

Activation of the reward system ameliorates passive cutaneous anaphylactic reaction in mice

To the Editor,

Positive emotional states are often associated with better physical health outputs, including cancer survival.¹ Some allergic diseases may also benefit from positive emotional states, as a high placebo effect has frequently been observed in clinical trials of allergic diseases such as allergic rhinitis.² However, the causal link between positive emotional states and allergic reaction has not yet been investigated.

The brain's reward system, consisting of the dopaminergic neurons (DNs) in the ventral tegmental area (VTA) and the dopaminergic projections from the VTA to components of the limbic system, mediates positive emotions, expectations, and motivation,³ which may contribute to the clinical benefit of placebo effects.⁴ Given the close relationships between positive emotional states and the reward system, we hypothesized that activation of the reward system may affect allergic reactions. To test this hypothesis, we examined the effects of chemogenetic, spontaneous, and pharmacological activation of the brain reward system on the passive cutaneous anaphylactic (PCA) reaction, a classical model of IgE/mast cell-mediated allergic skin reaction, in mice.

Six-week-old male C57BL/6 mice and tyrosine hydroxylase (Th)-Cre mice housed under 12-hour light/12-hour dark conditions with ad libitum access to food and water were used. All animal experiments were approved by the Institutional Review Board of the University of Yamanashi. For more information, see the Supplementary Methods section.

First, we used the designer receptor exclusively activated by designer drugs (DREADD) system to specifically control reward system activity as previously described.⁵ We injected the VTA of Th-Cre mice with a viral vector encoding DREADDs, mutated muscarinic receptors that respond to the synthetic ligand clozapine-*N*-oxide (CNO) but not their endogenous ligand, and the fluorescent reporter mCherry (AAV-hM3Dq); control mice were injected with a viral vector encoding mCherry but lacking DREADDs (AAV-control) (Figure 1A). Five weeks after the viral injections, the mice were treated intraperitoneally with CNO and subjected to the PCA reaction (Figure 1B).

After AAV injection, we assessed the efficacy of virus expression in the VTA by immunohistochemistry for Th, a marker for DNs, as

well as mCherry, an indicator of virus expression. DREADDs were specifically expressed in the VTA, but not in the substantia nigra pars compacta (SNc), which also contains DNs, as judged by the expression levels of mCherry and Th (Figure 1C). DREADD (AAV-hM3Dq) expression was observed in >60% of Th-positive area in the VTA, while DREADD expression was less than 10% of Th-positive area in the SNc (Figure S1). The expression levels of mCherry in the VTA were comparable between AAV-control injected mice and AAV-hM3Dq-injected mice (Figure 1C). DNs in the VTA were activated in response to CNO in AAV-hM3Dq-injected but not AAV-control-injected mice, as judged by c-fos expression (an indicator of neuron activation) (Figure 1D). Interestingly, the extent of PCA reaction was significantly smaller in VTA-activated mice than in control mice (Figure 1E). These results suggest a causal link between activation of the brain's reward system and amelioration of the PCA reaction.

Next, we aimed to determine whether natural reward would affect allergic reactions. Rodents exhibit preferential saccharin intake when they are given an ad libitum two-bottle choice between saccharin solution and water, in association with activation of the VTA and the nucleus accumbens (NAcc), a dopamine projection area from the VTA.⁶ We used this experimental system for the present study (Figure 2A). Voluntary saccharin drinking markedly activated DNs in the VTA, as judged by c-fos expression (Figure 2B). The extent of PCA reaction was significantly smaller in mice that engaged in voluntary saccharin drinking than in control mice (Figure 2C). Importantly, administration of similar amounts of saccharin by gastric tube did not affect the extent of PCA reaction (Figure 2D). These results suggest that VTA activation triggered by voluntary saccharin drinking ameliorates the PCA reaction.

Finally, we examined the effects of the dopamine precursor levodopa (L-DOPA) on PCA reaction (Figure S2, A). L-DOPA is converted to dopamine in the brain and is to replenish depleted levels of dopamine in patients with Parkinson's disease. Normally, L-DOPA is administered with a dopamine decarboxylase inhibitor such as benserazide to prevent conversion of L-DOPA to dopamine in the bloodstream,⁷ as dopamine cannot cross the blood-brain barrier. We found that administration of L-DOPA with benserazide decreased the extent of the PCA reaction relative to control mice

Abbreviations: AAV, adeno-associated virus; CNO, clozapine-*N*-oxide; DNs, dopaminergic neurons; DREADD, designer receptor exclusively activated by designer drugs; L-DOPA, levodopa; NAcc, nucleus accumbens; Th, tyrosine hydroxylase; VTA, ventral tegmental area.

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(Figure S2B). Administration of L-DOPA or benserazide alone did not affect the extent of PCA reaction (Figure S2C,D), suggesting that dopamine levels in the brain, but not in the periphery, are important for suppression of the PCA reaction.

Several studies have reported relationships between dopamine and IgE/mast cell-mediated allergic reactions, including the PCA reaction. However, most of these studies focus on the peripheral, but not brain (central), activity of dopamine/dopamine receptors, and some of their results are controversial. For instance, Mori et al reported that bone marrow-derived mast cells (BMMCs) express D1-like dopamine receptors and that the antagonist SCH23390 inhibited IgE-mediated degranulation in BMMCs and the PCA reaction in mice,⁸ suggesting that dopamine promotes

allergic reactions. By contrast, Casale et al reported that intradermal or intravenous dopamine suppressed histamine-mediated

Highlights

The activation of the brain's reward system by chemogenetics, natural reward, or pharmacological approaches ameliorates IgE/mast cell-mediated allergic reaction in mice. These findings suggest a functional connection between a mood-regulating neuronal circuit and allergic reaction.

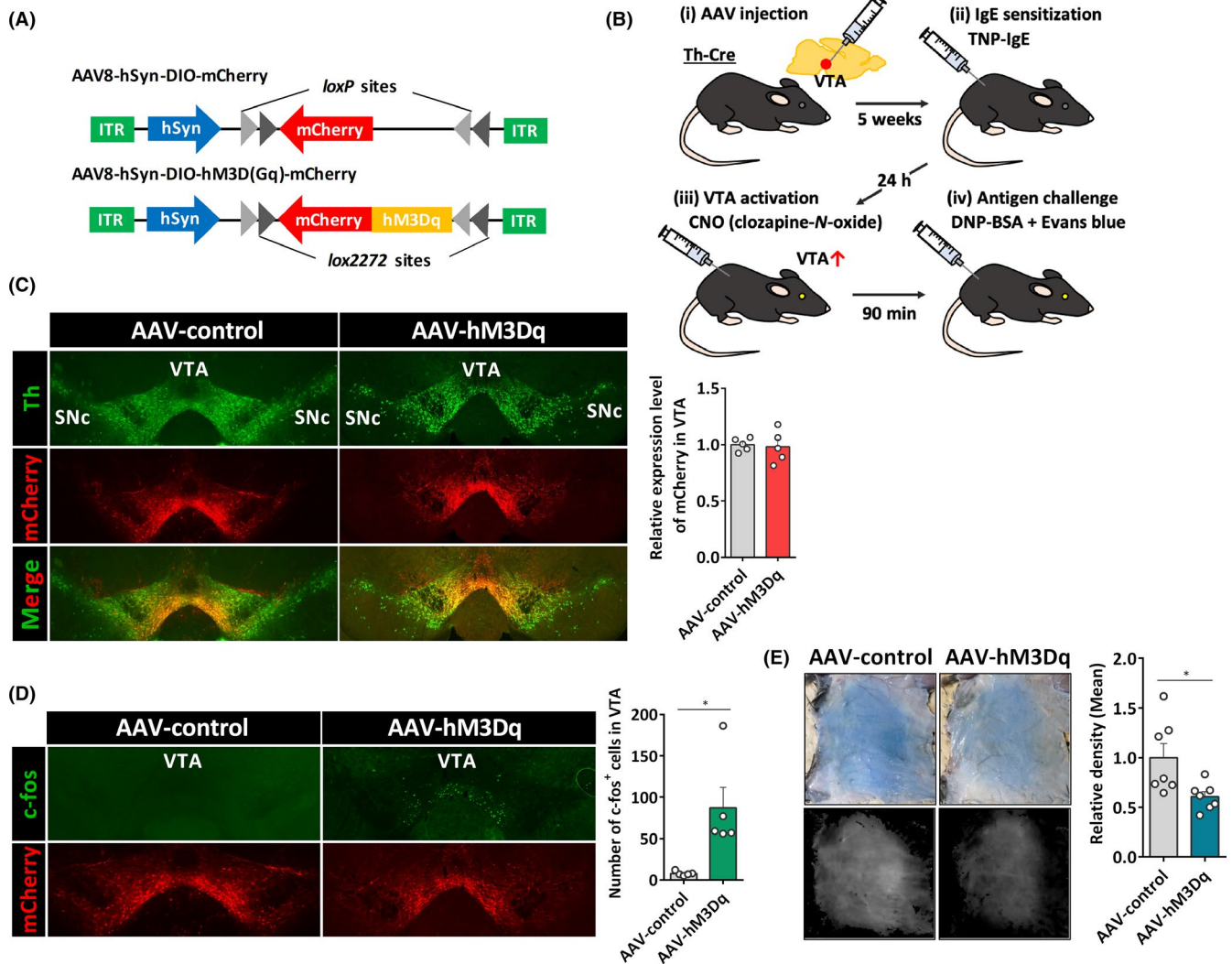


FIGURE 1 Amelioration of PCA reaction by activation of DNs in the VTA using DREADDs. A, Construction of the Cre-dependent DREADD AAV. B, Schematic illustration of the experimental design. C, Representative images of DREADD expression (mCherry: red) in VTA neurons (Th: green) in control (AAV-control) and VTA-activated mice (AAV-hM3Dq) (left). Quantitative analysis of mCherry expression in the VTA in control mice (AAV-control) and VTA-activated mice (AAV-hM3Dq) (n = 5) (right). D, Representative images of c-fos expression in VTA neurons 90 min after CNO administration in control (AAV-control) and VTA-activated mice (AAV-hM3Dq) (left). Quantitative analysis of c-fos⁺ neurons in the VTA of control mice (AAV-control) and VTA-activated mice (AAV-hM3Dq) (n = 5) (right). E, Representative images of PCA reaction in the skin of control mice (AAV-control) and VTA-activated mice (AAV-hM3Dq) (upper panels), and digitized images used for density value evaluations (black and white) (lower panels) (left). Quantitative analysis of the images of PCA reaction (right). Graph shows relative density (n = 7). C-E, Values are shown as means ± SEM. Data were analyzed by Student's t test; *P < .05

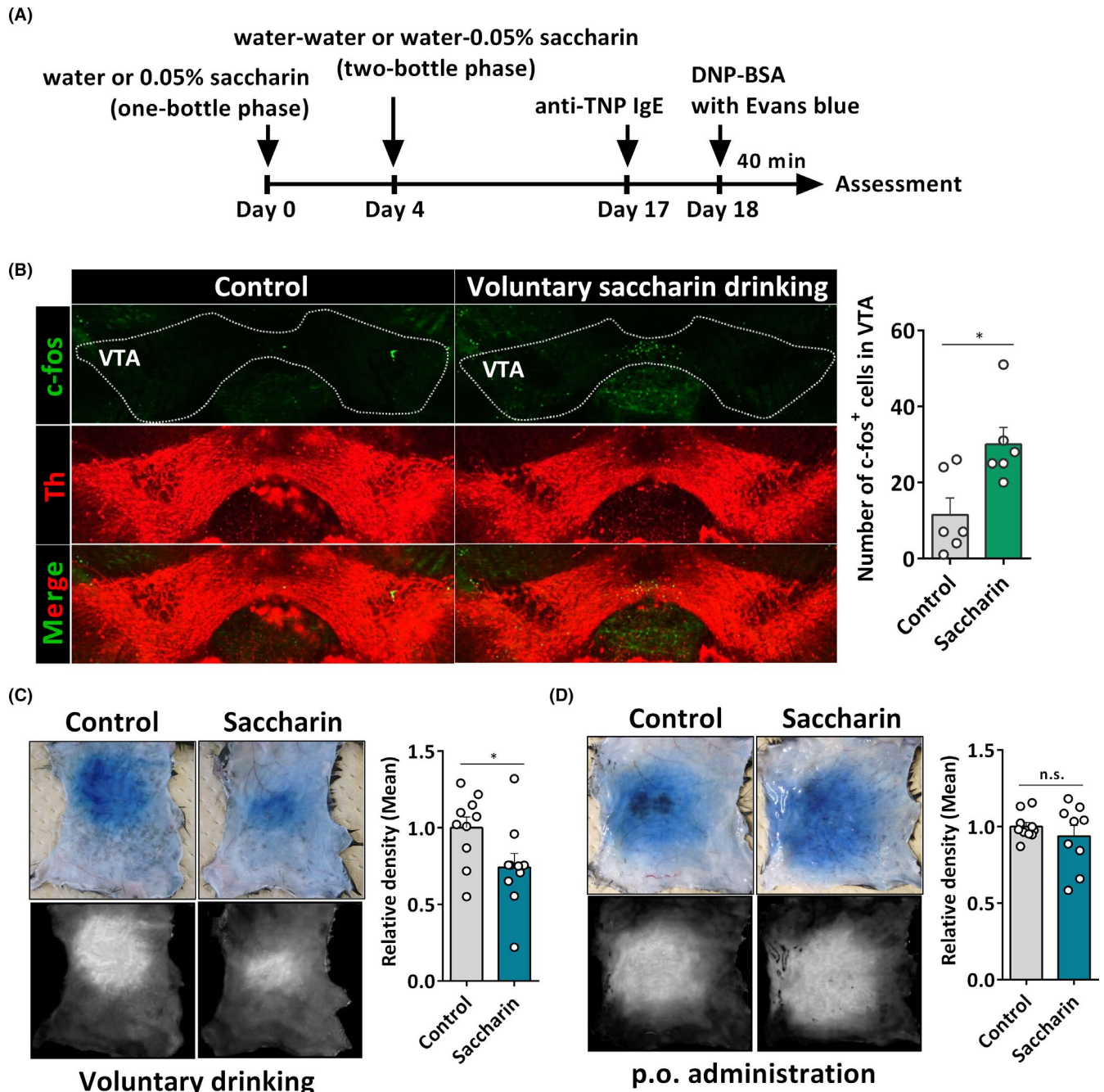


FIGURE 2 Amelioration of PCA reaction by spontaneous activation of the reward system with voluntary saccharin drinking. A, Schematic illustration of the experimental design. B, Representative images of c-fos expression in VTA neurons 3 days after voluntary saccharin drinking with a two-bottle choice (*left*). Quantitative analysis of c-fos⁺ neurons in VTA in control mice and voluntarily saccharin-drinking mice (*right*) ($n = 6$). C, Representative images of PCA reaction in the skin of control mice and voluntarily saccharin-drinking mice (*upper panels*), and digitized images used for density value evaluations (black and white) (*lower panels*) (*left*). Quantitative analysis of images of the PCA reaction (*right*). ($n = 9-10$). D, Representative images of PCA reaction in the skin of control mice and mice that received the same amount of saccharin by gastric tube (*left*). Quantitative analysis of images of the PCA reaction (*right*). ($n = 9-10$). B-D, Values are shown as means $\pm$ SEM. Data were analyzed by Student's t test; * $P < .05$

cutaneous responses in humans,⁹ suggesting that dopamine antagonizes allergic reaction. We wish to emphasize that in this study, we used several independent approaches—DREADDs, spontaneous saccharine drinking, and L-DOPA with benserazide—that selectively or preferentially target the reward system in the mouse brain. This allows us to exclude peripheral or net (ie, both

peripheral and central) effects of dopamine/dopamine receptors on the PCA reaction.

The mechanisms linking the reward system and allergic reaction remain to be determined. We observed that plasma histamine levels upon PCA reaction were comparable between VTA-activated and control mice, as well as between voluntarily saccharin-drinking

mice and control mice (Figure S3A,B), suggesting that the reward system might affect PCA reaction downstream of mast cell activation. Consistently, activation of the brain's reward system by voluntary saccharin drinking slightly but significantly suppressed plasma extravasation by intradermal injection of histamine in the skin (Figure S4). In addition, we did not find any significant effects of 6-hydroxydopamines (6-OHDA) that chemically ablate peripheral catecholaminergic neurons of the sympathetic nervous system (SNS) on the inhibition of PCA reaction in VTA-activated mice by voluntary saccharin-drinking mice (Figure S5). 6-OHDA did not affect basal PCA reaction in mice (Figure S5). These data suggest that the brain's reward system might affect PCA reaction in part through inhibition of histamine-induced vascular permeability, independent on the SNS.

The extent of control PCA reaction appears to be smaller in virus-injected mice than mice without virus-injected mice (Figure 1E, Figure 2C,D, Figure S6). Thus, viral injection might affect PCA responses perhaps because viral injection affects innate immune response.

The limitations of this study are as follows;

1. Relationships between the reward system and positive emotional states are complex. In addition, the reward function associated with emotions is difficult to measure/interpret reliably in animals. Thus, we emphasize that it is the effects of VAT activation (the reward system activation), but not positive emotion, on PCA reaction that are directly demonstrated in this study.
2. Dopamine neurotransmission exerts different influences on neuronal processes and behaviors at different timescales. Thus, VTA activation by DREADDs, voluntary saccharin drinking, and L-dopa treatment will also affect other brain or physiological system at different time scales, which can also contribute to the observed effects.
3. Affective, cognitive, and reward-related brain circuits that activate VTA and inhibit PCA reaction should be investigated in future studies since the identification of such variables are relevant to real world.

In summary, this study shows that activation of the reward system in the brain by chemogenetic, natural reward, or pharmacological approaches can ameliorate PCA reaction in mice. These findings suggest a functional connection between a mood-regulating neuronal circuit, specifically the reward system, and type I allergic reactions.

KEYWORDS

dopamine, mast cells, passive cutaneous anaphylactic (PCA) reaction, reward system, ventral tegmental area (VTA)

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING INFORMATION

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REFERENCES

1. Pressman SD, Jenkins BN, Moskowitz JT. Positive affect and health: what do we know and where next should we go? *Annu Rev Psychol*. 2019;70:627-650.
2. del Cuvillo A, Sastre J, Bartra J, et al. Placebo effect in clinical trials involving patients with allergic rhinitis. *J Invest Allergol Clin Immunol*. 2011;21(Suppl 3):40-45.
3. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. *Nat Rev Neurosci*. 2013;14:609-625.
4. de la Fuente-Fernández R, Ruth TJ, Sossi V, Schulzer M, Calne DB, Stoessl AJ. Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. *Science*. 2001;293:1164-1166.

5. Ben-Shaanan TL, Azulay-Debby H, Dubovik T, et al. Activation of the reward system boosts innate and adaptive immunity. *Nat Med*. 2016;22:940-944.
6. Dudek M, Abo-Ramadan U, Hermann D, et al. Brain activation induced by voluntary alcohol and saccharin drinking in rats assessed with manganese-enhanced magnetic resonance imaging. *Addict Biol*. 2015;20:1012-1021.
7. Shimozaawa A, Fujita Y, Kondo H, et al. Effect of L-DOPA/Benserazide on propagation of pathological α -synuclein. *Front Neurosci*. 2019;13:595.
8. Mori T, Kabashima K, Fukamachi S, et al. D1-like dopamine receptors antagonist inhibits cutaneous immune reactions mediated by Th2 and mast cells. *J Dermatol Sci*. 2013;71:37-44.
9. Casale TB, Shelhamer JH, Parrillo JE, Kaliner MA. Dopamine inhibition of histamine-mediated cutaneous responses. *J Allergy Clin Immunol*. 1984;73:837-841.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Supporting Information

Activation of the reward system ameliorates passive cutaneous anaphylactic reaction in mice

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Supplemental Methods

Mice

Tyrosine hydroxylase (Th)-Cre mice [B6.Cg-Tg(Th-cre)1Tmd/J]¹⁰ were purchased from Jackson Laboratory. Six-to-eight-week-old male C57BL/6 mice were purchased from Japan SLC (Tokyo, Japan). The mice were housed under 12-hour light/12-hour dark conditions. All animal experiments were approved by the Institutional Review Board of the University of Yamanashi and carried out according to the guidelines.

Designer Receptor Exclusively Activated by Designer Drugs (DREADDs)

expressed in adeno-associated virus (AAV)-based vectors

DREADDs (hM3D[Gq]) are mutated muscarinic receptors that no longer respond to their endogenous ligand, but are activated upon exposure to a synthetic ligand, clozapine-N-oxide (CNO). Activated DREADDs then stimulate intracellular signaling leading to neuronal activation. To express DREADDs and a fluorescent reporter marker in VTA dopaminergic neurons, we infected a viral vector that expresses a Cre-dependent DREADD construct (AAV8-hSyn-DIO-hM3D[Gq]-mCherry) (Addgene, Watertown, MA, USA) under the control of the human synapsin I (*Syn I*) promoter in mice that express *Cre* under the tyrosine hydroxylase (Th) promoter (which we refer to as Th-Cre mice), as

previously described.⁵ As a control, we infected a sham virus that expresses only the fluorescent reporter (AAV8-hSyn-DIO-mCherry). Please note that the vectors have loxP sites and we used Th-Cre mice for this experiment (Fig. 1A).

Stereotaxic injection

Stereotaxic adeno-associated virus (AAV) injection was performed as previously described.⁵ Briefly, male adult Th-Cre mice were anesthetized with chloral hydrate (500 mg/kg, i.p.) (Nacalai Tesque, Kyoto, Japan) in sterile saline solution and injected stereotaxically into the ventral tegmental area (VTA) with recombinant AAV8-hSyn-DIO-hM3D(Gq)-mCherry or AAV8-hSyn-DIO-mCherry as a control. Bilateral injection sites were as follows: anterior–posterior -3.28 mm; medial–lateral $\pm$ 0.5 mm; dorsal–ventral 4.2 mm from bregma. After 30 days, the mice were i.p. administered 1 mg/kg clozapine-*N*-oxide (CNO) (Tocris Bioscience, Bristol, UK), the ligand of DREADDs, to activate dopaminergic neurons (DNs) in the VTA.

Immunofluorescence staining

Murine brain sections fixed in 4% paraformaldehyde were permeabilized with 0.3% Triton X-100 in PBS (PBST) and blocked with 3% donkey serum (Sigma-Aldrich, St.

Louis, MO, USA) in PBST for 30 minutes at room temperature (RT). The sections were then stained with anti-Th antibody (Merck Millipore, Jaffrey, NH, USA), anti-mCherry antibody (Abcam), or anti-c-Fos antibody (Santa Cruz Biotechnology, Dallas, TX, USA) at 4°C overnight. The sections were washed and incubated for 2 hours with Alexa Fluor 488- or Alexa Fluor 568-conjugated anti-mouse secondary antibodies and/or Alexa Fluor 488- or Alexa Fluor 568-conjugated anti-rabbit secondary antibodies. Nuclei were stained with DAPI (Sigma-Aldrich) for 10 minutes. Finally, slides were mounted using ProLong Diamond antifade reagent (Invitrogen, Carlsbad, CA, USA), and images were obtained using the BZ-X800 microscope (KEYENCE, Tokyo, Japan). Staining areas of mCherry and Th in the VTA and the SNc of VTA-activated mice (AAV-hM3Dq) were measured by imageJ software. The DREADD expressing areas were divided by the Th-positive areas to calculate the percentages of DREADD expression in the both regions. The expression of c-fos (fluorescent dots) in the VTA region were counted to represent the number of c-fos positive cells in the VTA. Brightness of mCherry in the VTA was measured using imageJ software to compare the expression level of mCherry in the VTA between control mice and VTA-activated mice.

Induction of the passive cutaneous anaphylactic (PCA) reaction

Induction of the PCA reaction was performed as previously described.¹¹ Mouse anti-TNP IgE (125 ng; unless otherwise indicated) (BD Bioscience, San Jose, CA, USA) was intradermally injected into dorsal skin, and 24 hours later the mice were challenged i.v. with 50 µg of DNP-BSA (Code-number LG3017; Cosmo Bio, Tokyo, Japan) with 0.2% Evans blue (Sigma-Aldrich), the standard dye used for monitoring PCA reactions in the skin. Vascular permeability was visualized 40 minutes later based on blue staining of the injection sites on the reverse side of the skin. Staining sites were digitized using a high-resolution color camera (digital camera IXY3; Canon, Tokyo, Japan), and images were saved in Windows Photo Viewer as 8-bit color-scale JPEG files. The open-source software package ImageJ 1.43 (NIH, USA) was used for image analysis, as previously described.^{2,3} Briefly, color-scale images exported from Windows Photo Viewer were converted to HSB (hue/saturation/brightness) stack-type images using the Image tool. Thereafter, the HSB stack images were split into hue, saturation, and brightness images, and blue-stained areas were selected from the hue image using the threshold tool. These images were then combined with the saturation image and density values for the blue-stained areas, and measured using the analysis tool. Mean density was normalized against brightness and saturation, and relative density was plotted.

Plasma extravasation by intradermal injection of histamine

Histamine (500 ng) (Nacalai Tesque) was intradermally injected into dorsal skin, and then the mice were i.v. administered with 0.2% Evans blue. Vascular permeability was visualized 40 minutes later based on blue staining of the injection sites on the reverse side of the skin and calculated as describe above.

Voluntary consumption and oral administration of saccharin

Voluntary saccharin-drinking experiments using a two-bottle choice were performed as previously described.⁶ Briefly, mice were given water (control group) or 0.05% (w/v) saccharin (Sigma-Aldrich) solution (saccharin group) during the first 4 days. Thereafter, mice of the saccharin group were offered a choice between 0.05% saccharin solution and water for 2 weeks. Mice of the control group always had access to water in two bottles during this period. Every 3 days, the bottles were filled with fresh solutions, and the left–right position of the bottles was changed. In voluntary saccharin-drinking experiments, mice took 5.5 mL/mouse/day of 0.5 mg/mL saccharin solution, suggesting that mice took 2.75 mg saccharin per day. Therefore, mice were orally administered 2.75 mg saccharin in a final volume of 200 μ L using a gastric tube once a day for 2 weeks for oral saccharin administration experiments.

Administration of levodopa (L-DOPA)

Male C57BL/6 mice were sensitized with anti-TNP IgE in the dorsal skin; 24 hours after sensitization, the mice received intraperitoneal injection of 100 mg/kg levodopa (L-DOPA) (Sigma-Aldrich) with 12 mg/kg benserazide (Sigma-Aldrich), or saline as a control. A second injection (100 mg/kg L-DOPA with 12 mg/kg benserazide) was administered 45 minutes after the first injection. After 30 minutes of the second injection, the mice were challenged i.v. 24 hours later with 50 µg of DNP-BSA.

Sympathetic denervation

We used 6-hydroxydopamines (6-OHDA) to chemically ablate peripheral catecholaminergic neurons of the sympathetic nervous system (SNS) as previously described.⁵ Briefly, male C57BL/6 mice were i.p. administered with 100 mg/kg 6-OHDA (Sigma-Aldrich) for sympathetic denervation, or saline as a control (Day 0). A second administration (100 mg/kg 6-OHDA) was performed two days after the first injection (Day 2). On Day 3, the mice were sensitized with anti-TNP IgE in the dorsal skin, and 24 hours later the mice were challenged i.v. with 50 µg of DNP-BSA with 0.2% Evans blue.

Plasma histamine levels

Plasma samples were collected 10 minutes after the induction of the PCA reaction in mice. Concentrations of histamine in mouse plasma were determined using a specific ELISA kit (Oxford Biomedical Research, Rochester Hills, MI, USA).

Statistical analysis

All the data are expressed as means $\pm$ s.e.m. For two-group comparisons, statistical analyses were performed using the unpaired Student's *t*-test. For multigroup comparisons, we applied one-way ANOVA with *post hoc* testing using Tukey–Kramer multiple comparisons. A value of $p < 0.05$ was considered to be significant.

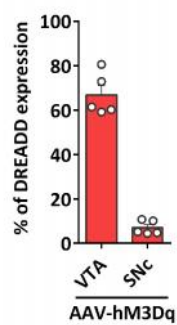
References for the supplemental methods

10. Savitt JM, Jang SS, Mu W, Dawson VL, Dawson TM. Bcl-x is required for proper development of the mouse substantia nigra. *J Neurosci* 2005;25:6721-8.

11. Nakamura Y, Nakano N, Ishimaru K, Hara M, Ikegami T, Tahara Y, Katoh R, Ogawa H, Okumura K, Shibata S, Nishiyama C, Nakao A. Circadian regulation of allergic reactions by the mast cell clock in mice. *J Allergy Clin Immunol*. 2014;133:568-75.

Supplemental Figure 1

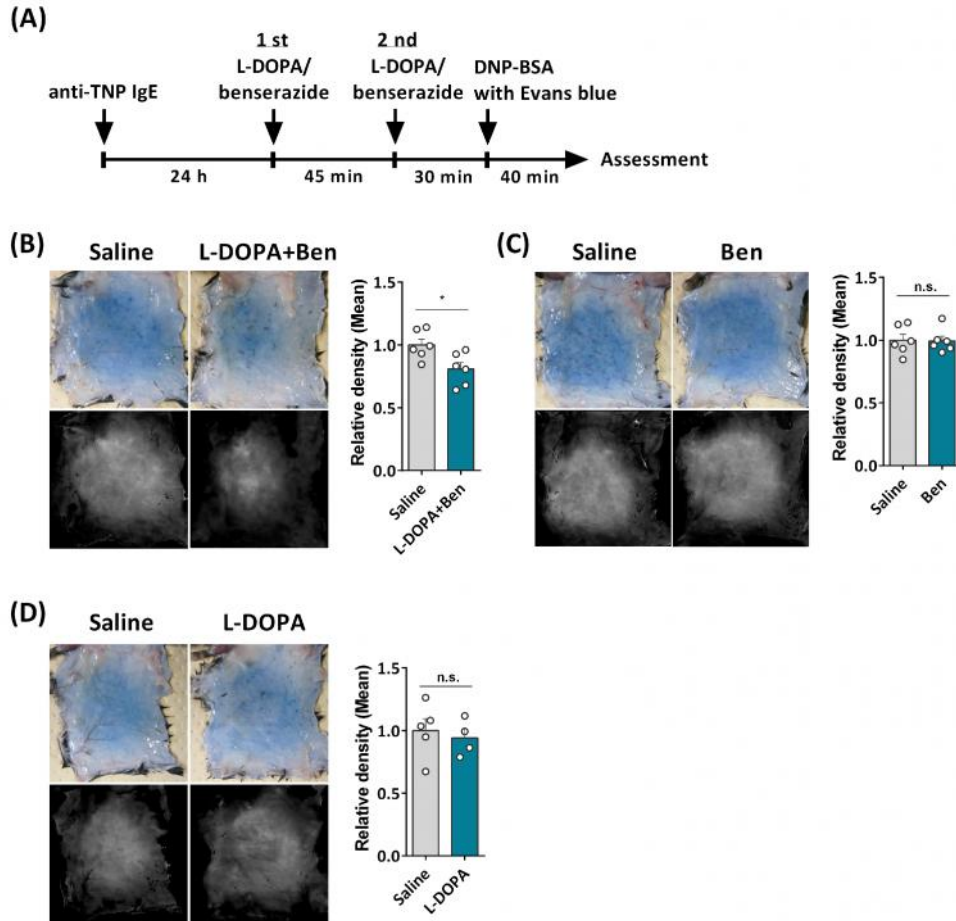
Selective DREADD expression in the VTA, but not the SNc.



Percentages of DREADD (AAV-hM3Dq) expression in the VTA and the SNc of VTA-activated mice (n=5) are shown. Values are shown as means $\pm$ SEM.

Supplemental Figure 2

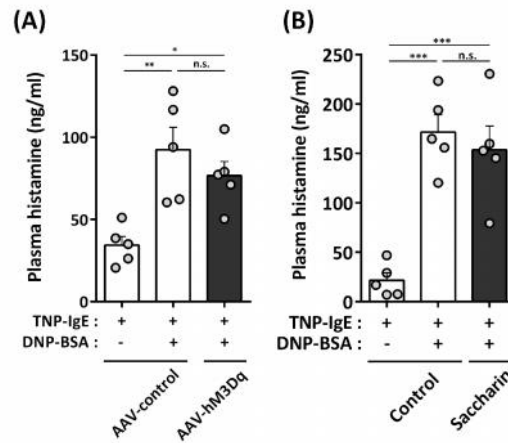
Amelioration of PCA reaction by L-DOPA with the dopamine decarboxylase inhibitor benserazide



(A) Schematic illustration of the experimental design. (B–D) Representative images of the PCA reaction in the skin of mice intraperitoneally treated with saline or L-DOPA plus benserazide (Ben) (B), with saline or benserazide (C), or with saline or L-DOPA (D) (*upper panels*), along with digitized images used for density value evaluations (black and white) (*lower panels*) (*left*). Quantitative analysis of images of the PCA reaction (*right*) (n=4-6). Graph shows relative density. Values are shown as means $\pm$ SEM. Data were analyzed by Student's t test; * P < 0.05; n.s., not significant.

Supplemental Figure 3

Plasma histamine levels upon PCA reaction

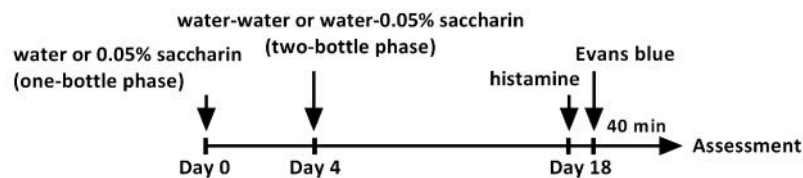


(A and B) Plasma histamine levels 10 minutes after induction of the PCA reaction [mouse anti-TNP IgE (500 ng) was used in this experiment] in control mice (AAV-control) vs. VTA-activated mice (AAV-hM3Dq) (n=5) (A), and mice allowed a two-bottle choice between saccharin solution and water vs. control mice (n=5) (B). (A and B) Values are shown as means $\pm$ SEM. Data were analyzed by one-way ANOVA with post hoc testing using Tukey-Kramer multiple comparisons; * P < 0.05, ** P < 0.01, *** P < 0.001; n.s., not significant.

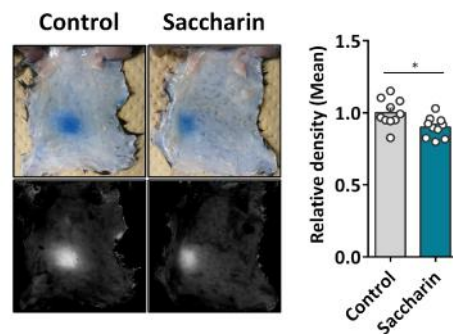
Supplemental Figure 4

Amelioration of histamine-induced plasma extravasation in the skin by spontaneous activation of the reward system with voluntary saccharin drinking

(A)



(B)

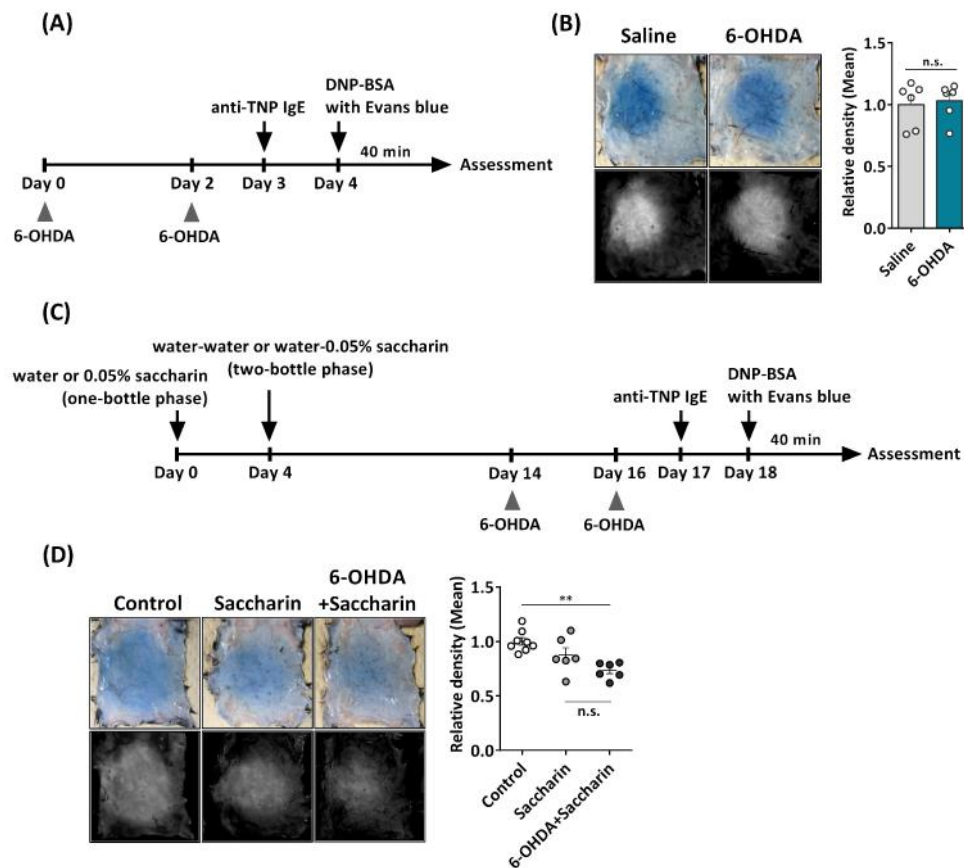


(A) Schematic illustration of the experimental design

(B) Representative images of plasma extravasation by intradermal injection with histamine in the skin of control mice and voluntary saccharin-drinking mice (*upper panels*), and digitized images used for density value evaluations (black and white) (*lower panels*) (*left*). Quantitative analysis of images of the PCA reaction (*right*) ($n=10$). Graph shows relative density. Values are shown as means $\pm$ SEM. Data were analyzed by Student's *t* test; $*P < 0.05$.

Supplemental Figure 5

The effects of sympathetic denervation on PCA reaction

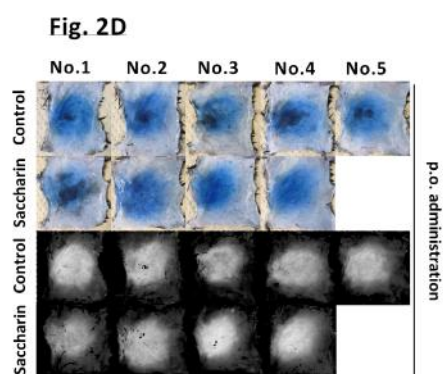
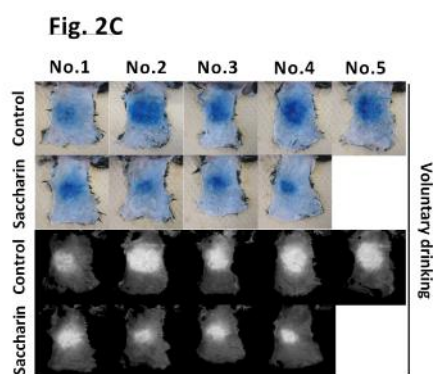
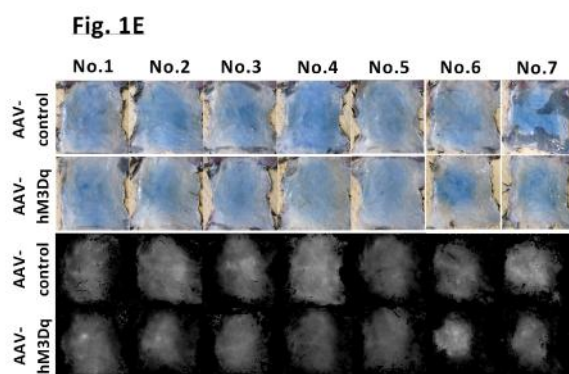


(A and B) Schematic illustration of the experimental design (A), and representative images of the PCA reaction in the skin of mice intraperitoneally treated with saline or 6-OHDA (*upper panels*), along with digitized images used for density value evaluations (black and white) (*lower panels*) (*left*). Quantitative analysis of images of the PCA reaction (*right*) (n=6) (B).

(C and D) Schematic illustration of the experimental design (C), and representative images of the PCA reaction in the skin of control mice and voluntary saccharin-drinking mice intraperitoneally treated with saline or 6-OHDA (*upper panels*), along with digitized images used for density value evaluations (black and white) (*lower panels*) (*left*). Quantitative analysis of images of the PCA reaction (*right*) (n=6-8) (D). (B and D) Graph shows relative density. Values are shown as means $\pm$ SEM. Data were analyzed by Student's t test (B) and one-way ANOVA with post hoc testing using Tukey-Kramer multiple comparisons (D); ** $P < 0.01$; n.s., not significant.

Supplemental Figure 6

Raw data of PCA reaction in Figure 1 and 2



Raw data of PCA reactions in Fig. 1E, and Fig. 2C and 2D, along with digitized images used for density value evaluations (black and white) (*lower panels*) are shown.