Journal of Pharmacological Sciences 157 (2025) 113–123

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Effect of antidepressants and social defeat stress on the activity of dorsal raphe serotonin neurons in free-moving animals

Masashi Koda [a], Hiroyuki Kawai [a,b], Hisashi Shirakawa [a], Shuji Kaneko [a], Kazuki Nagayasu [a,c,d,*]

[a] Department of Molecular Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto, 606-8501, Japan
[b] Department of Anatomy and Neuroscience, Graduate School of Medicine, Osaka Metropolitan University, 1-4-3 Asahi-cho, Abeno-ku, Osaka, 545-8585, Japan
[c] Laboratory of Molecular Neuropharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, 565-0871, Japan
[d] Project for Neural Networks, Drug Innovation Center, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, 565-0871, Japan

ARTICLE INFO

Keywords:
Serotonin
Antidepressant
Fiber photometry
Reward

ABSTRACT

Major depressive disorder (MDD) is among the most common mental disorders worldwide and is characterized by dysregulated reward processing associated with anhedonia. Selective serotonin reuptake inhibitors (SSRIs) are the first-line treatment for MDD; however, their onset of action is delayed. Recent reports have shown that serotonin neurons in the dorsal raphe nucleus (DRN) are activated by rewards and play a vital role in reward processing. However, whether antidepressant treatment affects the DRN serotonin neuronal response to rewards in awake animals remains unknown. In this study, we measured the activity of DRN serotonin neurons in awake mice and determined the effects of antidepressants and chronic stress on DRN serotonin neuronal activity. We found that acute treatment with citalopram, an SSRI, significantly decreased sucrose-induced activation of DRN serotonin neurons. The decrease in response to acute citalopram treatment was attenuated by chronic citalopram treatment. Acute treatment with (*S*)-WAY100135, a 5-HT_{1A} receptor antagonist, dose-dependently inhibited the response to acute citalopram treatment. These results indicate that autoinhibition by activating 5-HT_{1A} receptors via acute SSRI treatment may blunt the reward response, which can be recovered after chronic SSRI treatment.

1. Introduction

Major depressive disorder (MDD) is characterized by the persistence of negative thoughts and emotions that disrupt mood, cognition, motivation, and behavior. It is among the most common mental disorders and is the leading cause of disability worldwide, affecting approximately 280 million people.[1] Although patients with MDD are often treated with antidepressants such as selective serotonin reuptake inhibitors (SSRIs), chronic administration for 4–12 weeks is required to achieve therapeutic effectiveness.[2] Moreover, approximately one-third of the patients with MDD are resistant to conventional drug treatments.[3–5] Therefore, based on the biological insights of MDD and its therapeutics, the development of rapid-acting antidepressants with higher efficacy is urgently needed.

Accumulated evidence has implicated the critical role of 5-HT_{1A} receptors in the pathophysiology of depression. Although both serotonin neurons and their postsynaptic neurons express 5-HT_{1A} receptors, presynaptic and postsynaptic 5-HT_{1A} receptors have contrasting roles in stress response.[6] Postsynaptic 5-HT_{1A} receptors mediate serotonergic neurotransmission, thereby inducing antidepressive effects. In fact, a 5-HT_{1A} receptor agonist, buspirone, is used to treat anxiety and effective for depression.[7] In contrast, presynaptic 5-HT_{1A} receptors (autoreceptors) inhibit the activity of serotonin neurons and limit the efficacy of antidepressants.[6] Antidepressants such as SSRIs increase the extracellular serotonin concentration by inhibiting serotonin reuptake, leading to the stimulation of autoreceptors and postsynaptic receptors.[8] Previous electrophysiological experiments on anesthetized animals have demonstrated that slow- and regular-firing neurons in the dorsal raphe nucleus (DRN), presumably serotonin neurons, are suppressed by acute treatment with SSRIs via the activation of 5-HT_{1A} autoreceptors, which reverts after chronic SSRI treatment.[9–11] Consistent with this evidence, mice expressing high 5-HT_{1A} autoreceptor levels are more vulnerable to stress than those expressing low levels.[12] Therefore, downregulation of autoreceptors is thought to underlie the mechanism of action of the antidepressants. Although the electrophysiological experiments

* Corresponding author. Laboratory of Molecular Neuropharmacology, Project for Neural Networks, Drug Innovation Center, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, 565-0871, Japan.
E-mail address: nagayasu@phs.osaka-u.ac.jp (K. Nagayasu).

https://doi.org/10.1016/j.jphs.2025.01.001
Received 2 December 2024; Received in revised form 28 December 2024; Accepted 1 January 2025
Available online 2 January 2025

described above presumably identified serotonin neurons based on electrophysiological characteristics, other reports suggest the difficulty of performing such experiments on the DRN,[13,14] indicating the necessity for analyzing genetically identified serotonin neurons.

Moreover, serotonin neurons also play a critical role in reward processing. Researchers have demonstrated that optogenetic activation of serotonin neurons in the DRN, especially those projecting to the ventral tegmental area (VTA), acts as positive reinforcement in operant conditioning[15–17] and induces an antidepressant-like effect in rodents.[18,19] Moreover, activity measurements using genetically encoded calcium sensors, such as GCaMP6,[20] have revealed that DRN serotonin neurons are activated by rewards, including sucrose and interaction with other mice, especially those of the opposite sex.[21] These observations highlight the importance of DRN serotonin neurons in reward processing. However, it is unclear whether the reward response of DRN serotonin neurons is affected by acute and chronic antidepressant treatment because previous electrophysiological studies analyzed anesthetized animals, making it impossible to determine the response to rewards.

Stress modulates the activity of DRN serotonin neurons. *Ex vivo* electrophysiological experiments have shown that chronic social defeat stress (CSDS) decreases DRN serotonin neuronal activity in stress-vulnerable (susceptible) mice but not in stress-resilient mice.[22,23] Moreover, chronic stress attenuates the preference for rewards in rodents and humans, resulting in anhedonia.[24,25] However, the effect of chronic stress on the reward response of DRN serotonin neurons remains to be elucidated.

In this study, we investigated the effects of acute and chronic antidepressant treatment and chronic stress on the reward response of DRN serotonergic neurons in awake animals using fiber photometry. We found that acute treatment with citalopram, an SSRI, suppressed the reward response of DRN serotonin neurons via 5-HT_{1A} receptor activation, but this suppression disappeared after chronic citalopram treatment.

2. Materials and methods

2.1. Animals

The experiments were conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee (approval number: 19–41) and the guidelines of the Japanese Pharmacological Society. Adult male C57BL/6JmsSlc mice (8–12 weeks old, Nihon SLC, Shizuoka, Japan) were housed in a plastic cage with wooden bedding and allowed free access to food (MF, Oriental Yeast, Tokyo, Japan) and water unless otherwise stated. They were kept under constant ambient temperature (22 ± 2 °C) and humidity (55 ± 10%), with 12 h light–dark cycles. In this study, mice that showed abnormal behavior after stereotactic surgery, such as tilting their heads or rotating their bodies, were excluded from all experiments. Chronic social defeat stress was performed according to the previous reports[30–32] and described in Supplementary Methods.

2.2. Stereotactic surgeries

Stereotactic surgeries were conducted using a small-animal stereotactic frame (Narishige, Tokyo, Japan) according to the Brain Atlas.[27] The animals were anesthetized with a mixture of medetomidine hydrochloride (Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan; 0.75 mg/kg), midazolam (Astellas Pharma Inc, Tokyo, Japan; 4 mg/kg), and butorphanol tartrate (Meiji Seika Pharma Co., Ltd, Tokyo, Japan; 5 mg/kg). One microliter of AAV vector (Supplementary Methods; mTPH2::Venus, mTPH2::GCaMP6s, and mTPH2::axon-GCaMP6s) was microinjected targetting the ventral part of DRN (AP –4.6 mm, ML +1.15 mm, depth +3.7 mm at 20° angles from the bregma. After viral injection, the animals were implanted with a fiber-optic cannula above the DRN (AP –4.6 mm, ML +1.15 mm, DV +3.6 mm at 20° angles from the bregma) and VTA (AP –3.1 mm, ML +0.7 mm, DV +4.5 mm from the bregma).

2.3. Fiber photometry

Calcium transients were measured using a custom-built fiber photometry system fabricated according to previous reports.[28,29] The detail was described in Supplementary Methods. Fiber placement and AAV infection were confirmed by immunohistochemical analysis (Supplementary Methods).

2.4. Sucrose-licking test

The mice were deprived of water for 16–24 h before testing. On the test day, a mouse was placed in a chamber (136 × 208 × 115, W × L × H, in mm) equipped with a bottle filled with 10% sucrose solution (w/v).[26] The test was conducted for 10 min using a free-moving setup. The time points for sucrose licking were manually curated by analyzing the recorded movies.

2.5. Drug treatment

For acute antidepressant treatment, citalopram hydrobromide (FWD Chemicals, Shanghai, China, 10 mg/kg) was dissolved in saline and intraperitoneally injected into the mice 30 min before the behavioral tests. For chronic antidepressant treatment, citalopram hydrobromide was dissolved in drinking water (0.2 mg/mL) and administered for 28 days.[32,33] Water consumption was approximately 3–4 mL/day/mouse, resulting in doses of ~24 mg/kg/day. Drug-containing drinking water was shielded from light and changed every 3–5 days. For acute 5-HT_{1A} receptor antagonist treatment, (*S*)-WAY 100135 dihydrochloride (Tocris Bioscience, Bristol, UK) was dissolved in saline and intraperitoneally injected into mice 30 min before the behavioral tests.

2.6. Statistical analysis

Statistical analyses were performed using Prism 9 software (GraphPad Software, San Diego, CA, USA). The detail was described in Supplementary Methods.

3. Results

3.1. Serotonin neuron-specific gene expression transduced by AAVs bearing mouse TPH2 promoter

To confirm the specificity of the mouse TPH2 promoter, we injected AAV-expressing Venus[34] into the DRN under the control of the TPH2 promoter (AAV-mTPH2-Venus-WPRE).[17,26,35] The specificity of the promoter was immunohistochemically examined in AAV-mTPH2-Venus-WPRE (mTPH2::Venus)-injected mice (Fig. 1A and B), and high specificity (88.5 ± 2.7%) and coverage (90.1 ± 2.5%) were confirmed (n = 3 mice, Fig. 1C and D).

3.2. Reward activates DRN serotonin neurons

To measure the activity of serotonin neurons using fiber photometry, we injected AAV-mTPH2-GCaMP6s-WPRE (mTPH2::GCaMP6s) into the DRN and implanted a fiber optic cannula immediately above the DRN. After recovery, we measured GCaMP fluorescence before and after intake of 10% sucrose solution, a representative reward (Fig. 2A–C). GCaMP fluorescence increase was reliably observed shortly before the licking ($\Delta F/F_0 = 0.046 \pm 0.011$, mean ± SEM, $p < 0.05$ by permutation test, n = 4, Fig. 2D and E), consistent with previous reports.[21,39] The fluorescence intensity increased slightly but significantly decreased when the concentration of sucrose decreased ($R^2 = 0.1456$, $p < 0.0001$, Fig. 2F). Therefore, these results suggest successful recording of DRN

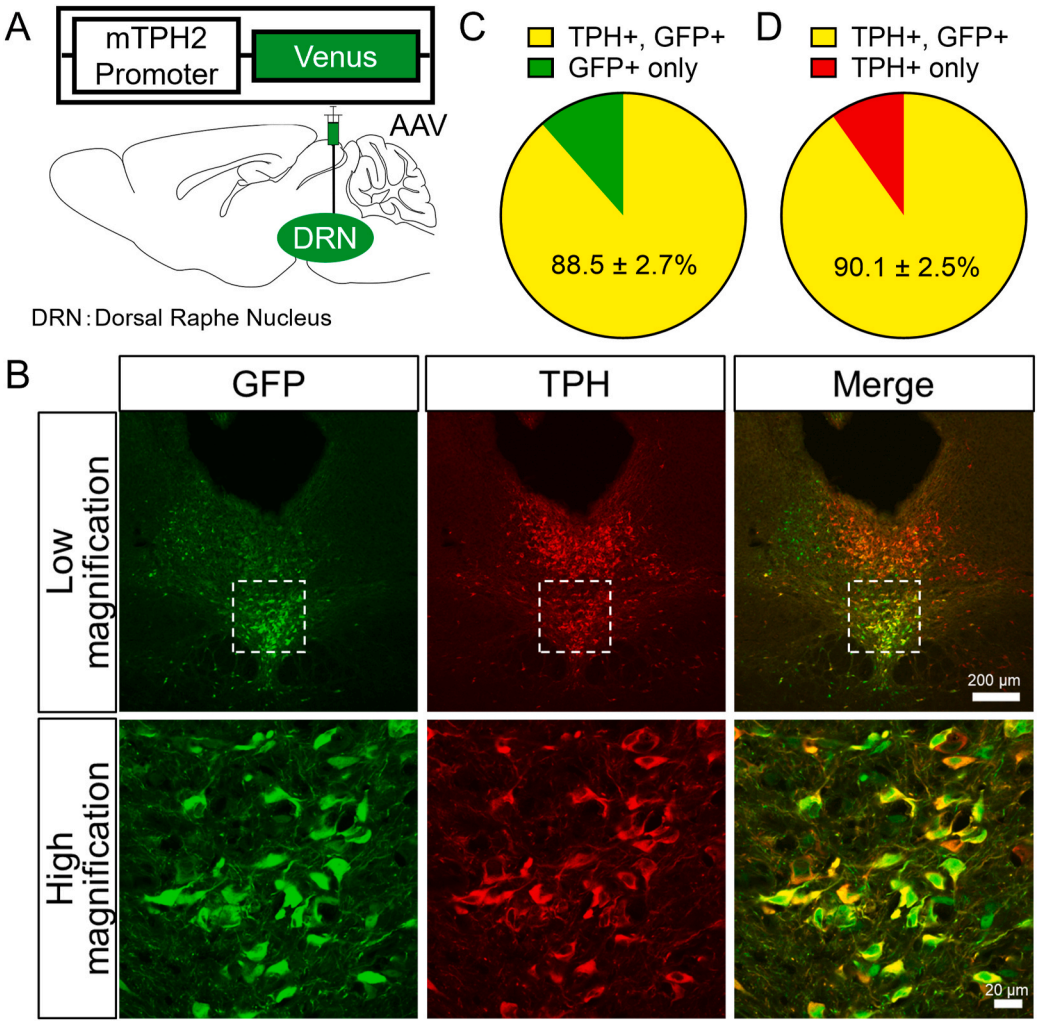


Fig. 1. Immunohistochemical validation of the AAV bearing the mouse TPH2 promoter. (A) Schema showing the sites of AAV (mTPH2Venus) injection. (B) Representative immunostaining images of DRN regions after AAV injection and staining with anti-green fluorescent protein (GFP) and anti-tryptophan hydroxylase (TPH) antibodies. Scale bars are 200 μm (Low) and 20 μm (High). (C) Data represent the mean ± SEM percentage of double-positive cells in GFP-positive cells ($n = 3$ mice). (D) Data represent the mean ± SEM percentage of double-positive cells in TPH-positive cells ($n = 3$ mice).

serotonin neurons in free-moving mice.

3.3. DRN serotonin neuronal activity after CSDS exposure

To determine whether the reward responses of DRN serotonin neurons were affected by CSDS, we measured the response of DRN serotonin neurons to spontaneous sucrose-licking behavior before and after CSDS exposure in free-moving mice using fiber photometry (Fig. 3A and B). We examined whether defeated mice showed depression-related behavior in the social interaction test (Supplementary Fig. 1A). Based on previous studies,[30,32] we classified the defeated mice into two groups based on the SI ratio, an index of antidepressant-like effects (Supplementary Fig. 1B): susceptible mice (Sus) and resilient mice (Res). We found that sucrose licking-induced Ca^{2+} responses were not significantly changed by CSDS in all groups (naïve: peak $\Delta F/F_0 = 0.0867 \pm 0.0167$ (before), 0.076 ± 0.0188 (after), $p = 0.649$, $t_9 = 0.471$ by two-tailed paired Student's *t*-tests, $n = 10$ mice; resilient: peak $\Delta F/F_0 = 0.0609 \pm 0.0140$ (before), 0.0757 ± 0.0184 (after), $p = 0.264$, $t_9 = 1.19$ by two-tailed paired Student's *t*-tests, $n = 10$ mice; susceptible: peak $\Delta F/F_0 = 0.0994 \pm 0.0181$ (before), 0.0812 ± 0.0239 (after), $p = 0.464$, $t_{10} = 0.761$ by two-tailed paired Student's *t*-tests, $n = 11$ mice, Fig. 3C). The percentage change in peak $\Delta F/F_0$ before and after the CSDS session and sucrose licking did not differ significantly among the three groups (naïve: 23.3 ± 23.4%; resilient: 37.7 ± 16.2%; susceptible: 23.6 ± 29.3%, $p = 0.891$, $F_{2,28} = 0.116$ by one-way ANOVA, $n = 10$–11 mice, Fig. 3C and D).

A previous study showed that the activity of DRN serotonin neurons is also induced by social interaction.[21] Therefore, we determined whether the responses of DRN serotonin neurons to social interactions were affected by CSDS. The ratio of peak $\Delta F/F_0$ after CSDS to that before CSDS in susceptible mice tended to be higher than that in naïve mice (naïve: −33.8 ± 12.0%; resilient: −20.5 ± 13.4%; susceptible: 43.7 ± 33.2%, $p = 0.0550$, $F2,27 = 3.61$ (susceptible vs. naïve) by one-way ANOVA, Bonferroni's multiple comparisons test, $n = 10$ mice, Supplementary Fig. 1E). These results suggest a negligible effect on the reward response of DRN serotonin neurons.

3.4. Acute SSRI treatment decreases the reward response of DRN serotonin neurons, whereas chronic SSRI treatment recovers the response

We measured GCaMP fluorescence in DRN serotonin neurons during sucrose licking (Fig. 4) and social interaction tests (Supplementary Fig. 2) before and after acute and chronic citalopram treatment in free-moving mice. First, we measured the response of DRN serotonin neurons to sucrose licking (before SSRI administration; Fig. 4B, C, and E). One day later, half of the mice were administered saline and the other half

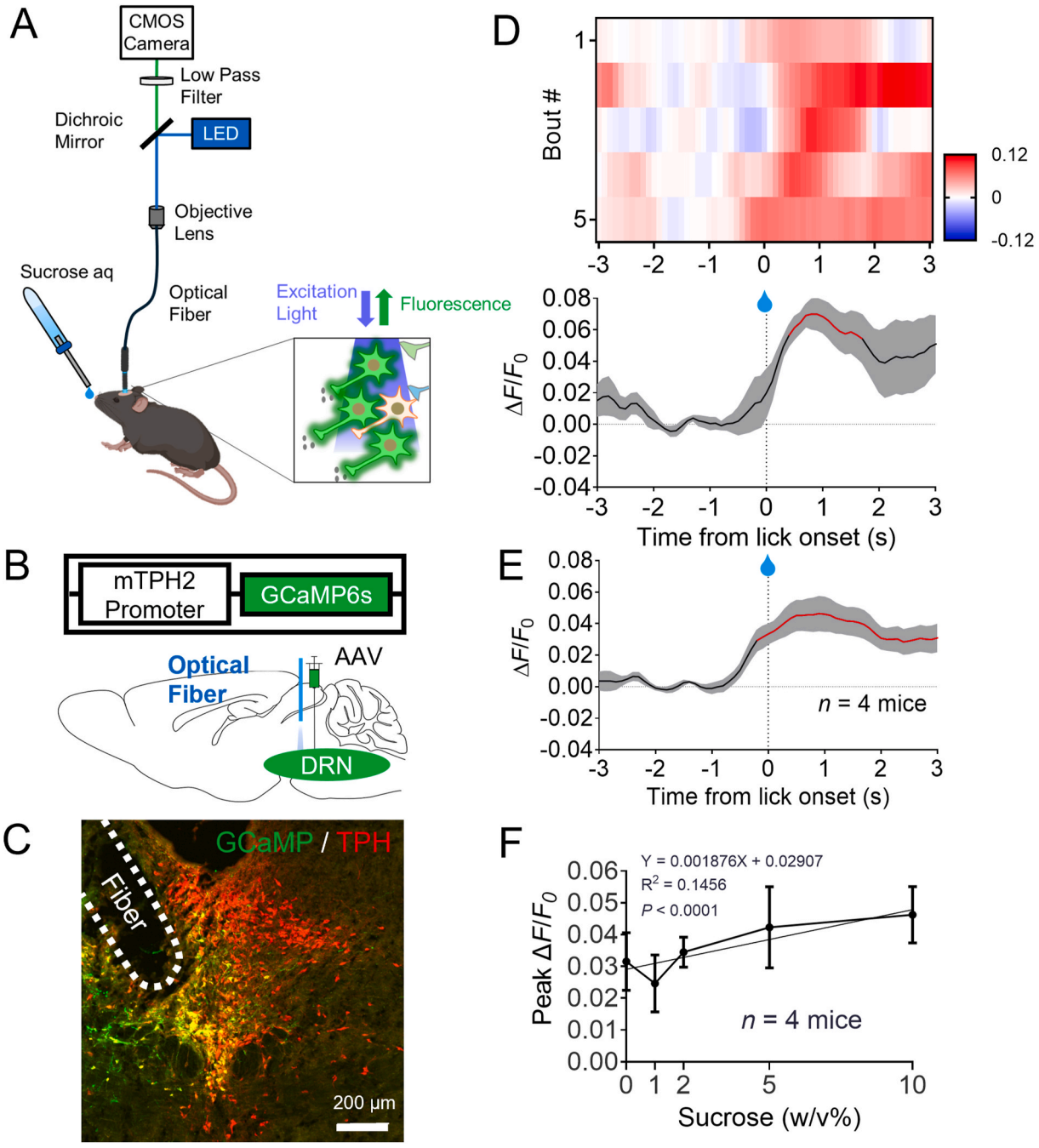


Fig. 2. Serotonin neuron-specific Ca^{2+} imaging in free-moving animals. (A) Schematic of the fiber photometry setup. Ca^{2+} transients were recorded from GCaMP6s-expressing DRN serotonin (5-HT) neurons in male C57BL/6JmsSlc mice that had free access to a sucrose solution in a test chamber. (B) Schema showing the sites of AAV (mTPH2::GCaMP6s) injection and optic fiber implantation. (C) Representative immunostaining image of the DRN after injection of mTPH2::GCaMP6s and fiber implantation (white line). Scale bar is 200 μm. (D) Ca^{2+} signals associated with sucrose lick bouts during behavioral sessions. Upper panel: heatmap illustration of Ca^{2+} signals aligned with the initiation of sucrose-licking bouts. Each row is plotted as a bout. Five bouts from one animal are shown. The color scale at the right indicates $\Delta F/F_0$. Lower panel: peri-event plot of average Ca^{2+} transients and lick frequencies. The thick lines and shaded areas indicate the mean and SEM, respectively. Red segments indicate a statistically significant increase from baseline ($p < 0.05$, permutation test). (E) Mean Ca^{2+} transients associated with sucrose licking in the entire test group ($n = 4$). Red segments indicate a statistically significant increase from baseline ($p < 0.05$, permutation test). (F) Dose-response curve of the fluorescence changes with sucrose concentration. Values are presented as the mean ± SEM.

were administered citalopram (10 mg/kg, i.p.). The sucrose-licking test was performed 30 min later (Fig. 4C and D). We found that acute citalopram treatment significantly attenuated the sucrose-induced increase in GCaMP fluorescence (change rate of peak $\Delta F/F_0$: saline: 39.2 ± 21.9%, acute citalopram: −42.5 ± 9.86%, $p = 0.0039$, $t_{15.3} = 3.40$ by unpaired *t*-test with Welch's correction; change rate of AUC: saline: 58.1 ± 34.3%, acute citalopram: −41.0 ± 20.2%, $p = 0.0209$, $t_{22} = 2.49$ by unpaired *t*-test; $n = 12$ mice, Fig. 4C and D).

Mice injected with saline were provided drinking water, and mice injected with citalopram were provided drinking water containing citalopram (0.2 mg/mL, ~24 mg/kg/day) for 28 days.[33] After this chronic treatment, the sucrose-licking test was performed again (Fig. 4E and F). We chose this dosing strategy based on our previous report showing that this protocol sustainedly increases active-coping behaviors in the tail

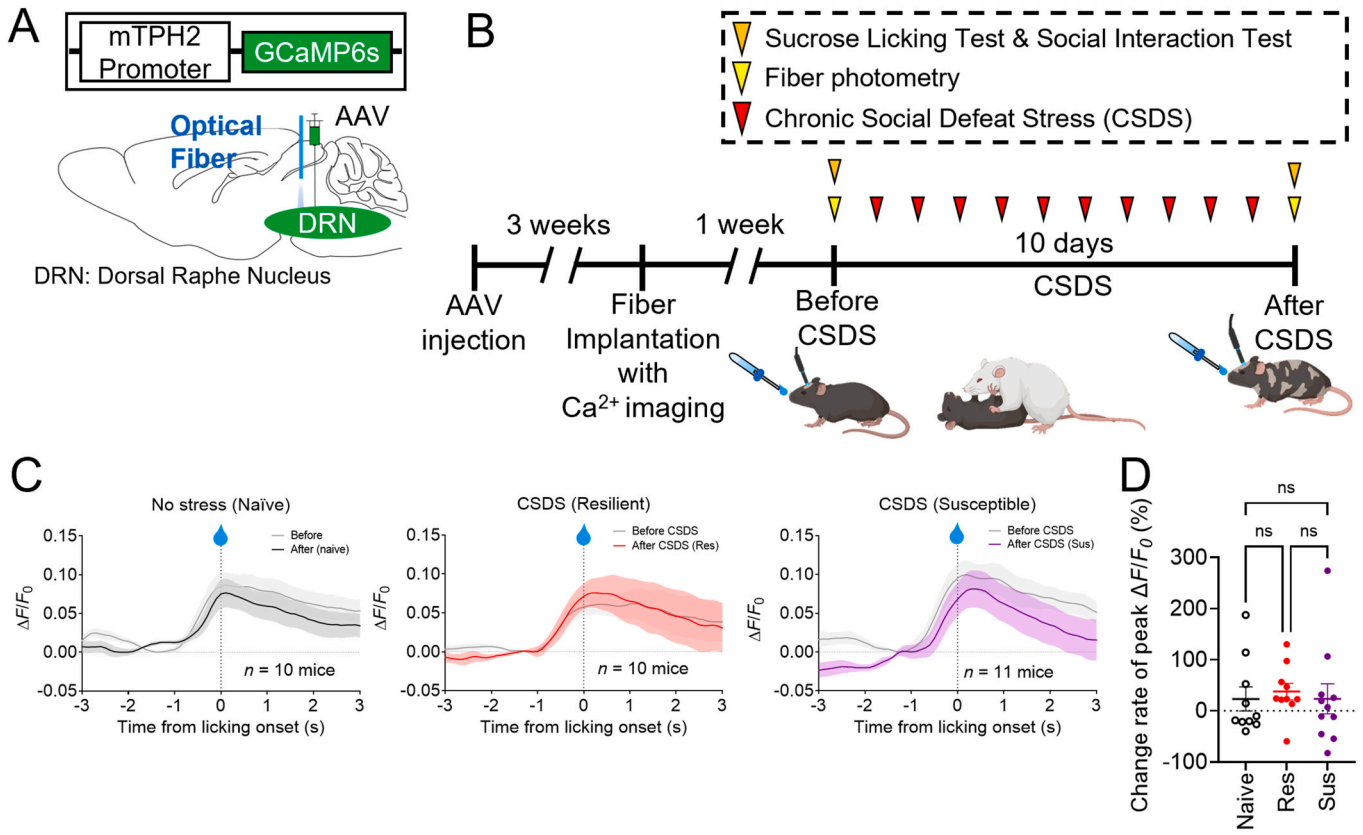


Fig. 3. Measurement of DRN serotonin neuronal activity after chronic social defeat stress (CSDS) based on sucrose licking. (A) Schema showing the sites of AAV (mTPH2::GCaMP6s) injection and optic fiber implantation. (B) Experimental timeline. For the CSDS, C57BL/6JmsSlc mice were exposed to 5 min of physical aggression by an aggressive ICR mouse for 10 consecutive days. (C) Ca^{2+} signals associated with bouts of sucrose licking in a behavioral session: left, naïve mice; middle, resilient mice; right, susceptible mice. The light and dark lines indicate the values before and after the CSDS session, respectively, in the sucrose-licking test. (D) Percentage change in peak $\Delta F/F_0$ before and after CSDS session followed by sucrose licking. Values are presented as the mean ± SEM. $p = 0.891$, $F_{2,28} = 0.1156$ by one-way ANOVA (n = 10–11 mice per group).

suspension test.[32] The sucrose-induced GCaMP fluorescence increase in mice with chronic citalopram treatment was similar to those in mice given water (change rate of peak $\Delta F/F_0$: water: 31.7 ± 21.3%, chronic citalopram: 6.91 ± 23.1%, $p = 0.437$, $t_{22} = 0.791$ by unpaired t-test; change rate of AUC: water: 52.1 ± 37.9%, chronic citalopram: 22.5 ± 31.0%, $p = 0.552$, $t_{22} = 0.604$ by unpaired t-test; $n = 12$ mice, Fig. 4E and F).

In contrast, the responses of the DRN serotonin neurons to social interactions were not significantly affected by acute or chronic citalopram treatment (Supplementary Figs. 2D–G). Collectively, our results suggest that the response of DRN serotonergic neurons to appetitive rewards, but not to social rewards, was suppressed by acute SSRI treatment, whereas the response recovered after chronic SSRI treatment. Moreover, we investigated whether the response changes to sucrose affected the number of sucrose licking behavior after acute and chronic citalopram treatment. However, there were no significant difference among groups (Supplementary Fig. 3A).

3.5. *5-HT_{1A}R antagonist reverses the reduced response to sucrose induced by acute SSRI treatment*

To elucidate how acute SSRI treatment reduced the response of DRN serotonin neurons to sucrose, we investigated the effects of 5-HT_{1A}R antagonists. (*S*)-WAY100135, a 5-HT_{1A}R antagonist, was co-administered with citalopram during the sucrose-licking test. A few days after recording the response to sucrose (before administering SSRI), citalopram (10 mg/kg, i.p.) were co-administered with increasing doses of (*S*)-WAY100135 (0–20 mg/kg, i.p., every alternate day) 30 min before the test (Fig. 5). This co-treatment restored the decreased response of DRN serotonin neurons to sucrose licking induced by acute citalopram treatment in a dose-dependent manner (Fig. 5C).

No significant difference was observed between mice with citalopram alone and those with citalopram and low dose (3.0 mg/kg) of (*S*)-WAY100135 (change rate of peak $\Delta F/F_0$: saline: 36.1 ± 36.5%, (*S*)-WAY (3.0 mg/kg): 20.7 ± 15.0%, citalopram: −63.1 ± 5.21%, citalopram + (*S*)-WAY (3.0 mg/kg): −26.4 ± 20.9%, $p = 0.0077$ (saline vs. citalopram), $p = 0.720$ (citalopram vs. citalopram + (*S*)-WAY), $p = 0.161$ (saline vs. citalopram + (*S*)-WAY), $F_{3,37} = 4.35$; change rate of AUC: saline: 49.9 ± 38.6%, (*S*)-WAY (3.0 mg/kg): 10.1 ± 21.5%, citalopram: −93.0 ± 9.11%, citalopram + (*S*)-WAY (3.0 mg/kg): −50.5 ± 36.1%, $p = 0.00530$ (saline vs. citalopram), $p > 0.999$ (citalopram vs. citalopram + (*S*)-WAY), $p = 0.106$ (saline vs. citalopram + (*S*)-WAY), $F_{3,37} = 5.12$ by one-way ANOVA, Bonferroni's multiple comparisons test, n = 10–11 mice, Fig. 5D and E). By contrast, co-treatment with citalopram and high dose (20 mg/kg) of (*S*)-WAY100135 significantly mitigated the reduced response of DRN serotonin neurons induced by citalopram only (change rate of peak $\Delta F/F_0$: saline: 7.80 ± 30.8%, (*S*)-WAY (3.0 mg/kg): −13.2 ± 8.82%, citalopram: −63.1 ± 11.6%, citalopram + (*S*)-WAY (3.0 mg/kg): 13.1 ± 22.0%, $p = 0.0370$ (saline vs. citalopram), $p = 0.0229$ (citalopram vs. citalopram + (*S*)-WAY), $p > 0.999$ (saline vs. citalopram + (*S*)-WAY), $F_{3,27} = 3.62$; change rate of AUC: saline: −0.12 ± 21.5%, (*S*)-WAY (3.0 mg/kg): −33.4 ± 9.88%, citalopram: −117.8 ± 21.3%, citalopram + (*S*)-WAY (3.0 mg/kg): −43.3 ± 17.6%, $p = 0.0006$ (saline vs. citalopram), $p = 0.0448$ (citalopram vs. citalopram + (*S*)-WAY), $p = 0.756$ (saline vs. citalopram + (*S*)-WAY), $F_{3,27} = 7.79$ by one-way ANOVA, Bonferroni's multiple

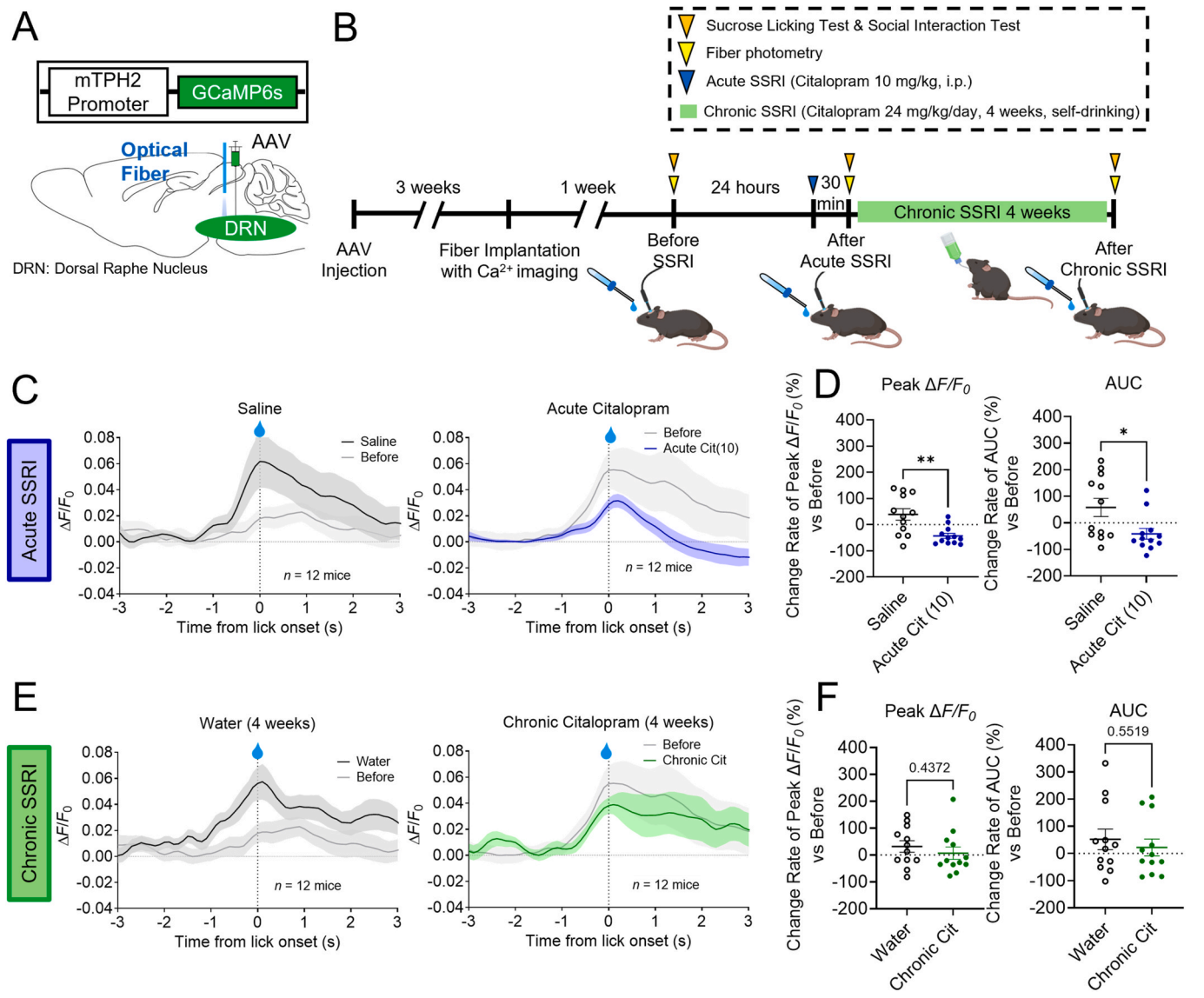


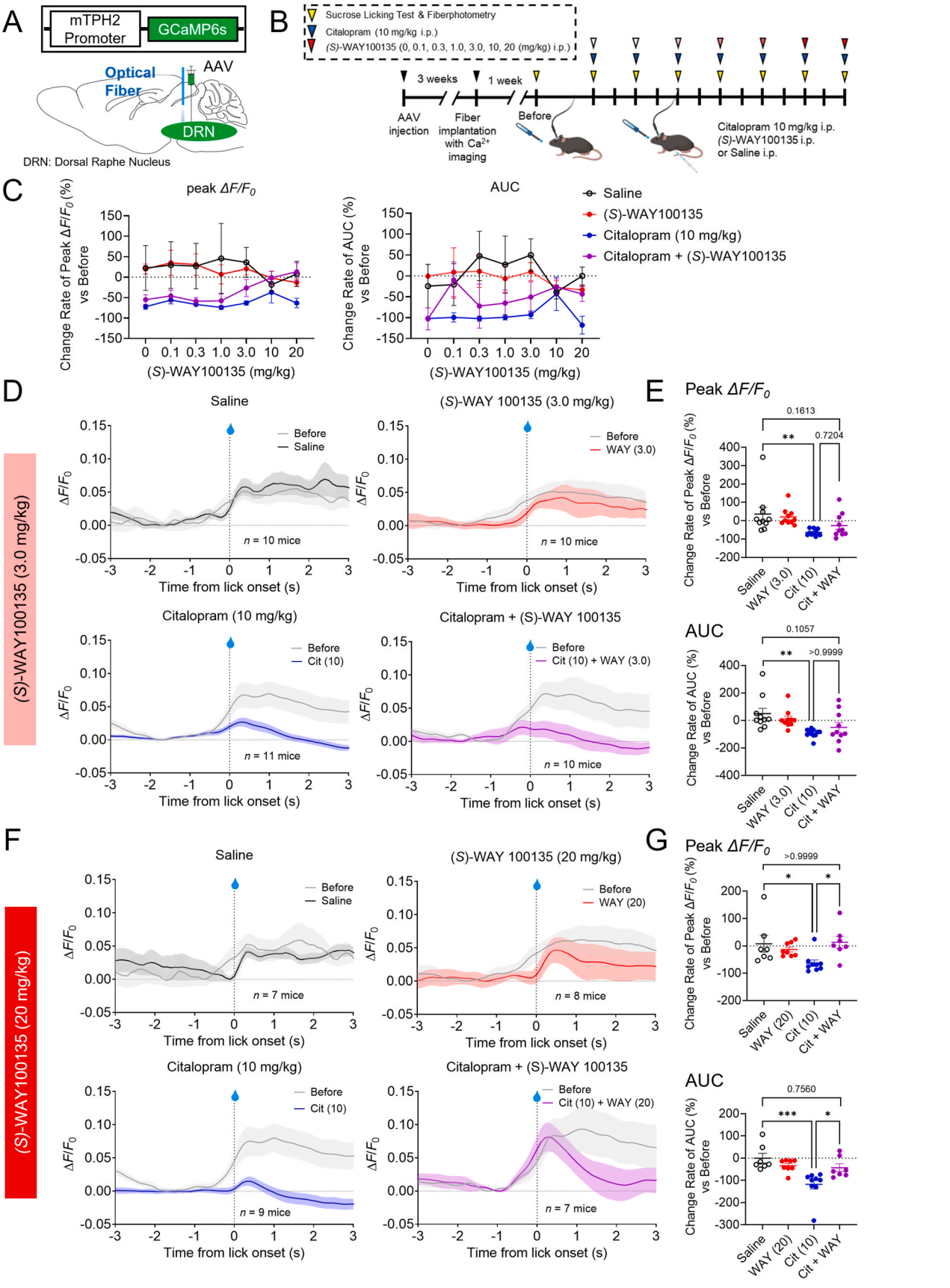
Fig. 4. Measurement of DRN serotonin neuronal activity after acute and chronic SSRI treatment and sucrose-licking test. (A) Schema showing the sites of AAV injection and optic fiber implantation. (B) Experimental timeline. (C,E) Ca^{2+} response patterns of the DRN serotonin neurons in the entire test group at the onset of sucrose licking. On day 1, naïve session without drug treatment was performed (C, E; "Before" group; light line). One day later, an acute SSRI treatment session in which citalopram (10 mg/kg, i.p.) was administered 30 min before the sucrose-licking test was performed (C, right; blue line). Chronic SSRI sessions were performed after administering 0.2 mg/mL citalopram in drinking water, p.o. for 4 weeks (E, right; green line). (D) Left: percentage change in peak ΔF/F0 before and after acute citalopram in sucrose licking. $^{**}p = 0.0039$, $t_{15.3} = 3.40$ by unpaired *t*-test with Welch's correction, $n = 12$ mice. Right: percentage change in AUC before and after acute citalopram in sucrose licking. $^{*}p = 0.0209$, $t_{22} = 2.49$ by unpaired *t*-test, $n = 12$ mice. (F) Left: percentage change in peak ΔF/F0 before and after chronic citalopram in sucrose licking. $p = 0.437$, $t_{22} = 0.791$ by unpaired *t*-test, $n = 12$ mice. Right: percentage change in AUC before and after chronic citalopram in sucrose licking. $p = 0.552$, $t_{22} = 0.604$ by unpaired *t*-test, $n = 12$ mice. Values are presented as the mean ± SEM.

comparisons test, n = 7–9 mice, Fig. 5F and G). Furthermore, we investigated whether the response changes to sucrose affected the number of sucrose licking behavior with or without 5-HT_{1A} receptor antagonist. However, there were no significant difference among groups (Supplementary Figs. 3B and C). These results suggest that acute SSRI treatment reduces sucrose licking-induced activation of DRN serotonin neurons by activating 5-HT_{1A} autoreceptors.

3.6. *Effect of SSRI on the reward response of VTA-projecting DRN serotonin neurons*

The DRN generates neuronal projections to various brain regions.[36,37] To compare the projection densities among brain regions that receive input from the DRN,[37] we measured the fluorescence intensities of mTPH2Venus in the VTA, lateral hypothalamuc (LH), central nucleus of amygdala (CeA), ventral pallidum (VP), and nucleus accumbens (NAc). The fluorescence intensity in the VTA was significantly higher than that in the other regions, indicating dense innervation of the VTA ($p < 0.0001$; VTA vs. other regions, $F_{4,17} = 32.3$, one-way ANOVA, Bonferroni's multiple comparison post hoc test; n = 4–5 mice, Fig. 6).

The VTA is among the most important brain nuclei involved in reward processing. The optogenetic stimulation of DRN serotonin neurons projecting to the VTA reinforces behaviors associated with stimulation.[16,17] Here, we measured the activity of DRN serotonin neurons projecting to the VTA to examine the effect of rewards on DRN serotonin neuronal activity. We injected AAV-mTPH2-axon-GCaMP6s-WPRE (mTPH2::axon-GCaMP6s), which is more efficiently transported to the axon-terminal region.[38] A fiber optic cannula was implanted above the VTA. We then measured the fluorescence changes in the VTA before and after sucrose consumption (Fig. 7A and B). Sucrose licking increased



(caption on next page)

Fig. 5. 5-HT_{1A}R antagonist restores the decreased response to sucrose induced by acute SSRI treatment. (A) Schema showing the sites of AAV injection and optic fiber implantation. (B) Experimental timeline. (*S*)-WAY100135 was administered every alternate day, starting with the lowest concentration. (C) Dose-response curve of the change rate of Ca^{2+} signals against (*S*)-WAY100135 concentration in sucrose-licking test. Values are presented as the mean ± SEM, $n = 7$–11 mice. (Left: peak $\Delta F/F_0$, Right: AUC). (D, E) Representative figure for low dose of (*S*)-WAY100135 (3.0 mg/kg i.p.). (F, G) Representative figure for high dose of (*S*)-WAY100135 (20 mg/kg i.p.). A single administration of citalopram (10 mg/kg, i.p., Cit) significantly decreased sucrose licking-induced activation of serotonin neurons in the DRN. Moreover, cotreatment with citalopram and (*S*)-WAY100135 (WAY) restored the decreased response to sucrose induced by acute citalopram treatment alone. Values are presented as the mean ± SEM, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ by one-way ANOVA and Bonferroni's multiple comparisons test; $n = 10$–11 (3.0 mg/kg) and $n = 7$–9 (20 mg/kg) mice.

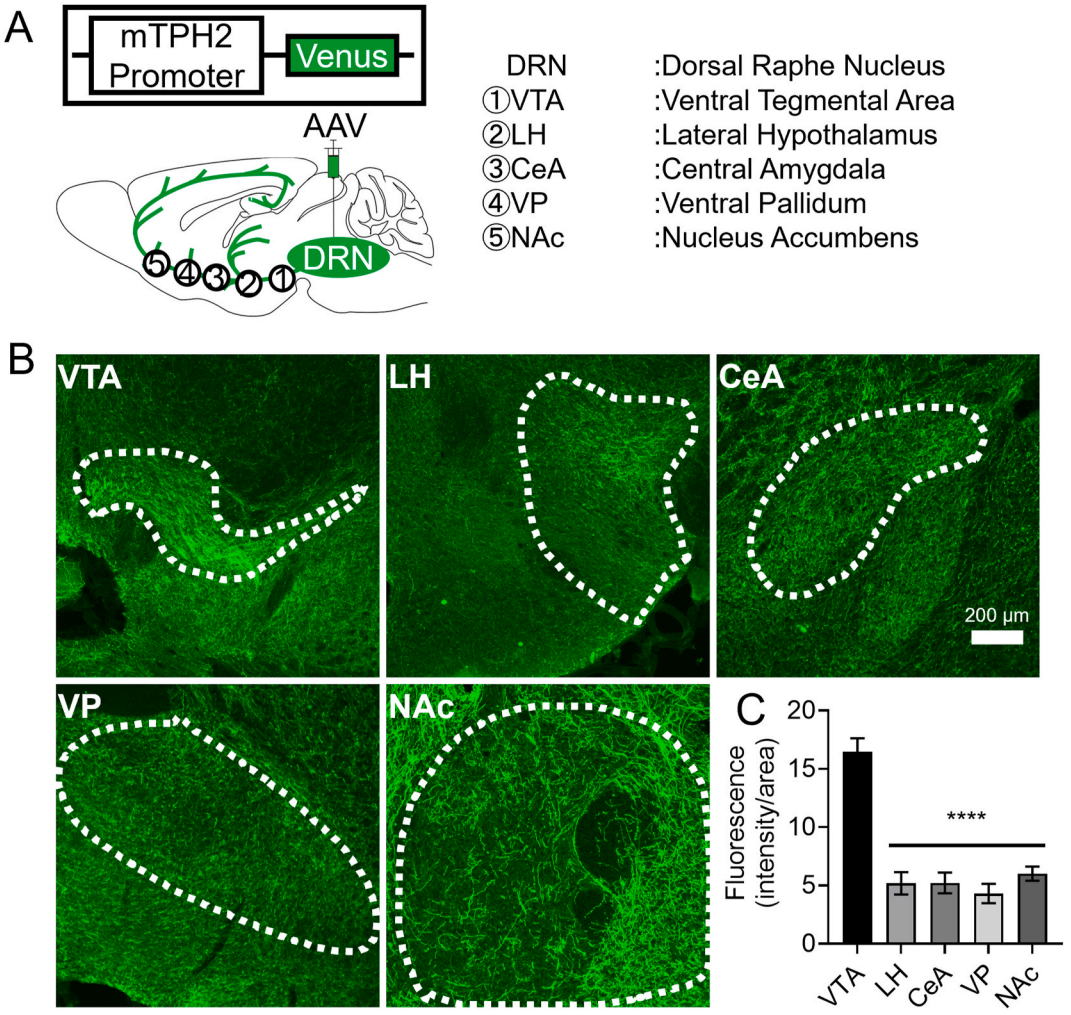


Fig. 6. Immunohistochemical examination of neuronal projection density of DRN serotonin neurons. (A) Schema showing the sites of AAV (mTPH2::Venus) injection and neuronal projection area. (B) Images of axon terminals of DRN serotonin neurons in the VTA, lateral hypothalamuc (LH), central nucleus of amygdala (CeA), ventral pallidum (VP), and nucleus accumbens (NAc). Scale bar is 200 μm. (C) Quantification of the fluorescence at each terminal area. Of the five regions, DRN serotonin neurons were projected the most densely into the VTA. $^{****}p < 0.0001$ vs. VTA by one-way ANOVA, Bonferroni's post hoc test; $n = 4$–5 mice. Values are presented as the mean ± SEM.

GCaMP fluorescence in naïve mice ($\Delta F/F_0 = 0.0025 \pm 0.00061$, $p < 0.05$ by permutation test, $n = 3$ mice, Fig. 7C). This result indicates that VTA-projecting serotonin neurons in the DRN are activated by rewards. Next, to determine the effects of acute and chronic antidepressant use on VTA-projecting DRN serotonin neuronal activity, we measured the response to rewards in SSRI-treated mice. The GCaMP fluorescence was not significantly changed during sucrose consumption in the mice acutely treated with citalopram (10 mg/kg, i.p.) ($\Delta F/F_0 = 0.0010 \pm 0.00078$, $p > 0.05$ by permutation test, $n = 3$ mice, Fig. 7C). In contrast, the GCaMP fluorescence was significantly increased during sucrose consumption in the mice chronically treated with citalopram (0.2 mg/mL, ~24 mg/kg/day, 28 days) ($\Delta F/F_0 = 0.0040 \pm 0.00060$, $p < 0.05$ by permutation test, $n = 3$ mice, Fig. 7C).

4. Discussion

DRN serotonin neurons are activated by various stimuli, including appetitive rewards, social rewards, and reward-associated cues after learning.[21,39] Although this has been confirmed in free-moving animals using fiber photometry, the effects of chronic stress and antidepressants on serotonin neuronal activity have been identified using *ex vivo* slice electrophysiology, in which the response to rewards cannot be examined.[22,33] Although fiber photometry has been widely used to measure the activity of certain sets of neurons in free-moving animals, relatively few studies have used this technique to investigate the effects of therapeutic/illicit drugs on neuronal activity.[40,41] In this study, we used fiber photometry to measure DRN serotonin neuronal response in

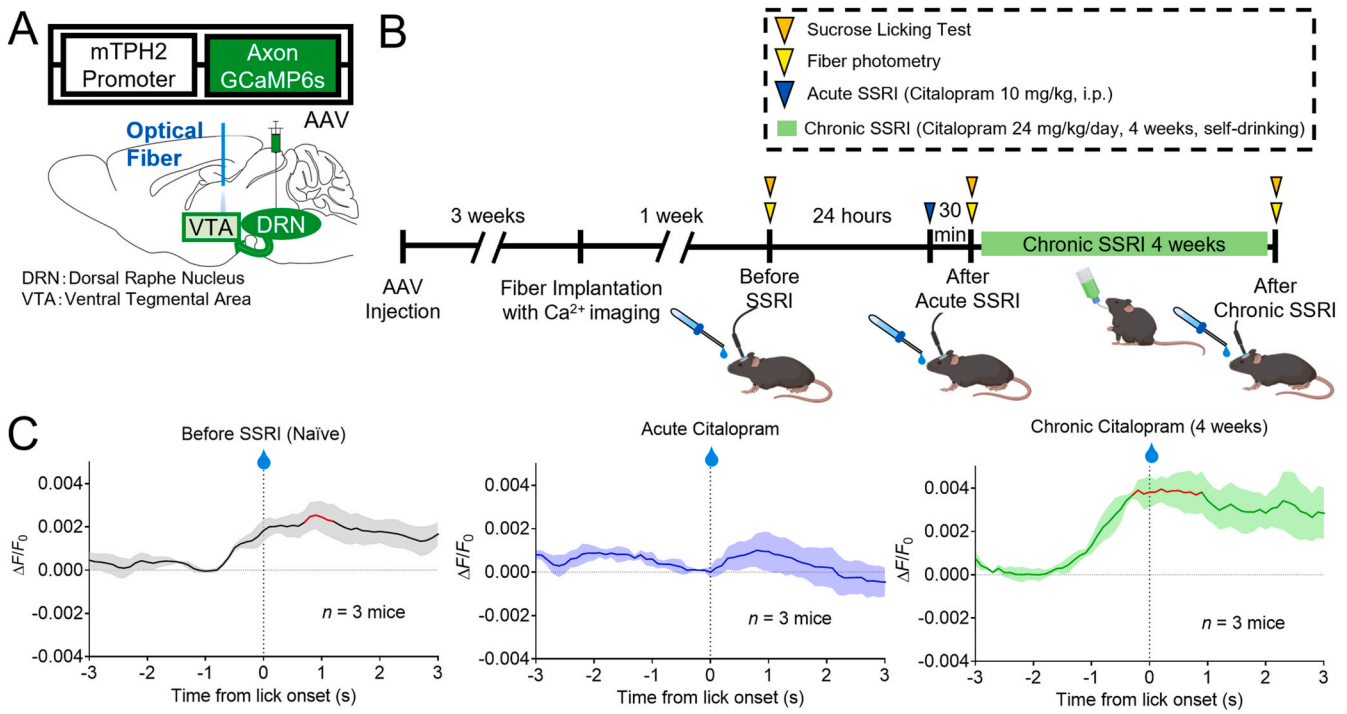


Fig. 7. Effect of antidepressants on the activity of VTA-projecting DRN serotonin neurons stimulated by rewards. (A) Schema showing the sites of AAV (mTPH2::Axon-GCaMP6s) injection and optic fiber implantation. (B) Experimental timeline. (C) Ca^{2+} response patterns of DRN serotonin neurons in the entire test group at the onset of sucrose licking. Acute treatment with SSRI decreased sucrose licking-induced activation of VTA-projecting DRN serotonin neurons (middle graph). Moreover, the decreased response to sucrose induced by acute SSRI treatment recovered after chronic treatment with SSRI for 4 weeks (right graph). Red segments indicate a statistically significant increase from baseline ($p < 0.05$, permutation test).

free-moving mice subjected to chronic stress and antidepressants. We found no significant differences in the activation of DRN serotonin neurons by rewards among naïve, susceptible, and resilient mice after CSDS. In contrast, Challis et al. demonstrated that DRN serotonin neuronal excitability decreased in susceptible mice after CSDS in *ex vivo* experiments involving slice electrophysiology.[22] This discrepancy may be explained by the possibility that the low excitability of DRN serotonin neurons may be compensated by excitatory input from other nuclei. Indeed, glutamatergic neuronal activation in the lateral habenula (LHb) was increased by social interactions with an aggressive mouse in susceptible mice after CSDS,[42] and optogenetic stimulation of LHb neurons increases the excitability of DRN serotonin neurons *ex vivo*.[43] Therefore, increased LHb activity may mask the low excitability of DRN serotonin neurons in susceptible mice; however, further analysis is necessary to manipulate LHb-DRN circuits.

Previous studies have indicated that acute SSRI treatment inhibits DRN serotonin neuronal activity via 5-HT_{1A} activation, which disappears after chronic SSRI treatment.[9–11] Although these studies measured the activity of neurons presumed to be serotonin neurons based on their electrophysiological characteristics, recent studies have raised questions regarding these conventional criteria,[13,14] indicating the necessity for analyzing genetically identified serotonin neurons. Moreover, it is unclear whether the suppression of baseline activity through 5-HT_{1A} autoreceptors also occurs in the reward response (activity changes) of serotonin neurons. In this context, we found that acute citalopram treatment significantly blunted the response of genetically defined DRN serotonin neurons to sucrose, and chronic treatment with citalopram for 4 weeks reversed the blunted response to sucrose in free-moving mice. Although these results aligned with previous findings in anesthetized animals, we have to note that different responses to sucrose may stem from different administration routes of citalopram between acute and chronic treatment. Moreover, acute co-treatment with (*S*)-WAY100135, a 5-HT_{1A} antagonist, mitigated the decreased response to sucrose following acute citalopram treatment in a dose-dependent manner. These results expand on the findings of previous *in vivo* electrophysiological studies in anesthetized animals,[9] revealing for the first time the decreased response of serotonin neurons to appetitive rewards after acute SSRI treatment and its recovery after chronic SSRIs treatment.

Although our results indicate that co-administration of SSRI and 5-HT_{1A}R antagonist recovered the DRN serotonin neuronal response similar to chronic SSRI administration, the temporal patterns of the increased response were apparently different. The 5-HT_{1A}R antagonist recovered the response in the early phase of sucrose-induced activity increase, whereas chronic SSRI treatment recovered the whole response. Therefore, in addition to 5-HT_{1A} autoreceptors, other factors may be involved in the increase in response following chronic SSRI treatment. Challis et al. have also shown that GABAergic neurons innervate serotonin neurons and inhibit their neural activity in the DRN.[22] Moreover, 5-HT_{2B} receptors and Gq-coupled GPCRs are expressed in GABAergic neurons and positively regulate their activity in the DRN.[44–46] High serotonin release induced by acute SSRI treatment may activate 5-HT_{1A} autoreceptors expressed on serotonin neurons and 5-HT_{2B} receptors expressed on GABAergic neurons in the DRN, which cooperatively inhibit serotonin neuronal activity. Although further examination is required, chronic SSRI treatment may also induce desensitization of 5-HT_{2B} receptors expressed on GABAergic neurons in the DRN, and thus treatment with SSRIs, 5-HT_{1A}R antagonists, and 5-HT_{2B}R antagonists may mimic the effects of chronic SSRI treatment. Moreover, we found that there were no significant differences in licking response among mice with or without SSRI and 5-HT1A receptor antagonist (Supplementary Fig. 3), while the response of DRN serotonin neurons were different. We assume that highly palatable 10% sucrose solution may interfere with behavioral changes induced by activity changes of the DRN serotonin neurons. Indeed, the sucrose preference test used for evaluation of anhedonia usually utilized 1–2% sucrose solution,[47] supporting this possibility and the necessity of analysis of activity changes in DRN serotonin neurons induced by 1–2% sucrose solution.

In this study, we conducted only experiments with citalopram as a

representative SSRI according to our previous studies,[32,33] although previous studies have used not only citalopram but also other SSRIs, such as fluoxetine or escitalopram.[2,9] Because these conventional SSRIs commonly inhibit the serotonin transporter, it is possible that the inhibition by acute treatment and recovery after chronic treatment, which we observed in this study, may be induced by other SSRIs. In line with this possibility, in our previous in vitro studies, we investigated the effects of acute treatment with and sustained exposure to a variety of antidepressants, including SSRIs (citalopram and fluoxetine), SNRIs (duloxetine, venlafaxine, and milnacipran), and tricyclics (imipramine and desipramine), on extracellular serotonin levels in rat raphe slice cultures, a proxy of serotonergic activity.[48] We found that chronic treatment with antidepressants, which inhibit serotonin transporter, commonly induced a robust increase in extracellular serotonin levels compared to acute treatment. These data indicate that the phenomena observed in this study may be caused by other antidepressants that inhibit serotonin transporter, although further experimental validation with other antidepressants is necessary.

Several reports have shown heterogeneity within DRN serotonin neurons from an anatomical perspective.[49,50] Specifically, brain-wide connectome analyses have revealed that dorsal/dorsolateral DRN serotonin neurons mainly project to subcortical areas, including the amygdala, LHb, and thalamic nuclei, whereas ventral subpopulations innervate anterior cortical regions, such as the orbitofrontal, entorhinal, and piriform cortices.[49] Notably, fiber photometry analyses indicated that aversive stimulation inhibits and activates cortex-projecting and amygdala-projecting DRN serotonin neurons, respectively,[49] suggesting the functional heterogeneity of DRN serotonin neurons. In this context, we confirmed that DRN serotonin neurons targeted by viral vectors bearing the TPH2 promoter,[17,18] have projection patterns similar to those described previously.[37,49] We found that DRN serotonin neurons innervate the VTA heavily compared to other brain areas, including the amygdala and hypothalamus.

Using axon-targeted GCaMP6,[38] we measured the reward response of DRN serotonin neurons projecting to the VTA. This subpopulation was activated by sucrose licking, thus indicating that VTA-projecting serotonin neurons in the DRN encode reward signals. Moreover, according to the results of the sugar-licking test, DRN serotonin nerve terminals in the VTA were affected by acute and chronic SSRI treatments in the same manner as the soma in the DRN. The VTA is one of the most critical brain nuclei involved in reward processing. It contains many neurons that synthesize dopamine, a key neurotransmitter for reward.[51] Electrical stimulation of the VTA increases extracellular dopamine levels in the nucleus accumbens and acts as positive reinforcement in the intracranial self-stimulation paradigm.[52] Electrophysiological experiments have suggested that the activity of midbrain dopamine neurons encodes a reward prediction error, i.e., the difference between received and predicted rewards, which acts as a teaching signal for learning.[53] Taken together, our histological analyses suggest that DRN serotonin neurons projecting to the VTA play a critical role in reward processing. Along with other researchers, we have consistently shown that stimulation and inhibition of DRN serotonin neurons projecting to the VTA have positive and negative valences, respectively.[16,17]

By using the fiber photometry technique in free-moving animals, this study demonstrated that acute SSRI treatment reduces sucrose licking-induced activation of DRN serotonin neurons via 5-HT_{1A} autoreceptors, and co-treatment with SSRIs and 5-HT_{1A}R antagonists partly mimic the effects of chronic SSRI treatment within a shorter timeframe. These findings provide insights into the development of rapid-acting antidepressants.

CRediT authorship contribution statement

Masashi Koda: Writing – original draft, Visualization, Investigation, Data curation. **Hiroyuki Kawai:** Visualization, Resources, Investigation. **Hisashi Shirakawa:** Visualization, Resources, Investigation. **Shuji Kaneko:** Supervision, Funding acquisition. **Kazuki Nagayasu:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Funding

This work was supported by JST FOREST (grant number JPMJFR2268 (K.N.)); JSPS KAKENHI (grant numbers JP20H04774 (K.N.), JP20K07064 (K.N.), JP24K22019 (K.N.), JP24K02352 (K.N.) and JP20H00491 (S.K.)); and AMED (grant numbers JP20ak0101088h0003 (S.K.) and JP21ak0101153h0001 (S.K.)). Grants were also obtained from the Mochida Memorial Foundation for Medical and Pharmaceutical Research (K.N.), the Suzuken Memorial Foundation (K.N.), the Lotte Research Promotion Grant (K.N.), and the Takeda Science Foundation (K.N.).

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

We thank Drs. Nagai and Miyoshi (pCSII-EF-Venus) for providing the construct.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphs.2025.01.001.

References

1. World Health Organization. Depressive disorder (depression). https://www.who.int/news-room/fact-sheets/detail/depression; 2023. Accessed December 3, 2024.
2. Kryst J, Majcher-Maślanka I, Chocyk A. Effects of chronic fluoxetine treatment on anxiety- and depressive-like behaviors in adolescent rodents – systematic review and meta-analysis. *Pharmacol Rep*. 2022;74:920–946. https://doi.org/10.1007/s43440-022-00420-w.
3. Rush AJ, Trivedi MH, Wisniewski SR, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry*. 2006;163:1905–1917. https://doi.org/10.1176/ajp.2006.163.11.1905.
4. Belmaker RH, Agam G. Major depressive disorder. *N Engl J Med*. 2008;358:55–68. https://doi.org/10.1056/nejmra073096.
5. Schlaepfer TE, Agren H, Monteleone P, et al. The hidden third: Improving outcome in treatment-resistant depression. *J Psychopharmacol*. 2012;26:587–602. https://doi.org/10.1177/0269881111431748.
6. Albert PR, Vahid-Ansari F. The 5-HT1A receptor: signaling to behavior. *Biochimie*. 2019;161:34–45. https://doi.org/10.1016/j.biochi.2018.10.015.
7. Dimitriou EC, Dimitriou CE. Buspirone augmentation of antidepressant therapy. *J Clin Psychopharmacol*. 1998;18:465–469. https://doi.org/10.1097/00004714-199812000-00009.
8. Tatsumi M, Jansen K, Blakely RD, Richelson E. Pharmacological profile of neuroleptics at human monoamine transporters. *Eur J Pharmacol*. 1999;368:277–283. https://doi.org/10.1016/s0014-2999(99)00005-9.
9. El Mansari M, Sánchez C, Chouvet G, Renaud B, Haddjeri N. Effects of acute and long-term administration of escitalopram and citalopram on serotonin neurotransmission: an in vivo electrophysiological study in rat brain. *Neuropsychopharmacology*. 2005;30:1269–1277. https://doi.org/10.1038/sj.npp.1300686.
10. Hajós M, Gartside SE, Sharp T. Inhibition of median and dorsal raphe neurones following administration of the selective serotonin reuptake inhibitor paroxetine. *Naunyn-Schmiedeberg's Arch Pharmacol*. 1995;351:624–629. https://doi.org/10.1007/BF00170162.
11. Czachura JF, Rasmussen K. Effects of acute and chronic administration of fluoxetine on the activity of serotonergic neurons in the dorsal raphe nucleus of the rat. *Naunyn-Schmiedeberg's Arch Pharmacol*. 2000;362:266–275. https://doi.org/10.1007/s002100000290.
12. Richardson-Jones JW, Craige CP, Guiard BP, et al. 5-HT1A autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron*. 2010;65:40–52. https://doi.org/10.1016/j.neuron.2009.12.003.
13. Allers KA, Sharp T. Neurochemical and anatomical identification of fast- and slow-firing neurones in the rat dorsal raphe nucleus using juxtacellular labelling methods in vivo. *Neuroscience*. 2003;122:193–204. https://doi.org/10.1016/S0306-4522(03)00518-9.
14. Kocsis B, Varga V, Dahan L, Sik A. Serotonergic neuron diversity: identification of raphe neurons with discharges time-locked to the hippocampal theta rhythm. *Proc*

Natl Acad Sci U S A. 2006;103:1059–1064. https://doi.org/10.1073/pnas.0508360103.
15. Liu Z, Zhou J, Li Y, et al. Dorsal raphe neurons signal reward through 5-HT and glutamate. *Neuron*. 2014;81:1360–1374. https://doi.org/10.1016/j.neuron.2014.02.010.
16. Wang HL, Zhang S, Qi J, et al. Dorsal raphe dual serotonin-glutamate neurons drive reward by establishing excitatory synapses on VTA mesoaccumbens dopamine neurons. *Cell Rep*. 2019;26:1128–1142.e7. https://doi.org/10.1016/j.celrep.2019.01.014.
17. Nagai Y, Takayama K, Nishitani N, et al. The role of dorsal raphe serotonin neurons in the balance between reward and aversion. *Int J Mol Sci*. 2020;21:2160. https://doi.org/10.3390/ijms21062160.
18. Nishitani N, Nagayasu K, Asaoka N, et al. Manipulation of dorsal raphe serotonergic neurons modulates active coping to inescapable stress and anxiety-related behaviors in mice and rats. *Neuropsychopharmacology*. 2019;44:721–732. https://doi.org/10.1038/s41386-018-0254-y.
19. Ohmura Y, Tsutsui-Kimura I, Sasamori H, et al. Different roles of distinct serotonergic pathways in anxiety-like behavior, antidepressant-like, and anti-impulsive effects. *Neuropharmacology*. 2020;167, 107703. https://doi.org/10.1016/j.neuropharm.2019.107703.
20. Chen TW, Wardill TJ, Sun Y, et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature*. 2013;499:295–300. https://doi.org/10.1038/nature12354.
21. Li Y, Zhong W, Wang D, et al. Serotonin neurons in the dorsal raphe nucleus encode reward signals. *Nat Commun*. 2016;7, 10503. https://doi.org/10.1038/ncomms10503.
22. Challis C, Boulden J, Veerakumar A, et al. Raphe GABAergic neurons mediate the acquisition of avoidance after social defeat. *J Neurosci*. 2013;33:13978–13988. https://doi.org/10.1523/JNEUROSCI.2383-13.2013.
23. Challis C, Beck SG, Berton O. Optogenetic modulation of descending prefrontocortical inputs to the dorsal raphe bidirectionally bias socioaffective choices after social defeat. *Front Behav Neurosci*. 2014;8:43. https://doi.org/10.3389/fnbeh.2014.00043.
24. Krishnan V, Han MH, Graham DL, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*. 2007;131:391–404. https://doi.org/10.1016/j.cell.2007.09.018.
25. Fawcett J, Clark DC, Scheftner WA, Gibbons RD. Assessing anhedonia in psychiatric patients. *Arch Gen Psychiatry*. 1983;40:79–84. https://doi.org/10.1001/archpsyc.1983.01790010081010.
26. Kawai H, Bouchekioua Y, Nishitani N, et al. Median raphe serotonergic neurons projecting to the interpeduncular nucleus control preference and aversion. *Nat Commun*. 2022;13:7708. https://doi.org/10.1038/s41467-022-35346-7.
27. Franklin KBJ, Paxinos G. *The Mouse Brain in Stereotaxic Coordinates*. third ed. New York, NY: Academic Press; 2007.
28. Gunaydin LA, Grosenick L, Finkelstein JC, et al. Natural neural projection dynamics underlying social behavior. *Cell*. 2014;157:1535–1551. https://doi.org/10.1016/j.cell.2014.05.017.
29. Kim CK, Yang SJ, Pichamoorthy N, et al. Simultaneous fast measurement of circuit dynamics at multiple sites across the mammalian brain. *Nat Methods*. 2016;13:325–328. https://doi.org/10.1038/nmeth.3770.
30. Golden SA, Covington HE, Berton O, Russo SJ. A standardized protocol for repeated social defeat stress in mice. *Nat Protoc*. 2011;6:1183–1191. https://doi.org/10.1038/nprot.2011.361.
31. Andoh C, Nishitani N, Hashimoto E, et al. TRPM2 confers susceptibility to social stress but is essential for behavioral flexibility. *Brain Res*. 2019;1704:68–77. https://doi.org/10.1016/j.brainres.2018.09.031.
32. Andoh C, Otani S, Noguchi T, et al. Transcriptional landscape of the dorsal raphe serotonin neurons rendering stress resiliency. *bioRxiv*. 2024;25. https://doi.org/10.1101/2024.03.21.586199. Published online March.
33. Asaoka N, Nishitani N, Kinoshita H, et al. Chronic antidepressant potentiates spontaneous activity of dorsal raphe serotonergic neurons by decreasing GABAB receptor-mediated inhibition of L-type calcium channels. *Sci Rep*. 2017;7, 13609. https://doi.org/10.1038/s41598-017-13599-3.
34. Nagai T, Ibata K, Park ES, Kubota M, Mikoshiba K, Miyawaki A. A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nat Biotechnol*. 2002;20:87–90. https://doi.org/10.1038/nbt0102-87.
35. Nagai Y, Kisaka Y, Nomura K, et al. Dorsal raphe serotonergic neurons preferentially reactivate dorsal dentate gyrus cell ensembles associated with positive experience. *Cell Rep*. 2023;42, 112149. https://doi.org/10.1016/j.celrep.2023.112149.
36. Azmitia EC, Segal M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J Comp Neurol*. 1978;179:641–667. https://doi.org/10.1002/cne.901790311.
37. Muzerelle A, Scotto-Lomassese S, Bernard JF, Soiza-Reilly M, Gaspar P. Conditional anterograde tracing reveals distinct targeting of individual serotonin cell groups (B5–B9) to the forebrain and brainstem. *Brain Struct Funct*. 2016;221:535–561. https://doi.org/10.1007/s00429-014-0924-4.
38. Broussard GJ, Liang Y, Fridman M, et al. In vivo measurement of afferent activity with axon-specific calcium imaging. *Nat Neurosci*. 2018;21:1272–1280. https://doi.org/10.1038/s41593-018-0211-4.
39. Zhong W, Li Y, Feng Q, Luo M. Learning and stress shape the reward response patterns of serotonin neurons. *J Neurosci*. 2017;37:8863–8875. https://doi.org/10.1523/JNEUROSCI.1181-17.2017.
40. Wei C, Han X, Weng D, et al. Response dynamics of midbrain dopamine neurons and serotonin neurons to heroin, nicotine, cocaine, and MDMA. *Cell Discov*. 2018;4:60. https://doi.org/10.1038/s41421-018-0060-z.
41. Yoshida K, Drew MR, Mimura M, Tanaka KF. Serotonin-mediated inhibition of ventral hippocampus is required for sustained goal-directed behavior. *Nat Neurosci*. 2019;22:770–777. https://doi.org/10.1038/s41593-019-0376-5.
42. Wang D, Li Y, Feng Q, Guo Q, Zhou J, Luo M. Learning shapes the aversion and reward responses of lateral habenula neurons. *Elife*. 2017;6, e23045. https://doi.org/10.7554/eLife.23045.001.
43. Hernandez Silva JC, Pausic N, Marroquin Rivera A, Labonté B, Proulx CD. Chronic social defeat stress induces pathway-specific adaptations at lateral habenula neuronal outputs. *J Neurosci*. 2024;44, e2082232024. https://doi.org/10.1523/jneurosci.2082-23.2024.
44. Cathala A, Devroye C, Drutel G, Revest JM, Artigas F, Spampinato U. Serotonin 2B receptors in the rat dorsal raphe nucleus exert a GABA-mediated tonic inhibitory control on serotonin neurons. *Exp Neurol*. 2019;311:57–66. https://doi.org/10.1016/j.expneurol.2018.09.015.
45. Cathala A, Lucas G, López-Terrones E, Revest JM, Artigas F, Spampinato U. Differential expression of serotonin$_{2B}$ receptors in GABAergic and serotoninergic neurons of the rat and mouse dorsal raphe nucleus. *Mol Cell Neurosci*. 2022;121, 103750. https://doi.org/10.1016/j.mcn.2022.103750.
46. Belmer A, Quentin E, Diaz SL, et al. Positive regulation of raphe serotonin neurons by serotonin 2B receptors. *Neuropsychopharmacology*. 2018;43:1623–1632. https://doi.org/10.1038/s41386-018-0013-0.
47. Primo MJ, Fonseca-Rodrigues D, Almeida A, Teixeira PM, Pinto-Ribeiro F. Sucrose preference test: a systematic review of protocols for the assessment of anhedonia in rodents. *Eur Neuropsychopharmacol*. 2023;77:80–92. https://doi.org/10.1016/j.euroneuro.2023.08.496.
48. Nagayasu K, Kitaichi M, Nishitani N, et al. Chronic effects of antidepressants on serotonin release in rat raphe slice cultures: high potency of milnacipran in the augmentation of serotonin release. *Int J Neuropsychopharmacol*. 2013;16:2295–2306.
49. Ren J, Friedmann D, Xiong J, et al. Anatomically defined and functionally distinct dorsal raphe serotonin sub-systems. *Cell*. 2018;175:472–487.e20. https://doi.org/10.1016/j.cell.2018.07.043.
50. Paquelet GE, Carrion K, Lacefield CO, Zhou P, Hen R, Miller BR. Single-cell activity and network properties of dorsal raphe nucleus serotonin neurons during emotionally salient behaviors. *Neuron*. 2022;110:2664–2679.e8. https://doi.org/10.1016/j.neuron.2022.05.015.
51. Ungerstedt U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol Scand Suppl*. 1971;82:1–48. https://doi.org/10.1111/j.1365-201X.1971.tb10998.x.
52. Fiorino DF, Coury A, Fibiger HC, Phillips AG. Electrical stimulation of reward sites in the ventral tegmental area increases dopamine transmission in the nucleus accumbens of the rat. *Behav Brain Res*. 1993;55:131–141. https://doi.org/10.1016/0166-4328(93)90109-4.
53. Schultz W. Dopamine reward prediction-error signalling: a two-component response. *Nat Rev Neurosci*. 2016;17:183–195. https://doi.org/10.1038/nrn.2015.26.