissue collected from animals intraperitoneally administered the monoamine oxidase-B inhibitor pargyline at 0.50 mmol/kg 10 min before administration between 09 : 00–12 : 00 in the dark period (feeding condition under lights on: 19 : 00–07 : 00, lights off: 07 : 00–19 : 00).

After weight measurement, the hypothalamus was frozen in liquid nitrogen and stored at −80°C until content measurement. For the measurements of the tissue histamine or t-MH content, the hypothalamus was combined with about 5× tissue weight of 0.2 mol/L perchloric acid and an internal reference, 3-methylhistamine, homogenized by ultrasonication and then centrifuged, and the supernatant was collected. The supernatant was subjected to ultrafiltration using the Microcon MMWL10K (Millipore, Billerica, MA), and the histamine level was measured in the filtrate as the analytical sample. Aliquots of 10 μL of the prepared samples were subjected to high-performance liquid chromatography (HPLC).

The histamine and t-MH contents were measured as follows. The substances were separated using a cation exchange column (Eicopak SC-5ODS; Eicom Corp., Kyoto, Japan) equipped with a guard column (Prepaset CA-ODS; Eicom), and measured by the column switching *o*-phthalaldehyde (OPA) post-column fluorescence derivation method with excitation and fluorescence wavelengths of 335 and 450 nm, respectively (27). The mobile phase, 0.1 mol/L acetate buffer-methanol solution (91 : 9, v/v) containing 220 mg/L sodium 1-octansulfonate, was passed at a flow rate of 0.5 mL/min. The reaction solutions, 2 v/v% ethanol solution containing 80 mg/L OPA and 40 μL/L 2-mercaptoethanol, and 0.5 mol/L potassium carbonate solution were passed at a flow rate of 0.1 mL/min. The tissue histidine content was measured in the samples prepared for histamine measurement using an amino acid analyzer (Model L-8500; Hitachi, Tokyo).

Novel object recognition test. Rodents explore novel objects in their habitat. This behavioral trait has been used extensively as a paradigm to examine learning, and it has been used to evaluate cognitive memory with an object recognition task (7, 10). In the present study, the object recognition task was conducted in an open-field arena (30 × 30 × 30 cm) made of polyvinyl chloride, plywood, and transparent acrylic. Three stimulus objects of similar size were used: a ceramic figure of a cat, a wooden cylindrical pen stand, and a square resin block.

Before their training, the mice were habituated to the experimental area by being allowed to freely explore it for 5 min. On the day following the habituation day, a familiarization phase was performed in which two of the three objects were placed in the arena. The objects were placed 8 cm from both sidewalls along the centerline of the floor. The objects were randomly selected to avoid bias among animals and between groups. At 60 min after the test sample or vehicle administration, the mouse was kept in the open field arena for 5 min and its behavior was recorded using a video camera. Twenty-four hours after this familiarization phase, the mouse was administered the second test sample or vehicle.

In the retention trial performed 48 h after the familiarization phase, two objects were placed in the open field arena, but one object was changed to the novel object not used in the familiarization phase. In the retention trial, the mouse was put inside the arena and its movements were recorded using a video camera. The time spent sniffing or touching with its nose or foreleg was measured as the time of exploration. Behaviors such as climbing on top of the object or walking around the object were not considered exploratory behaviors.

We analyzed the behaviors of each mouse over a 5-min retention period. The index for cognitive memory is expressed as the ratio between the total exploratory behavior and exploratory behavior for the novel object.

Y-maze spontaneous alternation behavior test. Rodents have a characteristic trait of selecting new routes over returning to routes that they have used before, and the Y-maze has been used to assess this spontaneous alternation as a means of monitoring spatial working memory (15, 23). In the present experiments we used a Y-maze consisting of three 40-cm-long arms with 12-cm-high walls, 3-cm-wide floor, and upper width of 10 cm set at an angle of 120° from each other. The Y-maze was placed at the center of the experimental room floor.

Each mouse received an intraperitoneal injection of either alpha-fluoromethylhistidine (FMH) (20, 25), which is an inhibitor of histidine decarboxylase (HDC) that is necessary for histamine biosynthesis, or saline. DBB or vehicle was orally administered

16 h later, and scopolamine or saline was administered intraperitoneally 30 min after that. The mouse was placed at the end of one arm of the Y-maze 30 min after the scopolamine or saline administration and allowed to freely explore the maze for 8 min. The arms that the mouse entered were recorded, as was the order of arm selection. The frequency of moving into each arm during the measurement period was counted and regarded as the total number of entries.

The selections of an arm different from the previous selection were identified, and the frequency of this behavior was regarded as the number of spontaneous alternation behaviors. The spontaneous alternation behavior was regarded as an index of short-term spatial working memory and was calculated as follows: Spontaneous alternation behavior (%) = Number of spontaneous alternation behaviors/(total number of entries −2) × 100.

Statistical analysis. Measured values are shown as means ± SEM. Novel object recognition was evaluated based as the discrimination index toward the novel object in the retention trial in each group. In each case, the vehicle administration group was considered the control, and a one-way analysis of variance (ANOVA) followed by Dunnett's test was performed between the test substance administration groups. An evaluation of the spontaneous alternation behavior ratio was performed with the only scopolamine administration group as a control, and a one-way ANOVA followed by Dunnett's test was performed to determine the significance of changes in the spontaneous alternation behavior ratio in each test group. The DBB administration and control groups were compared with regard to the levels of histamine and its metabolite t-MH. The Prism software package (GraphPad, San Diego, CA) was used for all statistical analyses, and the results were considered significant when the p-value was < 0.05.

RESULTS

Effects of dried bonito broth on novel object recognition

We calculated the discrimination index of the time spent exploring one object as an exploratory behavior during the familiarization phase. In the vehicle group, this index was 48.2%, and the corresponding values in the group administered thioperamide (as the positive control) (41) and the DBB group were 49.2% and 51.2%, respectively. There were no significant differences in exploratory behavior toward the two non-novel objects among the three groups.

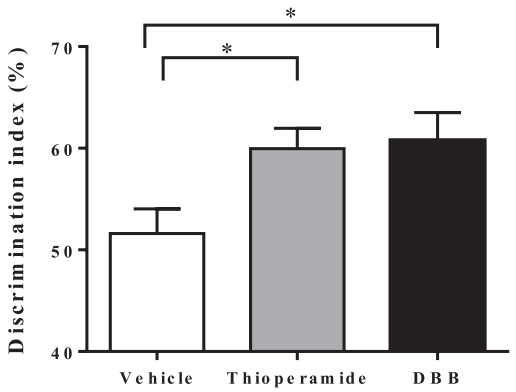

Fig. 1 Effects of DBB on the novel object recognition task. Vehicle, thioperamide, or DBB was administered 60 min before and 24 h after the familiarization phase, and the retention trial was performed 48 h after the familiarization phase. The discrimination index of exploratory behavior toward the novel object in the total exploratory behavior was calculated. Values are the means ± S.E. of 17 or 20 mice. $^{*}P < 0.05$ vs. vehicle.

Next, the ratio of the time spent exploring the novel objects in the retention trial 48 h after the familiarization phase was calculated. There were no significant differences in the total time of exploration toward each object in the retention trial among the three groups. However, the ratio of the time spent exploring the novel objects was 52.4% in the vehicle group, compared to 60.0% and 60.8% in the thioperamide and DBB administration groups, respectively, both of which were significantly higher than the vehicle group's ratio (Fig. 1).

Changes in histidine, histamine, and histamine metabolite after DBB administration

To examine the effects of DBB administration on the levels of histidine and histamine in the hypothalamus, we evaluated the changes in these levels over time after DBB administration. The plasma histidine concentration significantly increased 15 min after the administration of DBB (Fig. 2A), and the concentrations of histidine as well as histamine within the hypothalamus were significantly elevated after 60 min (Fig. 2B, C). The increased plasma histidine concentration decreased to the level seen prior to administration by 120 min after the DBB administration (Fig. 2A), and the concentrations of histidine and histamine within the hypothalamus also decreased to the levels seen prior to administration 6 h after administration (Fig. 2B, C).

As the hypothalamic histamine content was high at 120 min after DBB administration, to investigate

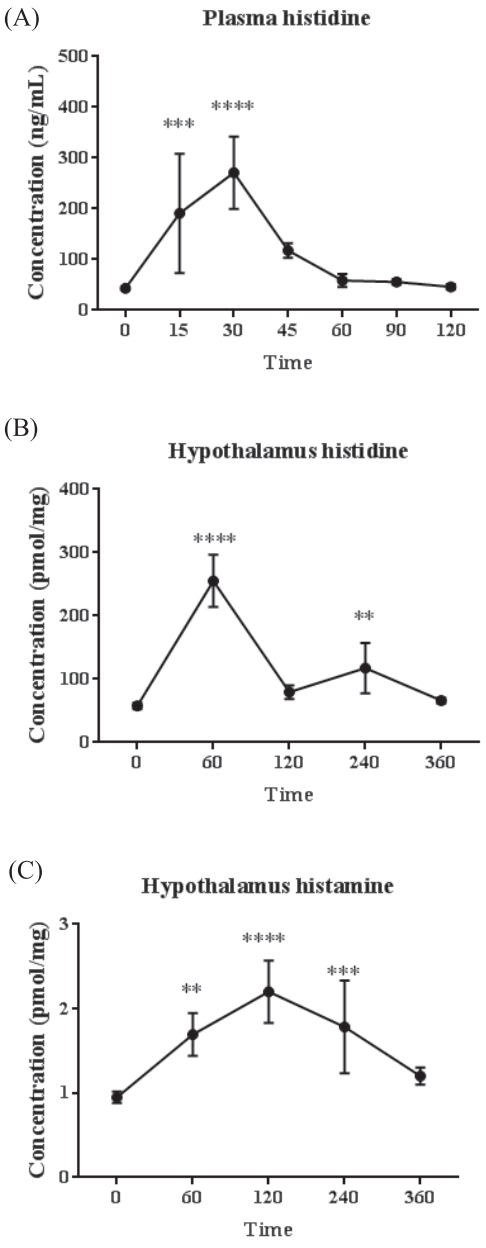

Fig. 2 Changes in the plasma histidine, hypothalamic histidine and histamine contents after oral DBB administration. (A) plasma histidine, (B) hypothalamus histidine, (C) hypothalamus histamine. Values are the means ± S.E. of 5 or 6 mice. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ vs. time zero.

the influence of DBB on the histaminergic system, we measured histamine and its metabolite, t-MH, at 2 h after oral vehicle or DBB administration during the dark period in which histaminergic neurons were activated. The histamine and t-MH contents were significantly higher in the DBB group compared to the vehicle group (Fig. 3).

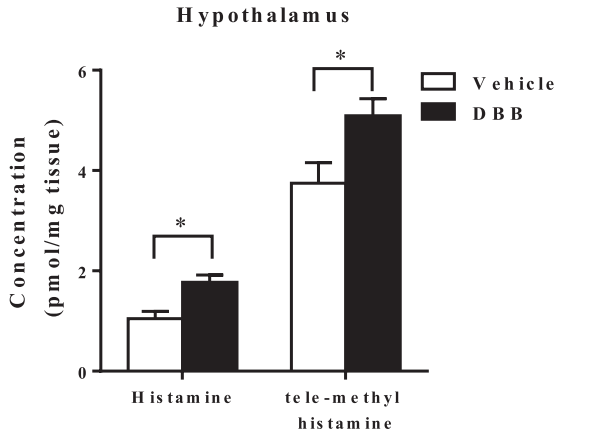

Fig. 3 The contents of histamine and its metabolite tele-methyl histamine in the hypothalamus at 2 h after vehicle or DBB administration in the dark period. Values are the means ± S.E. of 5 mice. $^{*}P < 0.05$ vs. vehicle.

Effects of dried bonito broth on spontaneous alternation behavior in the Y-maze

With mice that were or were not administered αFMH, which is an inhibitor of histamine biosynthesis, we evaluated the effects of DBB on scopolamine-induced amnesia based on changes in the spontaneous alternation behavior ratio in the Y-maze. Without αFMH administration, the alternation behavior ratio was 62.3% in the control group and 47.9% in the scopolamine group. The corresponding value was 60.6% in the DBB + scopolamine administration group, which is significantly higher than that in the scopolamine group.

On the other hand, with αFMH administration, the spontaneous alternation behavior ratios were 49.2% and 47.5% in the scopolamine and DBB + scopolamine groups, respectively. The alternation behavior ratio of the DBB administration group, which was higher than that in the scopolamine group without αFMH, was similar to the value in the scopolamine group when αFMH was administered (Fig. 4).

Effects of histidine on novel object recognition

We calculated the discrimination index of the time spent exploring one object in the animals' exploratory behavior during the familiarization phase. The index was 51.1% in the vehicle group, whereas the positive control group administered thioperamide and the groups with the oral administration of histidine 200 mg/kg and 500 mg/kg showed values of 50.9%, 52.0%, and 50.0%, respectively. There was no bias in the exploratory behavior of two objects among these four groups.

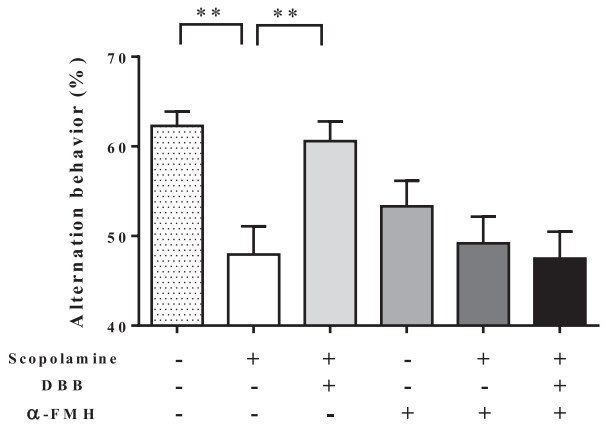

Fig. 4 Effects of DBB on the scopolamine-induced impairment of spontaneous alternation behavior. Under conditions with or without an inhibitor of histamine biosynthesis, α-FMH, the effects of vehicle or DBB on scopolamine-induced amnesia were evaluated based on the change in the spontaneous alternation behavior ratio in the Y-maze. Values are the means ± S.E. of 10 mice. $^{**}P < 0.01$ vs. scopolamine-alone group.

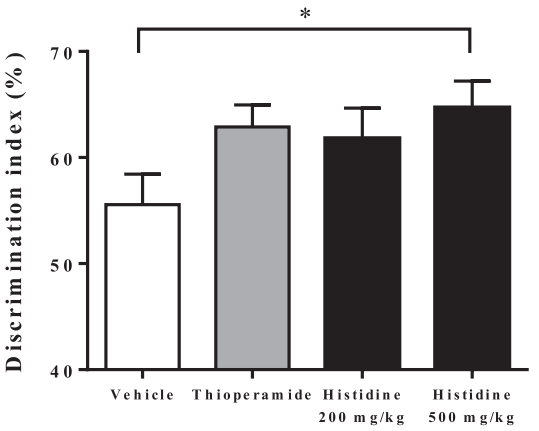

Fig. 5 Effects of histidine on the novel object recognition task. Vehicle, thioperamide, histidine 200 mg or histidine 500 mg/kg was administered 60 min before and 24 h after the familiarization phase, and the retention trial was performed 48 h after the familiarization phase. In the retention trial, the discrimination index of the time spent exploring the novel object in total exploratory behavior was calculated. Values are the means ± S.E. of 19 or 20 mice. $^{*}P < 0.05$ vs. vehicle.

We next calculated the discrimination index of the time spent exploring a novel object in the retention trial 48 h after the familiarization phase. The total time of exploration toward each object in the retention trial was similar among the four groups, but the ratio of time spent exploring the novel object was 55.2% in the vehicle group, 63.6% in the thioperamide group, and 61.8% and 64.7% in the histidine 200 mg/kg and 500 mg/kg groups. The value of the histidine 500 mg/kg group was significantly higher compared to the vehicle group (Fig. 5)

Effects of histidine on spontaneous alternation behavior in the Y-maze

We evaluated the effects of the oral administration of histidine on the scopolamine-induced memory impairment in the mice based on their spontaneous alternation behavior ratio in the Y-maze. As noted above, we calculated the spontaneous alternation behavior ratio using the number of alternation behaviors and the total number of entries into the maze arms.

The spontaneous alternation behavior ratios were 66.5% in the group without scopolamine administration, 51.0% in the mice administered scopolamine, 58.9% in the thioperamide + scopolamine group, 63.1% in the histidine 200 mg/kg + scopolamine group, and 62.4% in the histidine 500 mg/kg + scopolamine group. The mice administered 200 or 500 mg/kg histidine showed significantly higher values compared to the scopolamine group (Fig. 6).

DISCUSSION

We investigated the possible effect of the ingestion of DBB, which contains high levels of histidine, as well as the effects of the oral ingestion of L-histidine on cognitive function via activation of the histaminergic neuron system, using a mouse model. We used the novel object recognition task to evaluate recognition memory, based on the tendency of rodents to prefer novel objects (7, 10). Cognitive functions associated with the activation or impairment of the histaminergic neuronal systems by various compounds have been reported. As histamine H3 receptor is a presynaptic autoreceptor of the central histaminergic system and regulates the release of histamine, the administration of the H3 receptor antagonist thioperamide has reported to enhance learning memory function after memory acquisition (18, 41). In the present study, we observed that the administration of DBB significantly increased the ratio of time spent exploring the novel object in the Y-maze compared to the vehicle group, in which the exploratory behavior was almost the same for the two objects (Fig. 1). In the vehicle group, the memory of the object that was acquired during the familiarization phase was lost, and the exploratory behavior indicated that the mice recognized both objects as novel. In the DBB administration group, the memory of the object that was acquired during the familiarization phase was maintained, and therefore the mice

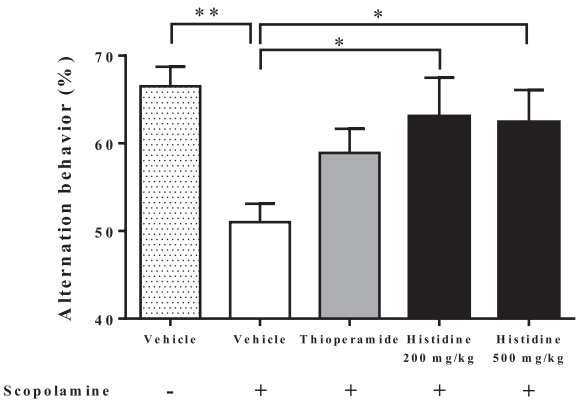

Fig. 6 Effects of histidine on the scopolamine-induced impairment of spontaneous alternation behavior. Vehicle, thioperamide, histidine 200 mg/kg, or histidine 500 mg/kg was administered 30 min before a scopolamine administration. Then, the number of alternation behaviors (number of times the mouse entered the three different arms consecutively) in the Y-maze was counted, and the alternation behavior ratio against total number entries into all arms was calculated. Values are the means ± S.E. of 19 or 20 mice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle + scopolamine.

were able to distinguish between the object that had been encountered previously and the novel object. These results suggested that DBB may enhance visual cognitive function and that DBB may be directly involved in improving cognitive function.

As shown in Table 1, DBB contains abundant histidine, which is characteristic of DBB (12). In the present study, after the DBB administration to mice, the hypothalamus was extracted so that the neural histamine which is biosynthesized from histidine by histidine decarboxylase in the tuberomammillary nucleus was included, and we measured the changes in histidine and histamine concentrations within the tissue over time. After the administration of DBB, plasma histidine increased, followed by an increase in the histidine concentration within the hypothalamus, and the histamine concentration then increased (Fig. 2). The histamine level in the brain changed within one day, and the histaminergic neuron system is known to be activated during the dark period in light/dark cycle (16, 30). When we administered DBB or vehicle to mice during the dark period, the levels of histamine as well as that of its metabolite t-MH within the brain were significantly higher when DBB was administered, indicating that DBB activates the histaminergic neuron system (Fig. 3). The elevated levels of histamine in the hypothalamus returned to the pre-administration level by 6 h after administration, suggesting that the increased histamine was metabolized under conditions of steady cerebral neural activity due to the DBB administration.

At 60 min after DBB administration, *i.e.*, while the hypothalamic histamine level was elevated, we used the Y-maze to examine the improvement in the spontaneous alternation behavior ratio after scopolamine-induced amnesia. Spontaneous alternation behavior is an established trait among rodents, and when a mouse is put into a Y-maze and allowed to explore, it tends to enter a different arm from the arm most recently visited. This is considered an index of spatial short-term working memory (15, 23). We used this trait to investigate the effect of DBB administration in mice. The DBB + scopolamine administration group showed significantly increased ratios compared to the scopolamine group, indicating that DBB had beneficial effects on the learning and memory deficits caused by scopolamine (Fig. 4). We also found that the administration of αFMH—an inhibitor of histidine decarboxylase (HDC) that is necessary for the biosynthesis of histamine—eliminated the beneficial effect on the spatial short-term working memory, and that the effect of DBB was mediated via histamine biosynthesis (Fig. 4). The scopolamine used in the Y-maze model is a muscarinic receptor antagonist, and is also known to cause learning and memory deficits (3, 39). The histaminergic neuron system and acetylcholine nervous system are thought to interact and mediate cognitive memory function (2, 5, 33). In the present study, we found that even when the memory learning pathway via acetylcholine was blocked by scopolamine, the degree of working memory impairment was reduced by activation of the histaminergic neuron system presumably due to the administration of DBB (Fig. 4), suggesting that histamine is involved in cognition in pathways that do not involve acetylcholine.

We also evaluated the effects of an oral histidine administration on cognitive function to determine whether histidine can substitute for DBB in behavioral models, as mentioned earlier. We found that the administration of 200 mg/kg histidine (which is equivalent to the quantity of histidine in DBB: 1.6 g/kg) and 500 mg/kg histidine increased the ratio of the time when the mice spent exploring the novel object, compared to the vehicle group in the novel object recognition task (Fig. 5). In the Y-maze spontaneous alternation test, a significant increase in the alternation behavior ratio was observed in the histidine 200 mg/kg and 500 mg/kg with scopolamine groups compared to the scopolamine-alone group (Fig. 6). Thus, the oral ingestion of histidine was confirmed to counteract the scopolamine-induced

amnesia of mice in the Y-maze test, a finding that is consistent with the observed improvement of amnesia in an elevated plus-maze test associated with the administration of histidine (28, 29).

As a brain neurotransmitter, histamine neurons are projected to almost all areas of the cerebral cortex and are thus involved in a variety of important nervous functions (14, 35). Further investigations are necessary to identify the optimal timing of administration and doses of DBB and histidine for recognition memory enhancement, but the results of the present study suggest that the oral administration of DBB activates the histaminergic neuron system. Our findings will contribute to the understanding of the mechanisms underlying the previously reported effects of DBB. In addition, the present results help elucidate the newly identified physiological activity of DBB.

Acknowledgments

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REFERENCES

1. Alvarez EO and Alvarez PA (2008) Motivated exploratory behaviour in the rat: the role of hippocampus and the histaminergic neurotransmission. *Behav Brain Res* **186**, 118–125.
2. Bacciottini L, Passani MB, Mannaioni PF and Blandina (2001) Interactions between histaminergic and cholinergic systems in learning and memory. *Behav Brain Res* **124**, 183–194.
3. Bartus RF and Johnson HR (1976) Short-term memory in the rhesus monkey: disruption from the anti-cholinergic scopolamine. *Pharmacol Biochem Behav* **5**, 39–46.
4. Bernaerts P, Lamberty Y and Tirelli E (2004) Histamine H3 antagonist thioperamide dose-dependently enhances memory consolidation and reverses amnesia induced by dizocilpine or scopolamine in a one-trial inhibitory avoidance task in mice. *Behav Brain Res* **154**, 211–219.
5. Blandina P, Efoudebe M, Cenni G, Mannaioni P and Passani MB (2004) Acetylcholine, histamine, and cognition: two sides of the same coin. *Learn Mem* **11**, 1–8.
6. Brioni JD, Esbenshade TA, Garrison TR, Bitner SR and Cowart MD (2011) Discovery of histamine H3 antagonists for the treatment of cognitive disorders and Alzheimer's disease. *J Pharmacol Exp Ther* **336**, 38–46.
7. Clarke JR, Cammarota M, Gruart A, Izquierdo I and Delgado-García JM (2010) Plastic modifications induced by object recognition memory processing. *Proc Natl Acad Sci USA* **107**, 2652–2657.
8. Dai H, Kaneko K, Kato H, Fujii S, Jing Y, Xu A, Sakurai E, Kato M, Okamura N, Kuramasu A and Yanai K (2007) Selective cognitive dysfunction in mice lacking histamine H1 and H2 receptors. *Neurosci Res* **57**, 306–313.
9. De Almeida MA and Izquierdo I (1986) Memory facilitation by histamine. *Arch Int Pharmacodyn Ther* **283**, 193–1981
10. Ennaceur A and Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res* **31**, 47–59.
11. Esbenshade TA, Browman KE, Bitner RS, Strakhova M, Cowart MD and Brioni JD (2008) The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. *Br J Pharmacol* **154**, 1166–1181.
12. Fuke S and Konosu S (1991) Taste-active components in some foods: a review of Japanese research. *Physiol Behav* **49**, 863–868.
13. Glowinski J and Iversen LL (1966) Regional studies of catecholamines in the rat brain. I. The disposition of [3H]norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain. *J Neurochem* **13**, 655–669.
14. Haas HL, Sergeeva OA and Selbach O (2008) Histamine in the nervous system. *Physiol Rev* **88**, 1183–1241.
15. Hughes RN (2004) The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neurosci Biobehav Rev* **28**, 497–505.
16. Itowi N, Yamatodani A, Kiyono S, Hiraiwa ML and Wada H (1991) Effect of histamine depletion on the circadian amplitude of the sleep-wakefulness cycle. *Physiol Behav* **49**, 643–646.
17. Jorgensen EA, Knigge U, Warberg J and Kjaer A (2007) Histamine and the regulation of body weight. *Neuroendocrinology* **86**, 210–214.
18. Köhler CA, da Silva WC, Benetti F and Bonini JS (2011) Histaminergic mechanisms for modulation of memory systems. *Neural Plast* **2011**, 328602.
19. Kohno K, Hayakawa F, Xichang W, Shunsheng C, Yokoyama M, Kasai M, Takeutchi F and Hatae K (2005) Comparative study on flavor preference between Japanese and Chinese for dried bonito stock and chicken bouillon. *J Food Sci* **70**, s193–198.
20. Kollonitsch J, Perkins LM, Patchett AA, Doldouras GA, Marburg S, Duggan DE, Maycock AL and Aster SD (1978) Selective inhibitors of biosynthesis of aminergic neurotransmitters. *Nature* **274**, 906–908.
21. Kuroda M, Ishizaki T, Maruyama T, Takatsuka Y and Kuboki T (2007) Effect of dried-bonito broth on mental fatigue and mental task performance in subjects with a high fatigue score. *Physiol Behav* **92**, 957–962.
22. Kuroda M and Nozawa Y (2008) Effect of dried-bonito broth on mood states: a pooled analysis of four randomized controlled human trials. *Biomed Res (Tokyo)* 29, 75–79.
23. Lalonde R (2002) The neurobiological basis of spontaneous alternation. *Neurosci Biobehav Rev* **26**, 91–104.
24. Leurs R, Bakker RA, Timmerman H and de Esch IJ (2005) The histamine H3 receptor: From gene cloning to H3 receptor drugs. *Nat Rev Drug Discov* **4**, 107–120.
25. Maeyama K, Watanabe T, Yamatodani A and Wada H (1982) Effect of α-fluoromethylhistidine, a suicide inhibitor of HDC, on histamine levels in mouse tissue. *Biochem Pharmacol* **31**, 2367–2370.
26. Masaki T and Yoshimatsu H (2010) Neuronal histamine and its receptors: implication of the pharmacological treatment of

obesity. *Curr Med Chem* **17**, 4587–4592.
27. Miyamoto Y, Yoshimoto R, Yumoto M, Ishihara A, Takahashi K, Kotani H, Kanatani A and Tokita S (2004) Simultaneous fluorometric measurement of histamine and tele-methylhistamine levels in rodent brain by high-performance liquid chromatography. *Anal Biochem* **334**, 89–96.
28. Miyazaki S, Imaizumi M and Onodera K (1995) Effects of thioperamide, a histamine H3-receptor antagonist, on a scopolamine-induced learning deficit using an elevated plus-maze test in mice. *Life Sci* **57**, 2137–2144.
29. Miyazaki S, Imaizumi M and Onodera K (1995) Ameliorating effects of histidine on learning deficits in an elevated plus-maze test in mice and the contribution of cholinergic neuronal systems. *Methods Find Exp Clin Pharmacol* **17**, 57–63.
30. Mochizuki T, Yamatodani A, Okamura K, Horii A, Inagaki N and Wada H (1992) Circadian rhythm of histamine release from the hypothalamus of freely moving rats. *Physiol Behav* **51**, 391–394.
31. Nozawa Y, Ishizaki T, Kuroda M and Noguchi T (2008) Effect of dried-bonito broth intake on peripheral blood flow, mood, and oxidative stress marker in humans. *Physiol Behav* **93**, 267–273.
32. Nozawa Y, Yamada K, Okabe Y, Ishizaki T and Kuroda M (2009) The anti-fatigue effects of the low-molecular-weight fraction of bonito extract in mice. *Biol Pharm Bull* **32**, 468–474.
33. Passani MB, Bacciottini L, Mannaioni PF and Blandina P (2000) Central histaminergic system and cognition. *Neurosci Biobehav Rev* **24**, 107–114.
34. Passani MB, Blandina P and Torrealba F (2011) The histamine H3 receptor and eating behavior. *J Pharmacol Exp Ther* **336**, 24–29.
35. Panula P and Nuutinen S (2013) The histaminergic network in the brain: Basic organization and role in disease. *Nat Rev Neurosci* **14**, 472–487.
36. Sakata T, Yoshimatsu H and Kurokawa M (1997) Hypothalamic neuronal histamine: implications of its homeostatic control of energy metabolism. *Nutrition* **13**, 403–411.
37. Sundvik M, Kudo H, Toivonen P, Rozov S, Chen YC and Panula P (2011) The histaminergic system regulates wakefulness and orexin/hypocretin neuron development via histamine receptor H1 in zebrafish. *FASEB J* **25**, 4338–4347.
38. Taylor KM and Snyder SH (1971) Brain histamine: rapid apparent turnover altered by restraint and cold stress. *Science* **172**, 1037–1039.
39. Tinsley CJ, Fontaine-Palmer NS, Vincent M, Endean EP, Aggleton JP, Brown MW and Warburton EC (2011) Differing time dependencies of object recognition memory impairments produced by nicotinic and muscarinic cholinergic antagonism in perirhinal cortex. *Learn Mem* **18**, 484–92.
40. Verdière M, Rose C and Schwartz JC (1977) Turnover of cerebral histamine in a stressful situation. *Brain Res* **129**, 107–119.
41. Yamamoto Y, Mochizuki T, Okakura-Mochizuki K, Uno, A and Yamatodani A (1997) Thioperamide, a histamine H3 receptor antagonist, increases GABA release from the rat hypothalamus. *Methods Find Exp Clin Pharmacol* **19**, 289–298.